Principles and Practice of Clinical Research
A GLOBAL JOURNAL OF CLINICAL RESEARCH

Leukocyte Telomere Length and Vitamin D: A Systematic Review of Observational Research

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Abstract

Background: *Interest in biological markers of healthy aging has grown, particularly vitamin D (VD) and leukocyte telomere length (LTL). Telomeres shorten with age and are linked to chronic disease, while VD may influence this process through effects on inflammation and oxidative stress. Clarifying the VD–LTL relationship could inform their value as biomarkers of longevity.*

Aim: *To clarify the relationship between vitamin D status and leukocyte telomere length as potential biomarkers of longevity.*

Methods: *A PRISMA-guided systematic review of observational studies was conducted, searching PubMed, EMBASE, Cochrane Library, and Scopus in April 2025. Eligible studies enrolled adults and reported serum/plasma VD and LTL measured with validated techniques. Risk of bias was appraised using the Newcastle–Ottawa Scale.*

Results: *From 521 records, 32 full texts were assessed, and 11 observational studies were included (10 cross-sectional, 1 cohort) spanning Europe, the Americas, and Asia (2007–2023). VD was measured via CLIA/RIA, ELISA, or LC-MS/MS; LTL predominantly by qPCR (one Southern blot). Findings were heterogeneous: 4 studies reported longer LTL with higher 25-hydroxyvitamin D [25(OH)D], 1 showed a U-shaped association (shorter LTL at very low and very high 25(OH)D), and 6 found no significant relationship. Positive signals appeared more often in female or middle-aged subgroups, but variability in exposure/outcome definitions and covariate adjustment limited comparability and precluded meta-analysis. Risk of bias was generally low-to-moderate (2 "very good," 8 "good," 1 "unsatisfactory" on NOS).*

Conclusion: *Based on current evidence, vitamin D supplementation cannot be recommended solely for telomere preservation, though the observed associations in specific subgroups warrant further investigation through well-designed longitudinal and interventional studies.*

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Received: September 4, 2025 **Accepted:** December 16, 2025
Published: January 16, 2026
Editor: Felipe Fregni
Reviewers: Maria Fe Velarde, Walter Marrou, Christianne Takeda, Aline Gruber, Antonio Macedo
Keywords: vitamin D, vitamin D deficiency, telomere shortening, longevity, biomarkers

Introduction

Vitamin D (VD) plays a well-established role in bone health and calcium homeostasis; however, emerging evidence suggests it may also contribute to broader physiological processes such as immune regulation,

DOI: https://doi.org/10.21801/ppcrj.2025.113.3

inflammation control, and cellular aging (Fantini et al., 2023). In recent years, the potential relationship between VD levels and leukocyte telomere length (LTL) has become of interest. Telomeres, which protect the ends of chromosomes, progressively shorten with each cell division and are considered markers of biological aging. Accelerated telomere shortening has been associated with increased risk of chronic diseases, frailty, and premature mortality (Victorelli & Passos, 2017).

Telomeres are sequences of nucleotides located at the ends of chromosomes. They protect against end-to-end fusions and chromosomal instability and play vital roles in cellular processes (Aksenova et al., 2019). On the other hand, after each cell division, 50-200 base pairs (bp) of telomere are lost, and certain regions are susceptible to oxidative damage. These processes lead to cellular senescence, so telomere loss and damage are key factors in aging processes (Srinivas et al., 2020; von Zglinicki et al., 2021). LTL is considered a marker of biological aging (Li et al., 2020). Furthermore, its shortening is associated with various pathologies such as coronary heart disease, atherosclerotic plaque instability, hypertension, and type 2 diabetes, which indicates its relationship with multiple components of metabolic syndrome. This could be explained by the increased oxidative stress that these pathologies have in common (Xu et al., 2019; Herrmann & Herrmann, 2020; Liu et al., 2019; Khalangot et al., 2020). Therefore, lifestyle changes and supplementation with various nutrients could provide benefits in maintaining and stabilizing telomeres.

Concurrently, VD deficiency has been linked to adverse health outcomes and higher mortality risk (Heath et al., 2019). These findings have prompted an investigation into whether VD levels might influence telomere length, thereby serving as a biomarker of healthy aging and longevity. Despite growing interest, this review is the first systematic review to focus exclusively on observational studies of circulating 25-hydroxyvitamin D [25(OH)D] and LTL in adults, in contrast to prior reviews that emphasized telomerase activity or combined heterogeneous designs and outcomes. By isolating observational evidence, the review clarifies what can and cannot be inferred about naturally occurring variation in VD status and telomere biology. Given VD's roles in inflammation, oxidative stress, and immune regulation, processes implicated in telomere attrition (Srinivas et al., 2020), the study aims to explore the relationship between circulating VD levels and LTL, measured with validated assays, among populations aged 18 years or older without comparison groups based on evidence from observational studies.

Materials and Methods

Search Strategy

A comprehensive literature search was conducted between April 25th and 28th, 2025. The search strategy combined MeSH/Emtree terms and free-text synonyms. The full search details are provided in Supplementary Table S1. Electronic searches were carried out in four databases: PubMed, Cochrane Library, EMBASE, and Scopus.

All citations were imported into Covidence®, which automatically removed duplicates. The remaining records proceeded to screening.

Inclusion and Exclusion Criteria

Eligibility parameters were established a priori and applied uniformly during screening.

The following eligibility criteria were adopted:

1. Observational studies, including cross-sectional, cohort, or case-control studies;
2. Articles published in the English language;
3. Articles published from January 2005 onward to ensure methodological comparability and reduce non-biological heterogeneity;
4. Studies involving adults aged 18 years or older of any gender;
5. Studies reporting serum or plasma 25(OH)D concentrations (in ng/mL or nmol/L);
6. Studies measuring LTL using validated molecular techniques: quantitative PCR or Southern blot, informing β coefficients, kilobase pairs (kbp), odds ratios, or mean differences.

The following were excluded:

1. Studies based on in vitro experiments, animal models, or genetic data without primary observational findings;
2. Studies involving participants with serious medical conditions: cancer, systemic lupus erythematosus, or advanced type 2 diabetes, unless separate data for healthy participants were reported;
3. Articles assessing telomere length in non-leukocyte tissues, using non-standard assays, or reporting only composite biological-age indices;
4. Studies with clinical subgroups comprising more than 30% of the sample or stratified results by disease status.

Screening Procedure

During the screening process, the participating researchers were randomly assigned into pairs, which remained fixed throughout the entire process. In case of disagreement, a third reviewer assisted in reaching a decision. Reasons for exclusion were recorded in Covidence.

Selection of Studies and Data Extraction

The following variables of interest were extracted from each study: publication date, country, aims of the study, study design, population sampled, number of participants, mean age of sample, sex distribution, VD measurement method, LTL measurement method, covariates assessed, outcomes, statistical method used, associations informed, confidence interval (CI), and p-value. To aid interpretation across heterogeneous designs, Table 1 summarizes, for each study, the population characteristics, LTL assay, key covariates, and the reported association. A narrative summary using content analysis was chosen. Due to the substantial variability among statistical analysis, conducting a meta-analysis was not considered appropriate.

Risk of Bias Assessment

The Newcastle–Ottawa Scale (NOS) quality assessment tool was used to assess the quality of studies, applying the version adapted for cross-sectional studies (maximum 10 points) and the standard version for cohort studies (maximum 9 points), as appropriate (Modesti et al., 2016; Ottawa Hospital Research Institute, n.d.).

Each study was assessed for risk of bias by two independent reviewers. If there was any disagreement or confusion, a third reviewer helped to clarify and resolve the issue. All reviewers used the same criteria to keep the assessment consistent.

Assessment of Reporting Bias and Certainty of Evidence

Because no quantitative synthesis was conducted, formal small-study tests, such as funnel plots or Egger's regression, were not applicable (Page et al., 2021; Higgins et al., 2024; Egger et al., 1997). To minimize the risk of missing results, searches included conference abstracts and Google Scholar in addition to standard databases, with backward and forward citation searches (Page et al., 2021; Campbell et al., 2020). Gray literature beyond these sources was not systematically searched. Certainty of evidence was appraised narratively using GRADE domains adapted for observational syntheses, considering risk of bias, inconsistency across populations and laboratory methods, indirectness, imprecision, and potential reporting bias (Balshem et al., 2011; Schünemann et al., 2013).

Results

The search process retrieved 521 articles. After removing duplicates and screening titles and abstracts, 32 articles were included for full-text assessment. Of these, 21 articles were excluded for prespecified reasons (Supplementary S2), and eleven studies met the inclusion criteria and were incorporated into this review (Figure 1, PRISMA flowchart).

The eleven studies included in this review were conducted across diverse populations in Europe, America, and Asia, and published between 2007 and 2023. Ten had a cross-sectional and one a cohort study design (Schöttker et al., 2019). Table 1 summarizes the characteristics and main results of the studies, as well as the correlation type, coefficient, and confidence interval or standard error of the chosen studies (Supplementary Figure S3 shows a bubble scatterplot of the results).

Regarding our study population, the majority of studies included community adults across a mean age range from 30 to 64 years, with sample sizes varying from under 100 to over 140,000 participants. Most of the participants across studies were selected from a prior cohort study (ESTHER Cohort, UK Twin Cohort, NFBC 1996, HPFS cohort, USRT cohort, UK Biobank, Pro-Saude Study, US NHANES, and US Nurses Health Study). Population details, mean age, and sample size are shown in the second column of Table 1. All papers considered as inclusion criteria having the measurements on VD and LTL available, Mazidi et al. (2017) excluded patients with diabetes and established cardiovascular disease (coronary heart disease, angina, myocardial infarction, stroke, or congestive heart failure), and Jelmila et al. (2020) excluded patients with chronic illness. Most studies included male and female participants; only the papers by Liu et al. (2016) and Richards et al. (2007) were specifically on females (Nurses' Health Study and UK Twin Registry), and one in men (HPFS cohort, Julin et al., 2017). One study in particular focused on a specific ethnic population: Minangkabau women from Indonesia (Jelmila et al., 2020).

Serum 25(OH)D was the exposure in all studies,

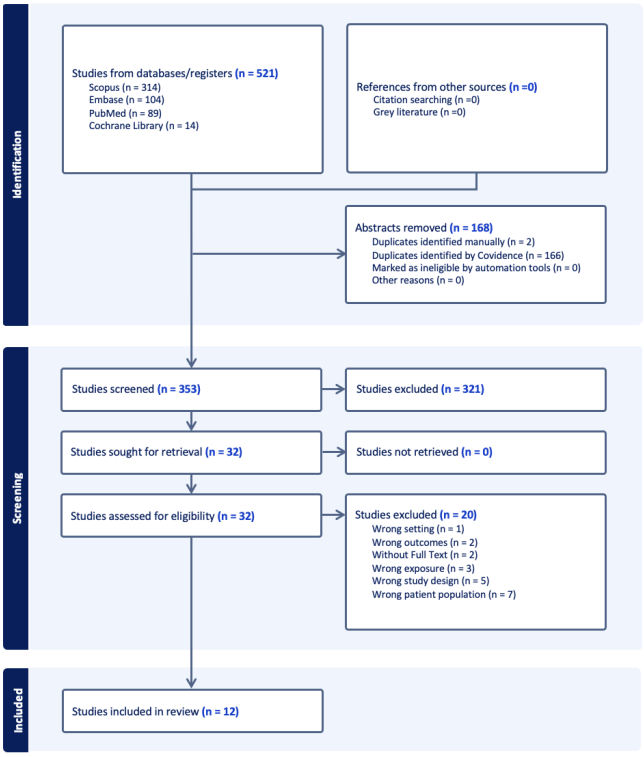

Figure 1: *PRISMA flow diagram.*

measured by chemiluminescence immunoassays (CLIA) or radioimmunoassay (RIA) kits (8/11 studies) (Beilfuss et al., 2017; Julin et al., 2017; Kuo et al., 2023; Liu et al., 2013, 2016; Mazidi et al., 2017; Normando et al., 2020; Richards et al., 2007), liquid chromatography–tandem mass spectrometry (LC-MS/MS) (2/11 studies) (Schöttker et al., 2019; Williams et al., 2016), or enzyme-linked immunosorbent assay (ELISA) (1/11 studies) (Jelmila et al., 2020).

Julin et al. (2017) and Liu et al. (2016) categorized VD levels into quartiles, Richards et al. (2007) into tertiles, Beilfuss et al. (2017), Kuo et al. (2023), Liu et al. (2013), and Normando et al. (2020) used thresholds predefined by the authors, and the rest analyzed VD as a continuous variable (Mazidi et al., 2017; Schöttker et al., 2019; Williams et al., 2016).

In terms of outcome, LTL was predominantly assessed using quantitative PCR (qPCR), with the telomere-to-single copy gene (T/S) ratio as the main metric. Only Richards et al. (2007) used the Southern blot method, which provides absolute telomere length in kbp. Despite methodological variability, most studies converted results to comparable units or accounted for measurement techniques in their analyses.

Most studies applied multivariable regression models adjusting for potential confounders, which were chosen by authors because of biological plausibility. Age, sex, and body mass index (BMI) were consistently included. Additional covariates commonly adjusted for included lifestyle factors, socioeconomic indicators, nutritional factors, inflammatory markers, and hormonal and reproductive factors in female-only studies. Because 25(OH)D varies by sex and across seasons, sex and season of blood draw were prespecified as potential confounders. However, reporting was inconsistent among included studies, and hormonal panels, menopausal status, and hormone therapy were not available. Only Normando et al. (2020) and Richards et al. (2007) accounted for the season of blood collection. Consequently, seasons could not be uniformly harmonized across studies, and sex-stratified effect estimates were rarely reported. These constraints are acknowledged a priori, and study-specific adjustment sets are summarized in Table 1.

Main Results

The results across the 11 included studies were heterogeneous. Jelmila et al. (2020), Liu et al. (2013), Richards et al. (2007) and Beilfuss et al. (2017) found a positive association between serum 25(OH)D levels and LTL, Kuo et al. (2023) showed a U-shaped association between LTL and the lowest and highest

Author, year	Population, excluded participants, mean age, sample size (n), country, type of study	Vitamin D measuring method	LTL measuring method	Covariates	Main result	Association	Statistical analysis / p-value / Confidence Interval or Standard error	Quality Assessment (NOS scale)
Richards et al., 2007	Community women adults from the UK twin cohort Mean age: 49.4 years ± 12.9 100% female (n=2160) Country: UK Cross-sectional study	Radioimmuno assay kit (DiaSorin) (nmol/L)	Southern blot; kbpb	Age, BMI, physical activity, fasting insulin and serum leptin concentrations, smoking, CRP, season of Vit. D sample, menopausal status and hormone replacement therapy.	Higher serum 25(OH)D concentrations were associated with longer LTL	Pearson's coefficient (age adjusted) = 0.09 LTL difference highest-lowest Vitamin D quartiles: 107.1 bp	Linear regression p-value <0.0001; CI: not reported p-value 0.0009; CI: not reported	8
Liu et al., 2013	Participants from previous nested case-control sets (US–Nurses' Health Study) Mean age: 59.5 years 100% female (n=1337) Country: US Cross-sectional study	Radio immunoassay (ng/mL)	qPCR; (T/S) ratio	Age, BMI, physical activity, smoking.	Higher plasma 25(OH)D levels were significantly associated with longer LTL	Linear regression: β = 0.005 Log regression (High Vitamin D >43 ng/mL): OR = 1.59	Linear regression p-value 0.05; SE: 0.003 Logistic regression p-value 0.01; CI: 1.11, 2.29	8
Liu et al., 2016	Adults from the USRT cohort Mean age: 63 years 38.4% men (n=1154) Country: US Cross-sectional study	Chemiluminescence immunoassay (DiaSorin) (nmol/L)	qPCR; (T/S) ratio	Age, sex, BMI, physical activity, smoking, Vit. D supplementation, cumulative occupational ionising radiation dose, menopausal state.	No significant associations between continuous 25(OH)D concentration and long LTL for the overall analysis in the population	OR per quartile of vitamin D: Q2 (45–65 nmol/L): 1.22 Q3 (65–85 nmol/L): 1.13 Q4 (>85 nmol/L): 0.98	Logistic regression p-trend: 0.440 CI: 0.86, 1.72 CI: 0.80, 1.61 CI: 0.69, 1.40	8
Williams et al., 2016	Community Finland adults recruited from the NFBC 1966 Mean age: 31.1 years (IQR 0.35) 48.2% men (n=5096) Country: Finland Cross-sectional study	High-performance liquid chromatography–tandem mass spectrometry (nmol/L)	qPCR; (T/S) ratio	Age, BMI, physical activity, sex, socio-economic position, diet quality, smoking, alcohol consumption, oral contraceptive use (women only), CRP.	25(OH)D was not associated with LTL	Mean difference in LTL (%): −4 × 10−5	Linear regression p-value: 0.97 CI: −0.2, 0.2	9
Beilfuss et al., 2017	Adults over 20 years from the US NHANES 2001–2002 cycle Age groups: 20–39 years (mean 30±0) (47% men); 40–59 years (mean 48±0) (53% men); >60 years (mean 71±0) (44% men) (n=4260) Country: US Cross-sectional study	Chemiluminescence immunoassay (Diasorin assay) (nmol/L)	qPCR; (T/S) ratio converted to kbpb by formula 3.274 + 2.413 × (T/S)	Age, BMI, physical activity, sex, race/ethnicity, total energy, sugar and calcium intakes, socioeconomic status, consumption of milk and supplement.	No association in the full cohort. Only middle-aged adults showed a positive association.	0.03 ± 0.01 kbpb per 10 nmol/L. In age group 40–59: Serum 25(OH)D >50 nmol/L: 0.13 ± 0.4 kbpb longer compared to 25(OH)D <50 nmol/L	Linear regression p-value 0.001; CI: not reported p-value 0.01; CI: not reported	8
Mazidi et al., 2017	Participants aged 18 and older from the US NHANES 2001–2002 cycle Excluded: DM, CV disease Mean age: 42.7 years 47% men (n=4347) Country: US Cross-sectional study	Radioimmuno assay (RIA) kit (ng/mL)	qPCR; (T/S) ratio	Sex, race, education, marital status, CRP, BMI, smoking, physical activity.	No association in adjusted models	β: −0.026	Linear regression p-value: not reported CI: −3.16, 1.67	8
Julin et al., 2017	Males aged 40–75 years recruited from the HPFS cohort Mean age: 64 100% men (n=2,483) Country: US Cross-sectional study	Radioimmuno assay and Enzyme Immunoassay (ng/mL)	qPCR; (T/S) ratio	Age, BMI, physical activity, smoking, alcohol intake, folate, calcium, retinol.	No association between 25(OH)D or 1,25-dihydroxyvitamin D [1,25(OH)2D] and LTL.	25(OH)D (β): −0.0008 OR per quartile of 25(OH)D: Q2: 0.94 Q3: 0.89 Q4: 1.02	Linear regression p-trend: 0.69; SE: 0.0020 Logistic regression p-trend: 0.92 CI Q2: 0.75–1.18 CI Q3: 0.71–1.12 CI Q4: 0.81–1.29	8
Schöttker et al., 2019	Adults aged 50–74 from the ESTHER Cohort Mean age: 62.1 ± 6.6 years Men: 45.1% (n=3,564) Country: Germany Cohort study	Liquid chromatography tandem-mass spectrometry (nmol/L)	qPCR; (T/S) ratio	Age, sex, education, smoking, physical activity, CV disease, and cancer.	25(OH)D levels were not associated with LTL	β: 0.564	Linear regression p-value: 0.382 SE: 0.635	9
Jelmila et al., 2020	Premenopausal women from Minangkabau ethnic (Indonesia) with menstrual disorders Excluded: Chronic illness Mean age: 46.52 ± 1.08 100% female (n=93) Country: Indonesia Cross-sectional study	ELISA kit from DBC Canada (ng/mL)	qPCR; bp	Not reported.	Significant correlation between serum 25(OH)D levels and telomere length	β: 0.267	Linear regression p-value: 0.01 SE: 0.220	4
Normando et al., 2020	Population from the Wave 4 of the Pro-Saude Study (2012–2013) (Brazil) Mean age: 51.4 years 48.5% men (n=464) Country: Brazil Cross-sectional study	Chemiluminescent immunometric assay (nmol/L)	qPCR; (T/S) ratio	Age, sex, marital status, educational attainment, smoking, physical activity, diagnosis of chronic conditions, BMI, CRP, month of blood collection.	No significant associations between categories of 25(OH)D concentrations and LTL.	SS: 3–50 nmol/L: 0.000 51–75 nmol/L: −0.019 >75 nmol/L: −0.028	Linear regression CI −0.039 to 0.038; p-value not reported CI −0.060 to 0.022; p-value not reported CI −0.091 to 0.036; p-value not reported	8
Kuo et al., 2023	Participants >60 from the UK Biobank protocol (2006–2010) Mean age: 64.13 years (SD: 2.85) 49.7% men (n=148,321) Country: UK Cross-sectional study	Chemiluminescence immunoassay (DiaSorin) (nmol/L)	qPCR; (T/S) ratio	Calcium, age, sex, ethnicity, education, BMI, Townsend deprivation index, smoking, alcohol intake, physical activity questionnaire.	U-shaped association in low, extremely low and high Vitamin D values.	β: Extremely low Vit D (≤16.6 nmol/L): −0.048 High Vit D (>95.9 nmol/L): −0.038	Extremely low Vit D: p-value 0.006; CI −0.083, −0.014 High Vit D: p-value 0.030; CI −0.072, −0.004	8

All reported coefficients and confidence intervals reflect the adjusted model. Unadjusted estimates are not displayed.

[a]: quantitative Polymerase Chain Reaction; [b]: kilobase pairs; [c]: Body Mass Index; [d]: Physical Activity; [e]: Not Informed; [f]: Diabetes Mellitus; [g]: Cardiovascular; [h]: C- Reactive Protein; [i]: Standar Error; [j]: base pairs.

Table 1: *Study details and main outcome.*

Author	Selection Max 5	Comparability Max 2	Outcome Max 3	Score	Study quality
*Beilfuss et al., 2017	3	2	3	8/10.	Good
*Mazidi et al., 2017	3	2	3	8/10.	Good
*Liu et al., 2013	3	2	3	8/10.	Good
*Jelmila et al., 2020	3	0	1	4/10.	Unsatisfactory
*Normando et al., 2020	3	2	3	8/10.	Good
*Kuo et al., 2023	3	2	3	8/10.	Good
*Liu et al., 2016	3	2	3	8/10.	Good
*Julin et al., 2017	3	2	3	8/10.	Good
*Williams et al., 2016	4	2	3	9/10.	Very good
*Richards et al., 2007	3	2	3	8/10.	Good
^Schöttker et al., 2019	4	2	3	9/9.	Very good

*: Cross sectional study; used NOS scale adapted for cross sectional (10 points max.); ^: Cohort studies; used NOS scale for cohort studies (9 points max.)

Very Good Studies: 9-10 points Good Studies: 7-8 points Satisfactory Studies: 5-6 points Unsatisfactory Studies: 0 to 4 points

Table 2: *Quality assessment: Newcastle-Ottawa Scale (NOS).*

VD levels groups; the others did not show a statistically significant relationship (Julin et al., 2017; Liu et al., 2016; Mazidi et al., 2017; Normando et al., 2020; Schöttker et al., 2019; Williams et al., 2016).

Five studies found a significant association between VD levels and longer telomere length. Richards et al. (2007) found that higher 25(OH)D concentrations (mean value for the highest tertile of the sample 124 ± 37.3 nmol/L) were associated with longer LTL, with a 107 bp difference between extreme tertiles (p-value=0.0009) and Liu et al. (2013) also showed higher plasma 25(OH)D (43.0 ng/mL for the highest quartile) was associated with longer LTL (OR=1.59, 95% CI: 1.11–2.29). The study by Jelmila et al. (2020) found a statistically significant positive association, indicating that higher VD levels were correlated with longer telomeres (β=0.267, p-value=0.01). Beilfuss et al. (2017) found a positive association through linear regression in which LTL increased 0.03 ± 0.01 kbp per 10 nmol/L increase in serum 25(OH)D (p-value=0.001) only in middle-aged adults (40–59 years (mean 48 ± 0) group. The paper by Kuo et al. (2023) was the only one that reported a U-shaped association of LTL between extremely low VD levels (β=−0.048; p-value=0.006) and high VD values (β=−0.038; p-value=0.030).

Six studies reported no statistically significant association between serum 25(OH)D levels and LTL. Three of these studies included cohorts of patients from the United States (Julin et al., 2017; Liu et al., 2016; Mazidi et al., 2017). Mazidi et al. (2017) excluded patients with diabetes mellitus and cardiovascular disease and did not find any association in the adjusted linear regression models (β=−0.026, CI:–3.16–1.67). The study by Liu et al. (2016) explored the data from adults from the US Radiologic Technologists (USRT) study, an occupational cohort composed of radiologic technologists and found no significant relationship between continuous 25(OH)D concentration and long LTL in the total population (p-trend=0.440) nor significant associations for quartiles of plasma 25(OH)D concentration and long LTL in the total population or among subgroups of race and sex for quartiles of plasma 25(OH)D. The study by Julin et al. (2017) focused exclusively on the male population from the Health Professionals Follow-up Study (HPFS) cohort, which included white men aged 40 to 75 years (mean age 64). For the linear multivariable-adjusted regression model neither 25(OH)D (β=−0.0008 [SE 0.0020]; p-trend=0.69) or $1,25(OH)_2D$ (β=0.0015 [SE 0.0018]; p-trend=0.41) were associated with LTL.

Outside the United States, three studies found no significant association: one from Latin America and two from Europe. Normando et al. (2020) evaluated the data from the population Pro-Saude Study (2012–2013), a cohort of civil servants at a university campus in Rio de Janeiro, Brazil. They primarily looked for an association between single-nucleotide polymorphisms in VD metabolic pathway genes and LTL, but also described no statistically significant associations in multiple regression models between categories of serum 25(OH)D concentrations and LTL. Williams et al. (2016) used data collected at 31 years of age on 5,096 male and female participants from the Northern Finland Birth Cohort 1966 (NFBC1966), all of white European ethnic origin. They described no association of 25(OH)D with LTL, for a mean

difference of -4×10^{-5} (CI:−0.2–0.2; p-value=0.97) in relative percent change in LTL (T/S ratio) per 1-nmol/L increase in 25(OH)D in adjusted models. Finally, Schöttker et al. (2019) examined participants from the German ESTHER cohort study, where no significant relationship was found between VD and LTL (β=−0.564 [SE 0.635]; p-value=0.382).

Assessment of Risk of Bias in Individual Studies

Two of the included studies were rated as very good quality (scores of 9/9 or 9/10 on the NOS scale), including one cross-sectional and one cohort study (Williams et al., 2016; Schöttker et al., 2019). Eight studies achieved scores between 7 and 8 and were therefore classified as good quality (Beilfuss et al., 2017; Julin et al., 2017; Kuo et al., 2023; Liu et al., 2013, 2016; Mazidi et al., 2017; Normando et al., 2020; Richards et al., 2007). Only the study by Jelmila et al. (2020) was classified as unsatisfactory, due to no adjustment for confounding and insufficient methodological reporting. Table 1 shows the total points graded to each study, and Table 2 summarizes the detailed quality assessment for each paper.

Reporting Bias and Certainty of Evidence

Formal small-study tests were not performed. The narrative appraisal found no consistent signals of selective reporting or small-study exaggeration. Several included studies reported negative or non-significant results, which reduces concern about exclusive capture of positive findings; however, the power to detect reporting bias remains limited.

Overall certainty was judged low to moderate. Heterogeneity in outcome measurement and exposure specification, modest sample sizes with imprecise estimates, and observational design constraints temper the strength of inference in this literature.

Discussion

This systematic review identified six studies reporting no statistically significant association (Julin et al., 2017; Liu et al., 2016; Mazidi et al., 2017; Normando et al., 2020; Schöttker et al., 2019; Williams et al., 2016), four reporting positive associations (Jelmila et al., 2020; Liu et al., 2013; Richards et al., 2007; Beilfuss et al., 2017), and one reporting a U-shaped relationship (Kuo et al., 2023). The absence of a priori power calculations in the "null" studies, together with wide confidence intervals, assay-related variability, and heterogeneous adjustment sets, suggests that limited precision may underlie some non-significant findings. Because most studies adjusted for sex, confounding by sex is unlikely to explain the lack of statistical significance; however, adjustment for sex addresses confounding but not effect modification. Few studies tested an interaction between sex and 25(OH)D or reported sex-stratified estimates, and none captured menopausal status or hormone therapy. Prospectively focusing on more homogeneous, enriched populations, such as older adults with low baseline 25(OH)D or defined inflammatory burden, and prespecifying subgroup analyses by age band, sex/menopausal status, comorbidity strata, and baseline deficiency, may enhance feasibility and signal detection, thereby improving the meta-analyzability of future datasets.

The heterogeneity in findings may reflect differences in population characteristics, study quality, and statistical analysis. All three female-only cohorts reported positive associations (Jelmila et al., 2020; Liu et al., 2013; Richards et al., 2007). The NHS, UK twins, and premenopausal Indonesian women, predominantly middle-aged between 46 to 60 years, showed that higher 25(OH)D correlated with longer LTL. These female-only populations may be more homogeneous in health and lifestyle profiles, or may differ due to sex-specific biological factors such as hormonal status or VD metabolism that influence telomere dynamics. Consistently, a positive association was observed in middle-aged adults in Beilfuss et al., reinforcing that age and sex could modulate this relationship (Beilfuss et al., 2017; Díaz et al., 2024; Srinivas et al., 2020). Appropriate adjustment for true confounders is expected to reduce bias rather than increase it; observed differences across studies more likely reflect residual confounding from incomplete measurement or modeling, overadjustment due to conditioning on mediators or colliders, model misspecification including unmodeled nonlinearity, or genuine effect modification by age or sex. Future studies should prespecify minimally sufficient adjustment sets, assess nonlinearity, and test interaction terms or present sex- and age-stratified estimates to clarify these patterns.

On the other hand, the six studies reporting no association considered one men-only cohort (Julin et al., 2017), and five mixed-sex studies (Liu et al., 2016; Mazidi et al., 2017; Normando et al., 2020; Schöttker et al., 2019; Williams et al., 2016), all of them with a broader age range between 31 to 64 years, which makes them more heterogeneous in terms of the population analyzed. The null findings in male-only or male-dominant cohorts suggest

potential effect modification by sex. Adjustment for sex mitigates confounding but does not test interaction; few studies prespecified sex-stratified analyses or captured menopausal status/hormone therapy, limiting inference about sexual dimorphism in the VD–LTL relationship (Yeap et al., 2016). Sex may modify the relationship between VD status and LTL through differences in sex hormones, VD metabolism, and immune regulation. Yet only two of the included studies measured hormonal panels or menopausal status, limiting inferences about effect modification (Richards et al, 2007; Liu et al, 2016).

Kuo et al. (2023) reported a U-shaped association: both deficient ($<$ 25 nmol/L) and high ($>$ 125 nmol/L) 25(OH)D were linked to shorter LTL after multivariable adjustment for age, body-mass index, socioeconomic status, alcohol intake, smoking, and physical activity, including a balanced proportion of men and women. Low VD plausibly accelerates telomere attrition because insufficiency heightens systemic inflammation and oxidative stress, key drivers of telomeric damage (Renke et al., 2023). At the opposite extreme, overshooting the physiological range is not harmless: excessive VD can induce hypercalcemia, up-regulate fibroblast growth-factor 23, disrupt phosphate balance, and elevate reactive-oxygen species, any of which may erode telomeric DNA or repress telomerase activity (Tebben et al., 2016).

Regarding covariate control, ten of the eleven studies incorporated adjustment in their regression models; the sole exception was Jelmila et al. (2020), which reported unadjusted estimates. Commonly adjusted covariates included age, BMI, smoking, and physical activity, factors known to influence telomere length (Song et al., 2022; Srinivas et al., 2020). Studies reporting both positive and null associations between VD and LTL also adjusted for socioeconomic status, inflammatory markers, and chronic disease history (Chen et al., 2024; Alexeeff et al., 2019). These adjustments likely reduced residual confounding and improved internal validity. However, the season of blood draw was incompletely captured and seldom adjusted, and data on reproductive hormone panels and menopausal/hormone-therapy status were unavailable. These gaps limit assessment of sex-specific effects, permit residual confounding by seasonality, and may partly explain geographic heterogeneity across cohorts.

Three of the four studies reporting a positive association were conducted in the Northern Hemisphere (Richards et al, 2007; Beilfuss et al, 2017; Liu et al, 2013), whereas other studies from Europe, such as Germany and Finland, the United States, and South America did not detect differences. Taken together, these patterns do not indicate a consistent global geographic effect. Rather, incomplete control for seasonality and latitude-related UV exposure, often unreported or unadjusted, likely contributes to between-study variability. Because the season of blood draw was not uniformly available, residual confounding by seasonality remains plausible and may bias pooled interpretations.

Evidence for an association between VD status and LTL remains inconclusive in this review, a pattern reflected in the randomized literature. Per the pre-specified eligibility criteria, inclusion was limited to observational designs to synthesize evidence of associations and to characterize confounding; therefore, RCTs were not included in the analyses. Notably, two trials in distinct populations reported divergent findings: an ancillary analysis of the D-Health Trial (Australia; ages 60–84; n=1,519) tested monthly 60,000 IU cholecalciferol for $\geq$4 years and found no difference in mean LTL versus placebo (Rahman et al., 2023), whereas VITAL, including population with a mean age of 60 years, reported that daily VD supplementation, with or without omega-3, attenuated telomere attrition (Zhu et al., 2025). Taken together, both the observational synthesis and the available RCTs leave the overall evidence base inconclusive.

Observed heterogeneity is biologically plausible. Hormonal context may influence VD signaling and telomere maintenance; inflammatory-immune pathways and oxidative stress can modulate leukocyte telomere dynamics; and genetic variation in VD pathways may contribute to between-study differences. Clinically, the current observational evidence does not support VD supplementation solely to preserve telomeres; rather, VD should be managed according to established indications.

This review has several strengths. It is the first systematic review focused on the association between VD levels and LTL in observational studies. It used a rigorous and transparent methodology, following PRISMA guidelines, and included populations from Europe, the Americas, and Asia, making the findings applicable to diverse populations. Articles included in this systematic review are characterized by having high-quality data and low bias, based on the NOS scale (two studies catalogued as very good, eight as good, and one unsatisfactory).

However, important limitations must be acknowledged. First, the observational design of the included studies limits causal inference, and residual confounding is likely. Second, substantial heterogeneity across studies, regarding population characteristics, the covariates included for adjustment, and the statistical methods employed, complicates direct comparisons and synthesis of findings. Third, due to our predefined exclusion criteria, studies involving participants with serious medical conditions were not included, which limits the generalizability of the results to those populations. Fourth, given that only English publications were included, publication and language bias may also be present. Fifth, no included study provided a sample-size justification or minimal detectable effect for the VD-telomere analysis, and many were not primarily powered for telomere outcomes; this lack of design-based power likely reduced the ability to detect modest associations. Finally, this review was not prospectively registered with PROSPERO. The lack of preregistration may limit transparency and introduce a risk of selective reporting. To mitigate this risk, we provide supplementary materials, including the full search strategies and a list of excluded studies with reasons, and note that the eligibility criteria and outcomes were finalized a priori, before data synthesis.

Future investigations should prespecify sex and age-stratified analyses, incorporate hormonal panels such as estradiol, testosterone, and sex hormone-binding globulin, capture menopausal and hormone-therapy status, and explicitly test interactions to distinguish true effect modification from residual confounding. Study designs should ensure adequate power, control for season and latitude, standardize LTL assays and reporting, model potential nonlinearity, and use transparent, minimally sufficient adjustment sets. Notably, two randomized controlled trials conducted in distinct populations have reported divergent results, underscoring the need for additional, well-designed trials in enriched, clinically relevant subgroups such as older adults with low baseline 25(OH)D or a defined inflammatory burden; these trials should employ harmonized assays, standardized outcome timing focused on change in telomere length, rigorous adherence monitoring, and prespecified subgroup analyses. As trial evidence grows, pre-registered systematic reviews and meta-analyses, ideally using individual-participant data to harmonize sex, hormonal, and seasonal covariates, will be warranted. Finally, mechanistic studies should clarify hormonal, inflammatory, and immune, oxidative-stress, and genetic pathways through which VD may influence cellular aging biology.

Conclusion

Based on available observational studies, the association between VD status and LTL remains inconclusive. There is currently no evidence to recommend VD supplementation for telomere preservation.

Acknowledgments

We would like to express our gratitude to the authors of the included studies for their valuable contributions to the field. We also extend our sincere thanks to the PPCR program and the teaching assistants for providing the supportive framework and guidance that made this work possible.

Supplementary Materials

Search strategy on each database; full-text articles excluded from the review; bubble scatterplot of included studies.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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