Non-linear Association between Serum 25-Hydroxyvitamin D and All-cause Mortality in Adults with Inflammatory Bowel Disease in a Prospective Cohort Study

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CD, Crohn's disease; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; IBD, inflammatory bowel disease; MET, metabolic equivalent of the task; NHS, National Health Service; RCS, restricted cubic spline; SD, standard deviation; TDI, Townsend deprivation index; UC, Ulcerative colitis

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Abstract

Background: Non-linear association between serum 25-hydroxyvitamin D [25(OH)D] concentration and all-cause mortality has been widely reported for the general population, but this association has not been quantified for individuals with inflammatory bowel disease (IBD).

Objectives: This was to explore the association between serum 25(OH)D and all-cause mortality in individuals with IBD.

Methods: We identified 2690 females and 2532 males aged 40–69 with diagnosed IBD at baseline in the UK Biobank. Serum 25(OH)D concentration was measured by direct competitive chemiluminescent immunoassay. The outcome was all-cause mortality, ascertained via the death registry. Cox proportional hazard regression was used to evaluate associations between serum 25(OH)D in quintiles and all-cause mortality among individuals with IBD (Crohn's disease [CD, *n* = 1760] and ulcerative colitis [UC, *n* = 3462]). Restricted cubic splines (RCS) were used to investigate potential non-linearity.

Results: During the mean follow-up period of 11.9 years, 529 deaths (198 in CD and 331 in UC) were documented among 5222 individuals with IBD. Compared with the lowest quintile of serum 25(OH)D, hazard ratios and 95% confidence intervals for the second to the highest quintiles were 0.82 (0.63, 1.06), 0.63 (0.47, 0.83), 0.64 (0.48, 0.85), and 0.74 (0.55, 0.99), respectively. Non-linearity was detected in the dose-response association between serum 25(OH)D concentration and all-cause mortality (*P*-nonlinearity < 0.001), and 25(OH)D concentrations of 44–78 nmol/L were associated with a 50% lower risk of all-cause mortality (vs. 10 nmol/L). Subgroup analyses showed that the non-linear association mostly applied to females (*P*-nonlinearity < 0.001 vs 0.080 in males).

Conclusions: We observed a non-linear association, mostly applied to the females, between serum 25(OH)D concentrations and all-cause mortality among individuals with IBD. A

concentration range of 44–78 nmol/L of 25(OH)D can serve as a starting point for future research to confirm recommended 25(OH)D concentrations for individuals with IBD.

Keywords: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Vitamin D; 25-hydroxyvitamin D; All-cause mortality

Introduction

Individuals with inflammatory bowel disease (IBD) are widely reported to have a high prevalence of vitamin D deficiency (1), which is defined as a serum 25-hydroxyvitamin D [25(OH)D] concentration below 50 nmol/L (2). Low serum 25(OH)D concentration has been reported to be associated with worse clinical outcomes of IBD. A prospective single-center cohort study showed that a serum 25(OH)D concentration below 87.5 nmol/L in patients in remission was associated with a 25% increased risk of endoscopic-determined ulcerative colitis (UC) relapse compared with individuals with higher 25(OH)D concentrations (3). In addition, several studies have reported that increased latitude (4-6) and decreased ultraviolet light intensity (7, 8) were also associated with increased incidence and severity of IBD, which may relate to changes in serum 25(OH)D concentration. However, it remains unclear whether serum 25(OH)D concentration is associated with all-cause mortality in individuals with IBD. A non-linear association between serum 25(OH)D and all-cause mortality has been widely reported in the general population; thus, an optimal 25(OH)D range may exist (9-12). Individuals with IBD are at a higher risk of premature death than the general population (13-15). Given that serum 25(OH)D may be influenced by the pathological changes of IBD, the association between serum 25(OH)D concentration and all-cause mortality may differ for individuals with IBD. Therefore, it is necessary to explore the association between serum 25(OH)D and all-cause mortality among individuals with IBD and to estimate the protective effect of 25(OH) quantitatively.

This study aims to investigate the association between serum 25(OH)D concentration and all-cause mortality in individuals with IBD and provide a reference concentration for clinical practice.

Methods

Study population

The UK Biobank is a large prospective cohort study that enrolled approximately 500,000 individuals aged 40–69 from 2006 to 2010 (16). The project involved baseline questionnaires, physical measurements, blood and urine sample storage, and follow-up of medical or health-related records of individuals across the United Kingdom (17). All participants signed electronic informed consents at recruitment, and all available resources are listed on the UK Biobank website (18). The North West–Haydock Research Ethics Committee granted ethical approval for the UK Biobank (REC reference: 16/NW/0274).

Individuals with IBD were identified from primary care data and inpatient data that were embedded in the UK National Health Service (NHS). We also included self-reported diagnostic information at baseline. Participants were asked about the disease they had, which was confirmed in a verbal interview by trained staff at the UK Biobank and recorded according to a coding tree with specific mappings to the three-character ICD-10 codes (19). Participants with baseline IBD were coded as ICD-10 codes K50 and K51 or ICD-9 codes 555 and 556. We excluded participants who (1) did not have IBD subtype information (n = 164), (2) were simultaneously diagnosed with UC and CD (n = 18), or (3) did not have serum 25(OH)D concentration information (n = 558). More details are shown in **Supplementary Figure 1**.

Measurement of serum 25(OH)D concentration

Blood samples were collected at baseline and serum 25(OH)D concentration was measured using direct competitive chemiluminescent immunoassay on the platform Diasorin Liaison XL (detection range: 10–375 nmol/L). The coefficient of variation for known internal quality

control samples ranged from 5.04% to 6.14%, and the results of the external quality assurance were 100%. More details about the procedure have been published online (20). Outlying serum 25(OH)D concentration (n = 1, concentration = 230 nmol/L) was set as the second-highest concentration (148 nmol/L) (21).

Ascertainment of outcome

The outcome of interest was all-cause mortality. The date of death was extracted from NHS Digital for participants in England and Wales and from the NHS Central Register for participants in Scotland. These data sources record the deaths of all participants in the UK Biobank. Details of the linkage procedure are available online (22). Follow-up ended at the time of death, loss, or the end of follow-up (March 13, 2021), whichever came first.

Covariates

Covariates were selected based on a *priori* knowledge and previous studies (12, 23): sociodemographic characteristics (age, sex, ethnicity, Townsend deprivation index [TDI], and education level); factors directly associated with serum 25(OH)D concentration (season of blood sampling, time spent outdoors in summer and winter, and vitamin D and multivitamin supplement use); lifestyle factors (body mass index [BMI], smoking status, alcohol consumption, physical activity and consumption of fresh fruit, raw or salad vegetables, processed meat, and fish); inflammation indicator (serum C-reactive protein [CRP]); as well as baseline comorbidities, including self-reported heart disease (heart attack, angina, and stroke), hypertension, and cancer. More details are provided in **Supplementary Methods**.

Statistical analysis

Baseline characteristics of participants with IBD were summarized as means with standard

deviations (SD) for continuous variables, and percentages for categorical variables, stratified by serum 25(OH)D concentration in quintiles. Cox proportional hazard regression was applied to investigate the associations between serum 25(OH)D concentration and all-cause mortality in participants with IBD. We first evaluated the hazard ratios (HRs) of serum 25(OH)D concentration in quintiles and set the lowest quintile of 25(OH)D concentration as the reference group for the categorical model (24). The restricted cubic spline (RCS) method was used with three knots at the 10^{th}, 50^{th}, and 90^{th} percentiles to test the potential non-linear association (25-27).

RCS is a sum of polynomial functions that does not assume a linear association between variables and response. RCS can also provide ranges for risk function (28). The lowest value of 25(OH)D concentration (10 nmol/L) was taken as the reference level when using the RCS method. We also investigated the range of serum 25(OH)D concentrations associated with a 50% lower risk of all-cause mortality in the RCS dose-response curves. The overall significance of the spline curve was tested using the likelihood ratio test. Proportional hazard assumptions were confirmed using the weighted residuals method ($P = 0.15$) (29).

Four models were used to evaluate the association. Model 1 included sociodemographic characteristics: age, age squared, sex, ethnicity (White or non-White), TDI, and education level (college or below). Given the possible non-linear association between age and all-cause mortality, we included age-squared in the model. Model 2 was additionally adjusted for factors directly associated with the serum 25(OH)D concentration: season of blood sampling (spring, summer, autumn, or winter), time spent outdoors in summer, time spent outdoors in winter, use of vitamin D supplements (yes or no) and use of multivitamin supplements (yes or no). Model 3 was additionally adjusted for lifestyle factors: BMI, smoking status (never, previous or current), alcohol consumption status (current or not current), physical activity level (low, moderate, high, or unknown), consumption of fresh fruit and raw or salad

vegetables, and frequency of processed meat and fish intake. Model 4 was further adjusted for self-reported heart disease, hypertension, and cancer (yes or no) as a fully adjusted model. Missing values were imputed using medians for continuous variables and the most common categories for the categorical variables. The physical activity level was imputed using the missing-indicator method (30), due to excessive missing values (n = 1,057, 20.2%). The associations were also investigated separately among participants with CD and UC, using the fully adjusted model.

In the subgroup analyses, we explored the associations stratified by main covariates. We then tested the interaction between these covariates and serum 25(OH)D concentration by entering a cross-product interaction term in model 4.

We also conducted several sensitivity analyses to test the robustness of the results. Based on the fully adjusted model, we further: (1) excluded deaths in the first 1-, 2-, and 3-year periods, respectively, to reduce the influence of reverse causality; (2) adjusted for the serum CRP level to examine the impact of inflammation and disease activity; (3) reprocessed the missing values using multiple imputations to address the potential influence of the imputing method (31); (4) applied two different methods to address the seasonal variations in 25(OH)D concentration. The first approach was seasonal cut-off points (32, 33), which recategorized the exposure according to the season-specific quintiles of the measured concentrations. The second method, May-standardized serum 25(OH)D, regressed 25(OH)D on the month of sampling and adjusted for age and gender to yield a variable approximating 25(OH)D concentration, if blood was drawn in May (34, 35). It is comparable to using the mean values in May (46.1 nmol/L) as a proxy for the annual mean 25(OH)D concentration (45.6 nmol/L) among the participants.

All statistical analyses were performed using R version 3.6.3. All P values were 2-tailed and considered significant at 0.05.

Results

Baseline characteristics

A total of 5222 participants with IBD at baseline, including 1760 (33.7%) CD and 3462 (66.3%) UC, were included in the study. Their baseline characteristics are presented in **Table 1.** The mean age of the participants was 57.3 (SD 7.9). Among the participants, 2690 (51.5%) were female and 2532 (48.5%) male. Participants with lower 25(OH)D concentration were more likely to have higher TDI scores (indicating poor socioeconomic status), be non-White, have a lower level of physical activity, be current drinkers, and were less likely to take vitamin D or multivitamin supplements (all $P < 0.001$).

Primary analysis

We documented 529 deaths during a follow-up of 62,190 person-years (mean follow-up period: 11.9 years). Compared with participants in the lowest quintile (10.0–≤29.2 nmol/L), those with IBD in the third to the highest quintiles of serum 25(OH)D concentration had a 37% (95 %CI [17%, 53%]), 36% (95 %CI [15%, 52%]), and 26% (95 %CI [1%, 45%]) reduced risk of all-cause mortality in the fully adjusted model, respectively (**Table 2**). Similar associations between serum 25(OH)D concentrations and all-cause mortality were observed among CD and UC separately (**Table 3**). Compared with the lowest quintile, HRs and 95% CIs for the third and fourth quintiles in participants with CD were 0.58 (0.37, 0.91) and 0.42 (0.25, 0.70); and for the third quintile in participants with UC was 0.68 (0.47, 0.97). Non-linear associations were observed between serum 25(OH)D concentration and all-cause mortality in participants with IBD (**Figure 1A**), CD (**Figure 1B**), and UC (**Figure 1C)** (*P*-nonlinearity: <0.001, 0.002, and 0.036, respectively). The cut-off concentrations for serum 25(OH)D at which the mortality curves leveled off were 58, 55, and 58 nmol/L, respectively.

As the 25(OH)D concentration increased from the 10 nmol/L to the cut-off concentrations, the estimated risk (HR [95% CI]) of the mortality curves declined from 1 to 0.45 (0.32, 0.65), 0.37 (0.21, 0.64), and 0.55 (0.34, 0.89) in participants with IBD, CD, and UC, respectively. Mortality curves also indicated that 25(OH)D concentrations of 44–78 nmol/L and 34–90 nmol/L were associated with a 50% reduced all-cause mortality risk compared with the lowest concentration of 10 nmol/L for IBD and CD.

Subgroup analysis

The association between serum 25(OH)D concentrations and all-cause mortality was not altered by stratifications of covariates (*P* for interaction > 0.05), except for education level (*P* for interaction 0.035) and physical activity level (*P* for interaction 0.034), as shown in **Supplementary Table 1**. Subgroup analyses showed that the associations were strengthened among participants with high physical activity or below a college degree. In individuals with high physical activity or without a college degree, those in the highest quintile of 25(OH)D concentration were associated with 67% (95% CI [42%, 81%]) or 38% (95% CI [14%, 56%]) reduced risk of all-cause mortality, respectively, compared with those in the lowest quintile of 25(OH)D concentration.

No evidence of a non-linear association was observed in men (**Supplementary Figure 2B**), those reporting moderate physical activity (**Supplementary Figure 2D**), or those with a college degree (**Supplementary Figure 2G**, all *P*-nonlinearity > 0.05). Subgroup analyses demonstrated significant non-linear associations between serum 25(OH)D concentrations and all-cause mortality among females, those reporting high physical activity, and those without a college degree (all *P*-nonlinearity < 0.001). The range of 25(OH)D concentrations associated with a 50% reduction in mortality risk, compared with the participants with the lowest 25(OH)D concentration of 10 nmol/L, were 34–69 nmol/L, 27–69 nmol/L, and 37–69 nmol/L,

respectively.

When further stratified by the sampling season (**Supplementary Figure 3**), the associations disappeared in winter (**Supplementary Figure 3D**, *P*-nonlinearity = 0.13). For participants whose blood was drawn in spring (**Supplementary Figure 3A**), summer (**Supplementary Figure 3B**), and autumn (**Supplementary Figure 3C**), the mortality curves showed that the minimum estimates of the risk of mortality (HR [95% CI]) at the cut-off 25(OH)D concentrations compared with the lowest 25(OH)D concentration (10 nmol/L) were 0.46 (0.25, 0.82), 0.25 (0.11, 0.56), and 0.25 (0.11, 0.55), respectively. Moreover, 25(OH)D concentrations of 37–62, 31–69, and 30–69 nmol/L were associated with a 50% lower risk of mortality compared with the lowest 25(OH)D concentration of 10 nmol/L for participants stratified by sampling seasons of spring, summer, and autumn.

Sensitivity analysis

We performed sensitivity analyses by excluding participants who died in the first 1-, 2-, and 3-year periods to reduce the potential effect of reverse causality. Compared with participants in the lowest quintile of serum 25(OH)D concentration, those in the third to fourth quintiles were significantly associated with a reduced risk of all-cause mortality (**Table 4**) and the mortality curves (**Figure 2**) were consistent with the primary analysis. The results were similar to the primary findings, after adjusting for serum CRP concentration (**Supplementary Table 2**) or filling missing values using multiple imputations (**Supplementary Table 3**). In the two sensitivity analyses (Supplementary Table 2 and Supplementary Table 3), categorical models showed that, compared with participants in the lowest quintile of serum 25(OH)D, those in the third quintile of 25(OH)D concentration (>41–≤53 nmol/L) were associated with the smallest HRs of 0.62 (95% CI [0.47, 0.83]) and 0.52 (95% CI [0.36, 0.74]), respectively. The results were consistent with our primary analysis after applying season-specific cut-off

points (**Supplementary Table 4**) or using May-standardized 25(OH)D concentration (**Supplementary Table 5**). Categorical models with the two methods showed a 31% (95 %CI [9%, 48%]) and 28% (95 %CI [5%, 45%]) reduction in mortality risk for participants with the highest quintile of 25(OH)D concentration compared with those with the lowest quintile. When we assessed the non-linear association using May-standardized 25(OH)D, the findings were similar to our primary analysis (*P*-nonlinearity = 0.008, **Supplementary Figure 4**). As the 25(OH)D concentration increased from 10 nmol/L to the cut-off 25(OH)D concentration of 68 nmol/L, the HR for the mortality curve declined from 1 to 0.48 (95% CI [0.32, 0.72]).

Discussion

This study found a non-linear association between serum 25(OH)D concentration and all-cause mortality in individuals with IBD, and the non-linear associations mainly applied to the females. A 25(OH)D concentration of 44–78 nmol/L was associated with a 50% reduction in mortality risk compared with the lowest 25(OH)D concentration of 10 nmol/L. Categorical models showed serum 25(OH)D concentrations ranging from 41 to 66 nmol/L were associated with more than 35% reduced risk of all-cause mortality in participants with IBD compared with the lowest quintile of serum 25(OH)D concentration. The dose-response curve showed a 55% reduction in mortality risk at the serum 25(OH)D concentration of 58 nmol/L compared with the lowest 25(OH)D concentration of 10 nmol/L. Similar results were observed in subgroup and sensitivity analyses.

Several observational studies have reported similar non-linear associations, mostly J-shaped, between serum 25(OH)D concentration and all-cause mortality in the general population. Results from analysis with RCS in a 9-year follow-up of the Third National Health and Nutrition Examination Survey showed a J-shaped inverse association between serum 25(OH)D concentration and all-cause mortality with the lowest relative risk at 81 nmol/L of

25(OH)D concentration (9). In a large Danish cohort of 247,574 adults with a 3-year follow-up in primary care, the mortality curve showed that the lowest mortality risk was at 25(OH)D concentration of 50–60 nmol/L using the RCS method (10). Such associations have also been observed in populations with specific disease like colorectal cancer. In a pooled dose-response meta-analysis of 17 studies in individuals with colorectal cancer, mortality curves showed the HR stopped decreasing after 25(OH)D concentration reached 20 nmol/L (36). A meta-analysis of studies in the general population (11) showed a 31% reduction in risk of all-cause mortality at a 25(OH)D concentration of 77.5 nmol/L compared with the lowest 25(OH)D category (median value = 27.5 nmol/L). Similarly, our findings showed a consistent non-linear association between serum 25(OH)D and all-cause mortality among individuals with IBD. But the dose-response curve was steeper for IBD (smallest HR of the curve, 0.45) than the meta-analysis in the general population (smallest relative risk of the dose-response curve, 0.69). This may imply that 25(OH)D will have extra protective effects for individuals with IBD.

There are several potential explanations for the protective effect of serum 25(OH)D against premature death. First, 25(OH)D may influence cell growth, proliferation, apoptosis, and the immune system (2, 37, 38). Second, a low serum 25(OH)D status has been shown to be associated with adverse outcomes such as cancer and coronary heart disease in the general population (39), which may explain the reduced risk of mortality in individuals with higher 25(OH)D concentration. Furthermore, elevated serum 25(OH)D concentrations are associated with longer leukocyte telomere length, a possible determinant for overall longevity (40, 41). Thirdly, vitamin D may have a role in preventing premature death in individuals with IBD. Compared with the general population, individuals with IBD have higher premature mortality rates because of intestinal inflammation and comorbidities such as colorectal cancer, *Clostridium difficile* infection, and fractures (14, 15, 42-44). Vitamin D may prevent the

release of inflammatory factors and apoptosis signals, regulating the transformation of immune cells, and activating tumor suppression pathways (45-47). Higher serum 25(OH)D concentrations are associated with lower inflammation status and disease severity (48, 49). Moreover, previous studies using self-report questionnaires have reported a positive association between serum 25(OH)D concentration and quality of life among individuals with IBD (50, 51).

The mortality curve in individuals with IBD showed a very wide confidence interval at a 25(OH)D concentration above 110 nmol/L, partly due to the small sample size. This makes it impossible to draw conclusions about harmful effects of high 25(OH)D concentration. However, the J-shaped non-linear association does not exclude the possibility that mortality risk may increase at higher 25(OH)D concentrations. It has been reported that a high 25(OH)D concentration may increase the risk of vascular calcification (52, 53). Additionally, a serum 25(OH)D concentration of 125–150 nmol/L has been associated with adverse outcomes such as breast cancer, pancreatic cancer, prostate cancer, falls, and fractures (54). Therefore, further studies are necessary to clarify the impact of high 25(OH)D concentration and the specific range for effect.

We noted that the association between 25(OH)D concentration and all-cause mortality was stronger in individuals with CD, but weaker in those with UC. There are several possible explanations. First, vitamin D deficiency was more prevalent in individuals with CD than in those with UC (55). Second, previous studies have reported that individuals with CD spend less time outdoors and perform less physical activity than those with UC (56-58). Third, the absorption of vitamin D was impaired in individuals with CD due to a decline in fat absorption (2, 59). Moreover, after small bowel resection, 37% of individuals with CD experience hyperthyroidism, resulting in an altered serum 25(OH)D concentration due to parathyroid hormone imbalance (55). In individuals with UC, some observational studies

have reported a null association between serum 25(OH)D concentration and disease severity (60, 61).

Subgroup analyses indicated that the associations between serum 25(OH)D and all-cause mortality were stronger among participants with a lower education level or with higher physical activity. And the non-linear association seems to apply mostly to the females in our study. Limited studies have reported a difference in association between serum 25(OH)D and mortality by sex. Data of the Swiss MONICA study has demonstrated opposite effects in women and men with respect to cardiovascular and cancer mortality (62). Whether our observation is a chance finding still needs confirmation by future studies.

There are various recommendations for the optimal range of serum 25(OH)D concentration in the general population. The Institute of Medicine (US) recommends a concentration above 50 nmol/L, while others recommend a concentration above 75 nmol/L (63-65). However, whether these are the optimal guidelines for managing individuals with IBD is unknown (66). Our findings suggest that a 25(OH)D concentration in the range of 44–78 nmol/L is associated with a 50% reduction in mortality risk compared with individuals in the lowest 25(OH)D concentration of 10 nmol/L. But further research is needed to confirm this finding.

To our knowledge, our study is the first cohort study to investigate the association between serum 25(OH)D concentration and all-cause mortality in individuals with IBD. This study has several strengths. First, data came from a cohort with a large sample size and linkages to external national datasets with reliable records of outcomes. Second, a well-designed quality control protocol was adopted to assess the serum 25(OH)D concentration data. Third, our analysis was conducted with long follow-up periods (mean 11.9 years) and showed a similar result after excluding deaths in the first 3 years. Therefore, reverse causation is less likely to bias our results. Fourth, using an RCS approach, we reported the dose-response curve between serum 25(OH)D concentration and all-cause mortality. Fifth, we applied two

approaches, season-specific cut-off points and May-standardized serum 25(OH)D, to explore the impact of seasonal variations in serum 25(OH)D concentration on mortality in individuals with IBD.

There are several limitations. First, the baseline serum 25(OH)D concentrations may not reflect the actual 25(OH)D status during a long follow-up. However, population-based studies have demonstrated a high correlation coefficient among measurements, suggesting that a single 25(OH)D measurement is a reliable tool in epidemiologic studies (67-69). Second, the UK Biobank did not use the liquid chromatography-mass spectrometry method, the recognized gold standard, to measure serum 25(OH)D concentration. However, the DiaSorin radioimmunoassay shows a good correlation with the gold standard (70, 71). Third, residual confounders could still exist though we considered confounders covering demographic, exposure measurement, lifestyle, inflammation, disease activity factors, and comorbidities. Fourth, although the parameters in the RCS model were set based on previous studies (25, 26), the desirable ranges of 25(OH)D concentrations can change if different statistical models are applied. The participants included in this study were nearly all White, aged 40–69, and living at high latitudes. Hence, the generalization of the results to other populations should be interpreted with caution. Finally, because of the observational nature of this study, causality cannot be determined, so validation with clinical trials is necessary.

In conclusion, our study described the dose-response association between serum 25(OH)D and all-cause mortality among individuals with IBD. Non-linearity was detected in our results and seemed to mostly apply to the females. Future studies are needed to confirm our findings and to investigate the recommended ranges of serum 25(OH)Ds concentration in individuals with IBD.

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Data sharing: Researchers can request the data from the UK Biobank (www.ukbiobank.ac.uk/).

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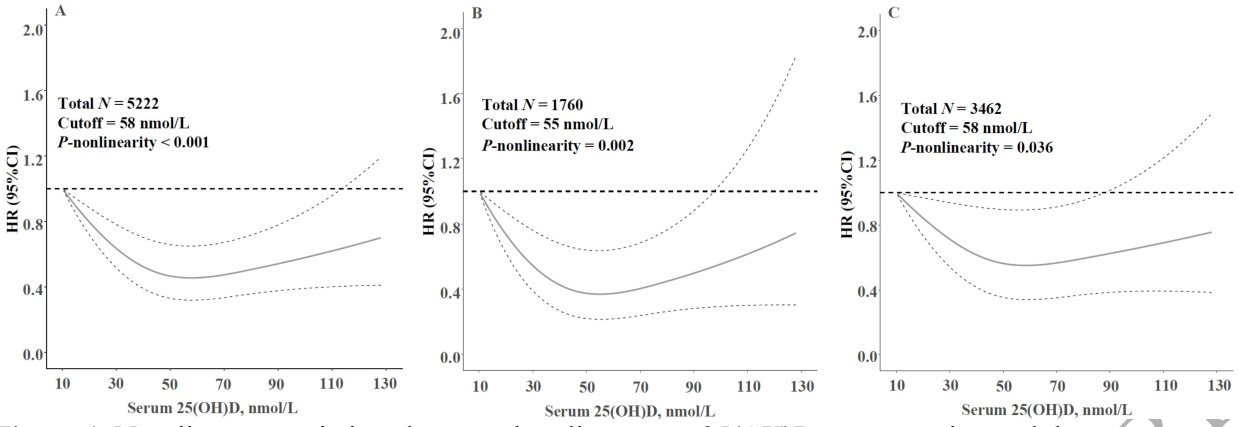

Figure 1. Non-linear associations between baseline serum 25(OH)D concentration and the risk of mortality in participants with IBD overall (A, *n* = 5222), CD (B, *n* = 1760), and UC (C, *n* = 3462). The associations were examined using fully adjusted Cox models with the restricted cubic spline method. The cut-off was defined as the corresponding serum 25(OH)D concentration at the cut-off of the curve. The horizontal axis represents the serum 25(OH)D concentration in nmol/L, and the vertical axis represents the HR (95% CI) of the mortality curve. The solid line represents HR estimates, and the dashed lines represent 95% CIs. 25(OH)D, 25-hydroxyvitamin D; CD, Crohn's disease; CI, confidence interval; HR, hazard ratio; IBD, inflammatory bowel disease; UC, ulcerative colitis.

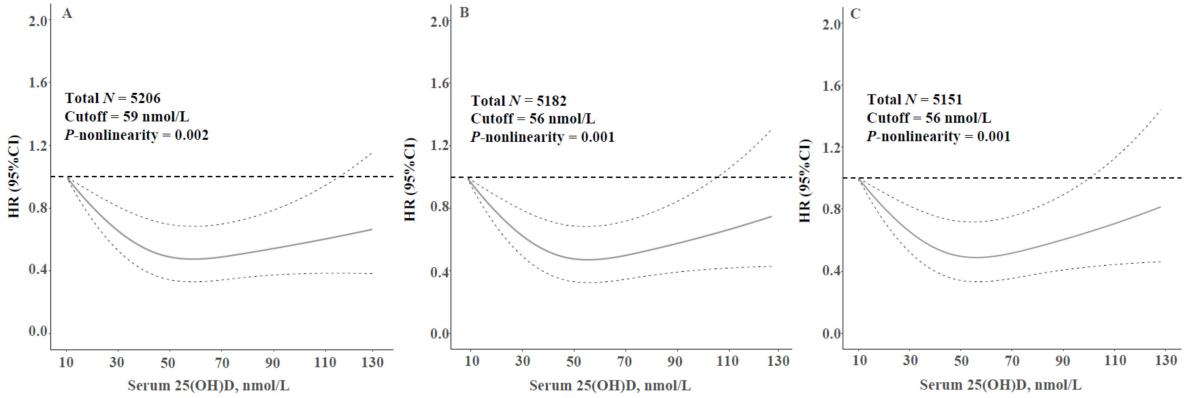

Figure 2. Sensitivity analyses of non-linear associations between baseline serum 25(OH)D concentration and all-cause mortality in participants with IBD excluding those who died in the first 1-year (A, *n* = 5206), 2-year (B, *n* = 5182), and 3-year (C, *n* = 5151) periods. The horizontal axis represents the serum 25(OH)D concentration in nmol/L and the vertical axis represents the HR (95% CI) of the mortality curve. The solid line represents HR estimates, and dashed lines represent 95% CIs. 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; HR, hazard ratio; IBD, inflammatory bowel disease.

Table 1. Baseline characteristics of participants with IBD in the UK Biobank according to quintiles of serum 25(OH)D concentration [1, 2]

Characteristics	All participants (*n* = 5222)	Serum 25(OH)D concentration in quintiles (nmol/L)				
		Quintile 1, 10.0–≤29.2 (*n* = 1047)	Quintile 2, >29.2–≤41.1 (*n* = 1043)	Quintile 3, >41.1–≤53.1 (*n* = 1051)	Quintile 4, >53.1–≤66.6 (*n* = 1040)	Quintile 5, >66.6–≤148.0 (*n* = 1041)
Sex, *n* (%)						
Female	2690 (51.5)	526 (50.2)	536 (51.3)	543 (51.7)	545 (52.4)	540 (51.9)
Male	2532 (48.5)	521 (49.8)	507 (48.7)	508 (48.3)	495 (47.6)	501 (48.1)
Age at baseline, y (mean ± SD)	57.3 ± 7.9	56.1 ± 8.0	56.7 ± 8.0	57.6 ± 8.0	57.8 ± 7.8	58.1 ± 7.5
TDI,[3] *n* (%)						
Low	1736 (33.3)	291 (27.8)	333 (32.0)	353 (33.7)	377 (36.3)	382 (36.7)
Medium	1740 (33.3)	290 (27.7)	351 (33.7)	356 (34.0)	357 (34.4)	386 (37.1)
High	1739 (33.3)	465 (44.5)	358 (34.4)	339 (32.3)	305 (29.4)	272 (27.2)
Education level, *n* (%)						
College	1483 (28.7)	338 (32.7)	313 (30.3)	278 (26.8)	284 (27.5)	271 (26.1)
Below college	3688 (71.3)	697 (67.3)	721 (69.7)	760 (73.2)	747 (72.5)	763 (73.9)

Ethnicity, *n* (%)						
White	5007 (96.3)	942 (90.7)	995 (95.9)	1021 (97.5)	1018 (98.2)	1031 (99.2)
Others	192 (3.7)	97 (9.3)	42 (4.1)	26 (2.5)	19 (1.8)	8 (0.8)
Physical activity level, [4] *n* (%)						
Low	901 (21.6)	227 (28.4)	211 (25.5)	166 (19.9)	155 (18.5)	142 (16.5)
Moderate	1669 (40.1)	333 (41.7)	352 (42.5)	345 (41.3)	337 (40.2)	302 (35.0)
High	1595 (38.3)	239 (29.9)	265 (32.0)	325 (38.9)	347 (41.4)	419 (48.6)
Smoking status, *n* (%)						
Previous or current smokers	2747 (52.8)	562 (53.8)	548 (52.8)	546 (52.2)	538 (51.9)	553 (53.4)
Never smoked	2453 (47.2)	482 (46.2)	490 (47.2)	500 (47.8)	499 (48.1)	482 (46.6)
Current alcohol consumption, *n* (%)						
Yes	4691 (90.1)	903 (86.5)	929 (89.4)	944 (90.0)	958 (92.3)	957 (92.1)
No	518 (9.9)	141 (13.5)	110 (10.6)	105 (10.0)	80 (7.7)	82 (7.9)

BMI, kg/m^2 (mean ± SD)	27.2 ± 4.7	28.2 ± 5.5	27.6 ± 4.8	27.5 ± 4.5	26.7 ± 4.2	25.8 ± 3.8
Prevalent heart disease, *n* (%)						
Yes	396 (7.6)	95 (9.1)	88 (8.5)	67 (6.4)	60 (5.8)	86 (8.3)
No	4810 (92.4)	947 (90.9)	951 (91.5)	982 (93.6)	976 (94.2)	954 (91.7)
Prevalent hypertension, *n* (%)						
Yes	1486 (28.5)	313 (30.0)	313 (30.1)	301 (28.7)	279 (26.9)	280 (26.9)
No	3720 (71.5)	729 (70.0)	726 (69.9)	748 (71.3)	757 (73.1)	760 (73.1)
Prevalent cancer, *n* (%)						
Yes	475 (9.1)	84 (8.1)	97 (9.4)	104 (9.9)	94 (9.1)	96 (9.2)
No	4720 (90.9)	956 (91.9)	939 (90.6)	943 (90.1)	940 (90.9)	942 (90.8)
Subtypes of IBD, *n* (%)						

CD	1760 (33.7)	386 (36.9)	366 (35.1)	352 (33.5)	337 (32.4)	319 (30.6)
UC	3462 (66.3)	661 (63.1)	677 (64.9)	699 (66.5)	703 (67.6)	722 (69.4)
Season of blood sampling, *n* (%)						
Spring	1547 (29.6)	445 (42.5)	368 (35.3)	297 (28.3)	246 (23.7)	191 (18.3)
Summer	1353 (25.9)	105 (10.0)	208 (19.9)	309 (29.4)	334 (32.1)	397 (38.1)
Autumn	1235 (23.6)	142 (13.6)	224 (21.5)	254 (24.2)	289 (27.8)	326 (31.3)
Winter	1087 (20.8)	355 (33.9)	243 (23.3)	191 (18.2)	171 (16.4)	127 (12.2)
Time outdoors in summer, h/day (mean ± SD)	3.8 ± 2.4	3.5 ± 2.2	3.6 ± 2.3	3.8 ± 2.5	4.1 ± 2.4	4.1 ± 2.3
Time outdoors in winter, h/day (mean ± SD)	1.9 ± 1.8	1.8 ± 1.6	1.8 ± 1.7	1.9 ± 1.8	2.0 ± 1.7	2.1 ± 1.9
Use of Vitamin D supplement, *n* (%)						

Yes	306 (5.9)	23 (2.2)	41 (4.0)	67 (6.4)	72 (7.0)	103 (10.0)
No	4882 (94.1)	1 012 (97.8)	995 (96.0)	980 (93.6)	963 (93.0)	932 (90.0)
Use of Multivitamin supplement, *n* (%)						
Yes	1161 (22.4)	112 (10.8)	192 (18.5)	234 (22.3)	295 (28.5)	328 (31.7)
No	4027 (77.6)	923 (89.2)	844 (81.5)	813 (77.7)	740 (71.5)	707 (68.3)

[1] 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CD, Crohn's disease; IBD, inflammatory bowel disease; MET, metabolic equivalent of task; SD, standard deviation; TDI, Townsend deprivation index; UC, ulcerative colitis.

[2] Continuous variables are presented as means ± SDs, and categorical variables are presented as numbers (percentages).

[3] Defined as TDI in tertiles.

[4] Physical activity levels were categorized as follows: high level refers to either vigorous-intensity physical activity for at least 3 days and achieving at least 1500 MET min/week or any combination of walking, moderate- intensity, or vigorous-intensity physical activity for ≥7 days achieving at least 3000 MET min/week; moderate level refers to ≥3 days of vigorous activity for at least 20 min per day or ≥5 days of moderate-intensity activity and/or walking for at least 30 min per day or ≥5 days of any combination of walking, moderate-intensity or vigorous-intensity physical activity achieving at least 600 MET min/week; and low level refers to no activity or insufficient activity to meet a moderate or high level.

Table 2. Associations between baseline serum 25(OH)D concentration in quintiles and all-cause mortality in participants with IBD (*n* = 5222) [1]

	Serum 25(OH)D concentration in quintiles (nmol/L)				
	Quintile 1, 10.0–≤29.2 (*n* = 1047)	Quintile 2, >29.2–≤41.1 (*n* = 1043)	Quintile 3, >41.1–≤53.1 (*n* = 1051)	Quintile 4, >53.1–≤66.6 (*n* = 1040)	Quintile 5, >66.6–≤148.0 (*n* = 1041)
Deaths/Person-years	131/12,281	111/12,316	91/12,623	89/12,522	107/12,418
Model 1, HR (95% CI) [2]	Reference	0.83 (0.65, 1.07)	0.61 (0.47, 0.81)	0.62 (0.47, 0.81)	0.74 (0.57, 0.96)
Model 2, HR (95% CI) [3]	Reference	0.81 (0.63, 1.04)	0.58 (0.44, 0.77)	0.59 (0.44, 0.78)	0.68 (0.51, 0.91)
Model 3, HR (95% CI) [4]	Reference	0.83 (0.64, 1.08)	0.61 (0.46, 0.81)	0.62 (0.47, 0.83)	0.74 (0.55, 0.99)
Model 4, HR (95% CI) [5]	Reference	0.82 (0.63, 1.06)	0.63 (0.47, 0.83)	0.64 (0.48, 0.85)	0.74 (0.55, 0.99)

[1] 25(OH)D, 25-hydroxyvitamin D; CI confidence interval; HR, hazard ratio; IBD, inflammatory bowel disease.

[2] Model 1 (minimally adjusted) was adjusted for sex, age, age squared, ethnicity, Townsend deprivation index, and education level.

[3] Model 2 was further adjusted for season of blood sampling, time spent outdoors in summer, time spent outdoors in winter, use of vitamin D supplements, and use of multivitamin supplements based on model 1.

[4] Model 3 was further adjusted for body mass index, smoking status, alcohol consumption status, physical activity level, fresh fruit consumption, raw or salad vegetable consumption, processed meat intake frequency, and fish intake frequency based on model 2.

[5] Model 4 (fully adjusted) was further adjusted for diagnoses of heart diseases, hypertension, and cancer based on model 3.

Table 3. Associations between baseline serum 25(OH)D concentration and all-cause mortality in participants with CD (*n* = 1760) or UC (*n* = 3462) [1, 2]

Subtypes of IBD	Serum 25(OH)D concentration in quintiles (nmol/L)				
	Quintile 1 (10.0–≤29.2)	Quintile 2 (>29.2–≤41.1)	Quintile 3 (>41.1–≤53.1)	Quintile 4 (>53.1–≤66.6)	Quintile 5 (>66.6–≤148.0)
CD					
Participants, *n*	386	366	352	337	319
Deaths/Person-years	57/4454	47/4307	32/4210	24/4074	38/3761
HR (95% CI)	Reference	0.80 (0.53, 1.19)	0.58 (0.37, 0.91)	0.42 (0.25, 0.70)	0.73 (0.46, 1.15)
UC					
Participants, *n*	661	677	699	703	722
Deaths/Person-years	74/7833	64/8016	59/8416	65/8,443	69/8,659
HR (95% CI)	Reference	0.85 (0.60, 1.19)	0.68 (0.47, 0.97)	0.82 (0.57, 1.19)	0.81 (0.55, 1.18)

[1] 25(OH)D, 25-hydroxyvitamin D; CD, Crohn's disease; CI, confidence interval; HR, hazard ratio; IBD, inflammatory bowel disease; UC, ulcerative colitis.

[2] Fully adjusted model adjusted for sex, age, age squared, ethnicity, Townsend deprivation index, education level, season of blood sampling, time

spent outdoors in summer, time spent outdoors in winter, use of vitamin D supplements, use of multivitamin supplements, body mass index, smoking status, alcohol consumption status, physical activity level, fresh fruit consumption, raw or salad vegetable consumption, processed meat intake frequency, fish intake frequency, and diagnoses of heart diseases, hypertension, and cancer.

Table 4. Sensitivity analyses of associations between baseline serum 25(OH)D concentration and all-cause mortality in participants with IBD excluding those who died in the first 1-year (*n* = 5206), 2-year (*n* = 5182), and 3-year (*n* = 5151) periods [1, 2]

Excluding deaths	Serum 25(OH)D concentration in quintiles (nmol/L)				
	Quintile 1 (10.0–≤29.2)	Quintile 2 (>29.2–≤41.1)	Quintile 3 (>41.1–≤53.1)	Quintile 4 (>53.1–≤66.6)	Quintile 5 (>66.6–≤148.0)
1-year period					
Participants, *n*	1043	1040	1050	1037	1036
Deaths/Person-years	127/12,287	108/12,323	90/12,621	86/12,517	102/12,420
HR (95% CI)	Reference	0.83 (0.64, 1.07)	0.65 (0.49, 0.86)	0.65 (0.48, 0.87)	0.75 (0.55, 1.00)
2-year period					
Participants, *n*	1038	1032	1046	1031	1035
Deaths/Person-years	122/12,280	100/12,310	86/12,615	80/12,506	101/12,418
HR (95% CI)	Reference	0.80 (0.61, 1.05)	0.65 (0.49, 0.87)	0.64 (0.47, 0.86)	0.78 (0.58, 1.06)
3-year period					
Participants, *n*	1028	1024	1042	1027	1030
Deaths/Person-years	112/12,254	92/12,286	82/12,608	76/12,488	96/12,399

HR (95% CI)	Reference	0.80 (0.60, 1.06)	0.67 (0.50, 0.91)	0.66 (0.48, 0.91)	0.82 (0.60, 1.12)

[1] 25(OH)D, 25-hydroxyvitamin D; CI confidence interval; HR, hazard ratio; IBD, inflammatory bowel disease.

[2] Fully adjusted model adjusted for sex, age, age squared, ethnicity, Townsend deprivation index, education level, season of blood sampling, time spent outdoors in summer, time spent outdoors in winter, use of vitamin D supplements, use of multivitamin supplements, body mass index, smoking status, alcohol consumption status, physical activity level, fresh fruit consumption, raw or salad vegetable consumption, processed meat intake frequency, fish intake frequency, and diagnoses of heart diseases, hypertension, and cancer.