

REVIEW

THE RELATIONSHIP BETWEEN VITAMIN D AND TELOMERE/TELOMERASE: A COMPREHENSIVE REVIEW

M. ZAREI¹, M. ZAREZADEH², F. HAMEDI KALAJAHI³, M.H. JAVANBAKHT⁴

1. Department of Cellular and Molecular Nutrition, School of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran; 2. Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran; 3. Department of Clinical Nutrition, Tehran University of Medical Sciences, Tehran, Iran; 4. Department of Cellular and Molecular Nutrition, School of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran.

Corresponding author: Mohammad Hassan Javanbakht, Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran, Email: Mhavan@sina.tums.ac.ir. Second corresponding author: Meysam Zarezadeh, Ph.D. of Nutritional Sciences, Department of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Attar-Neishaburi St., Golgasht Alley, Azadi Blvd., Tabriz, Iran. Fax Number: +984133340634, Phone Number: +989143319531, Email: zarezadehm@tbzmed.ac.ir

Abstract: Telomeres are repetitive nucleotide sequences that together with the associated shelterin complex protect the ends of chromosomes and maintain genomic stability. Evidences from various organisms suggests that several factors influence telomere length regulation, such as telomere binding proteins, telomere capping proteins, telomerase, and DNA replication enzymes. Recent studies suggest that micronutrients, such as vitamin D, folate and vitamin B12, are involved in telomere biology and cellular aging. In particular, vitamin D is important for a range of vital cellular processes including cellular differentiation, proliferation and apoptosis. As a result of the multiple functions of vitamin D it has been speculated that vitamin D might play a role in telomere biology and genomic stability. In this study, our main goal is investigating the relationship between telomerase enzyme and vitamin D. Findings of this study suggest that higher vitamin D concentrations, which are easily modifiable through nutritional supplementation, are associated with longer LTL, which underscores the potentially beneficial effects of this hormone on aging and age-related diseases. Vitamin D may reduce telomere shortening through anti-inflammatory and anti-cell proliferation mechanisms. Significant Low levels of telomerase activity create short telomeres, which in turn signal exit from the cell cycle resulting in cell senescence and apoptosis. In follow-up examination, the patients who remained vitamin D deficient tended to have shorter telomeres than those patients whose 25-hydroxyvitamin D levels were depleted. Increasing 25-hydroxyvitamin D levels in patients with SLE may be beneficial in maintaining telomere length and preventing cellular aging. Moreover, anti-telomere antibody levels may be a promising biomarker of SLE status and disease activity.

Key words: Vitamin D, telomere, telomerase.

J Frailty Aging 2020;in press
Published online July 7, 2020, <http://dx.doi.org/10.14283/jfa.2020.33>

Introduction

Telomeres appear to be of critical importance for genomic stability and cellular aging. Telomeres are repetitive nucleotide sequences that together with the associated shelterin complex protect the ends of chromosomes and maintain genomic stability. These end caps of chromosomes were first identified in 1938 by Hermann Müller (1). Since then telomere biology has been widely investigated and numerous studies indicate an involvement of telomeres in the process of aging. Telomere shortening and dysfunction have been proposed as indicators of cellular aging and are associated with age-related and inflammatory diseases including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), cancer or chronic obstructive pulmonary disease (2).

Telomeres are regions at the end of a chromosome, which protect the end of it from deterioration, the longer the better. The telomere regions reduce the degradation of genes near the ends of chromosomes by allowing for the shortening of chromosome ends, which necessarily occurs during

chromosome replication. Over time, due to numerous cell divisions over lifetime, the telomeres become shorter. During cell division, if cells divide without telomeres, they would lose the ends of their chromosomes and the necessary information they contain. The telomeres are disposable buffers blocking the ends of the chromosomes; they are consumed during cell division, but then replenished by an enzyme, telomerase. Telomerase deficiency is associated with aging, death, obesity, cardiovascular disease, depression and diabetes (3-5).

Micronutrients, such as vitamins and trace elements play an important role in cell metabolism and some studies suggest a direct effect of these micronutrients on telomere biology and cellular aging. Vitamin D, for example, is a steroid hormone with genomic and non-genomic activities that is involved in the regulation of cell proliferation, differentiation, apoptosis and aging (6). As a result of the multiple functions of vitamin D it has been speculated that vitamin D might play a role in telomere biology by regulating telomerase activity (2, 7, 8).

THE RELATIONSHIP BETWEEN VITAMIN D AND TELOMERE/TELOMERASE: A COMPREHENSIVE REVIEW

Historical Background

In the early 1930s, Hermann J. Muller and Barbara McClintock described the telomere (from the Greek word «telos», meaning end, and «meros», meaning part) as a protective structure at the terminal end of the chromosome. When this structure is absent, end-to-end fusion of the chromosome may occur, with ensuing cell death. In the 1970s, James D. Watson described what he called «end-replication problems». During DNA replication, DNA-dependent DNA polymerase does not completely replicate the extreme 5' terminal end of the chromosome, leaving a small region of telomere uncopied. He noted that a compensatory mechanism was needed to fill this terminal gap in the chromosome, unless the telomere would be shortened with each successive cell division (1, 3).

Meanwhile in the 1960s, Hayflick described a biological view of aging. He found that human diploid cells proliferate a limited number of times in a cell culture. The «Hayflick limit» is the maximal number of divisions that a cell can achieve in vitro. When cells reach this limit, they undergo morphologic and biochemical changes that eventually lead to arrest of cell proliferation, a process called «cell senescence» (9).

Then in the 1970s, Olovnikov connected cell senescence with end-replication problems in his «Theory of Marginotomy» in which telomere shortening was proposed as an intrinsic clocklike mechanism of aging that tracks the number of cell divisions before the arrest of cell growth or replicative senescence sets in (10). Greider and colleagues in 1988, corroborated this theory when they observed a progressive loss in telomere length in dividing cells cultured in vitro (4). In 1978, Elizabeth Blackburn found that the molecular structure of telomeres in *Tetrahymena pyriformis* contains long repeating units rich in thymine (T) and guanine (G) residues. In 1984, she and her colleagues isolated telomerase, the enzyme responsible for the maintenance and elongation of telomere length (11). In 1989, Gregg reported the existence of telomerase activity in human cancer cell lines, which was thought to contribute to the immortality of tumor cells (12). At about the same time, Greider and associates found that telomerase was nearly always absent in normal somatic cells (13).

In the 1990s, Shay and Harley detected telomerase in 90 of 101 human tumor cell samples (from 12 different tumor types), but found no activity in 50 normal somatic cell samples (from 4 different tissue types). Since then, more than 2600 human tumor samples have been examined and telomerase activity detected in about 90% of all tumor cells. The obvious implication is that telomerase may play a major role in the pathogenesis of cancer (14). Because of their role in physiologic aging, cancer pathogenesis, and premature aging syndromes (eg, progeria), telomeres and telomerase are currently under intensive investigation. Therefore, this review aims to underline the association between vitamin D and telomere and telomerase regarding to their molecular structure and associating proteins.

Telomeres, the Chromosome-End Protectors

The human telomeres consist of long, repetitive TTAGGG subunits, which are associated with a variety of telomere-binding proteins. These repeating sequences comprise a portion of the double-stranded telomeric DNA, which has an overhanging, single-stranded, G-rich 3' end. The human somatic cells will enter into replicative senescence after a limited number of cell replications. This phenomenon is attributed to the end-replication problem. At one or more concurrent sites within the replicating chromosome, DNA polymerase starts with a primer at the 3' end and runs toward the 5' end of the template, forming a 5' to 3' leading strand and a lagging daughter strand (15, 16). The leading strand runs toward the replication fork, whereas the synthesis of the lagging strand (consisting of Okazaki fragments) begins at the replication fork and runs in the opposite direction (Figure 1). When the synthesis is complete, the primers are degraded and internal gaps or spaces are formed at each site of replication.

The gaps between the newly replicated fragments of the lagging strand are filled by the action of DNA ligase; the terminal gap, the space left by the primer at the end of both strands, is not filled. The terminal gap is further enlarged by the action of putative 5' to 3' exonuclease, which degrades 130-210 nucleotides. Thus, the 5' end of the telomere is shortened with each replication (14, 17). After a finite number of replications, the telomere reaches a critical length, the senescence checkpoint, and either cell-proliferation arrest or cell death occurs (physiologic aging). If, however, the cell escapes or avoids this checkpoint, chromosomal instability or end-to-end fusion of the chromosome occurs, which may contribute to cell death or carcinogenesis.

Vitamin D

Metabolism and Physiologic Aspects of Vitamin D

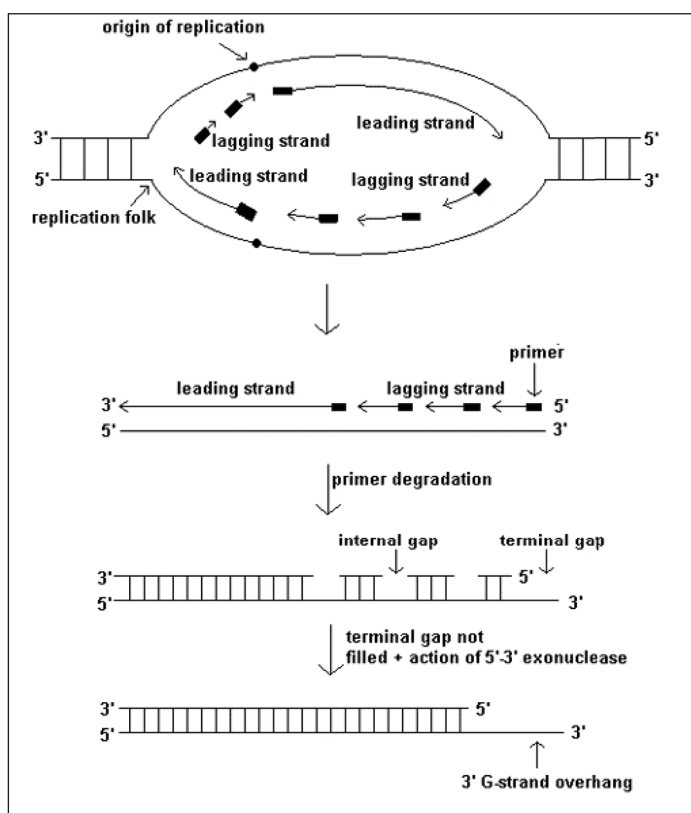
Vitamin D metabolism is a complex process involving the action of UV radiation and hydroxylation steps. Vitamin D₃ is primarily produced in the skin through the action of UV-B light on 7-dehydrocholesterol (18). In most individuals, dietary intake, from sources such as wild-caught fatty fish, provides only a small additional contribution to total vitamin D levels (19).

Vitamin D requires two hydroxylation steps to reach its active form. The first hydroxylation, occurs in the liver and produces 25-hydroxy vitamin D. Vitamin D in the form of 25(OH) D₃ is the predominant form of the vitamin found in the circulation. 25(OH) D₃ undergoes further hydroxylation at the C1 position in the proximal tubule of the kidney (20). The 1,25-dihydroxyvitamin D₃ [1,25(OH)₂ D₃] thus formed is responsible for most, if not all, of the biological actions of the vitamin. A number of extra-renal tissues have demonstrated the ability to convert 25(OH) D₃ to 1,25(OH)₂ D₃. The 1,25(OH)₂ D₃ produced by these tissues appears to act locally in an autocrine or paracrine fashion and does not contribute

significantly to the circulating 1,25(OH)₂ D₃ concentration (21).

Figure 1

Telomere length shortens after DNA replication. Forming replication fork, DNA polymerase reads the strands in the 3' to 5' direction which makes leading and lagging strands opposite each other. After the formation of Okazaki fragments in the lagging strand, the RNA primer of each section should be changed to DNA as well as sealing the internal gaps but It experiences a problem about the last RNA primer. This part would be destroyed instead, which leads to a terminal gap. Finally, telomere shortening happens due to the action of 5'3' exonuclease



Vitamin D Deficiency and Related Diseases

The classical role of vitamin D is in the maintenance of adequate calcium and phosphate status. Severe vitamin D deficiency causes rickets and osteomalacia, while more moderate deficiency is associated with osteoporosis and increased fracture risk (22). The discovery that VDR exists in multiple tissues unrelated to vitamin D's classical function, has led to intense interest in the role of vitamin D in diverse aspects of health. Epidemiological data support an association between vitamin D deficiency and numerous conditions. These include various parameters of muscle function, multiple autoimmune diseases, upper respiratory tract infection (URTI), tuberculosis, insulin resistance, T2DM, coronary heart disease, heart failure and peripheral vascular disease and all-cause

mortality (23, 24). Observational data suggest an association between hypovitaminosis D and cognitive function, depression, bipolar disorder and schizophrenia (25). Furthermore, there is an association between vitamin D and a number of cancers, including colorectal and prostate cancer (26). Despite these associations, there are limited data from randomized controlled trials demonstrating that supplementing with vitamin D in deficient individuals improves clinical outcomes. However, there is evidence that supplementation improves muscle function, prevents type 1 diabetes and multiple sclerosis, as well as diminishes clinical exacerbations in those with pre-existing multiple sclerosis (27). Supplementation studies have also shown accelerated clinical recovery from tuberculosis, improvement in depressive symptoms and, in males supplemented during the first year of life, a decreased risk of schizophrenia (28, 29). Results from studies addressing domains, such as mortality, and diabetes risk, URTI and cancer have been mixed (25, 28, 30-32).

Vitamin D and Telomere Biology

Research Method

In the present review study by searching the database: Pubmed, Scopes, Google Scholar, Science Direct, Medline, Web of Science (ISI WoK), Wiley Online Library & Magiran.

Using the vocabulary combination: «Vitamin D», cholecalciferol, «vitamin D 3», «vitamin D3» «Cholecalciferol»[Mesh], «Vitamin D»[Mesh], Telomere, Telomerase

The search was conducted in English-language articles, as well as review articles between 2010 - 2017. Out of 25 reviewed articles, 10 articles related to the purpose of this narrative review was selected and they went through the Narrative review study and The relationship between telomerase enzyme and vitamin D was assessed.

Studies in Humans

A summary of the literature data regarding the link between telomere biology and vitamin D is reported in Table 1. Richards et al. were the first to demonstrate a positive correlation between serum 25-OHD and LTL in humans, which remained significant after adjustment for age (33). In their study they analyzed 2160 women of the TwinsUK cohort. After multiple adjustments for age, season of vitamin D measurement, menopausal status, use of hormone replacement therapy and physical activity, the difference in telomere length between the highest (124±37.3 nmol/L) and lowest (40.9±11 nmol/L) tertile of 25-OHD concentrations was equivalent to 5 years of telomeric aging (33). In addition, the authors analyzed a subpopulation of 700 women that have consumed vitamin D supplements. On average, these women had longer telomeres than women who did not consume vitamin D supplements. The variations in type and dosage of supplements between subjects was not mentioned. However, this difference was not

THE RELATIONSHIP BETWEEN VITAMIN D AND TELOMERE/TELOMERASE: A COMPREHENSIVE REVIEW

Table 1
Review of researches: The Relationship between vitamin D & Telomere / Telomerase

First author, Year	Location	Study design	Sample size	Gender Male/ Female	Age (years)	Study population	Presence of relationship	Main outcomes	Reported results
Richards, 2007	United Kingdom	Cross-sectional	2160	0/2160	49.4±12.9	From a large population-based cohort of twins	Yes	Higher serum 25(OH)D concentrations are associated with longer LTL.	
Borras, 2012	Spain	Interventional case-control	62	40/34	>50	Hemodialysis patients & controls	Yes	Twenty-four HD patients who were treated with active vitamin D compounds had greater PBMC telomere length compared to the untreated patients.	
Zhu, 2012	USA	Double blind, randomized and placebo controlled clinical trial	37	13/24	19-50	Overweight African Americans	Yes	Vitamin D supplementation significantly increased PBMC telomerase activity by 19.2% in overweight African Americans.	
Hoffecker, 2013	USA	Case-controlled cross-sectional	118	0/118	39.86 ± 11.57	African American female SLE patients and unaffected controls from SLE Gullah Health cohort	Yes	Subjects with serum levels of 25(OH)D < 20 ng/ml had shorter LTL in cases and controls, however, no relationship between 25(OH)D levels and LTL was observed in all subjects.	
Liu, 2013	USA	Cross-sectional	1424	0/1424	30-55	Registered nurses from the NHS prospective cohort study	Yes	Higher quartiles of 25(OH)D, but not 1,25(OH)2 D was associated with longer LTL.	
Kim, 2018	South Korea	Cross-sectional	106	0/106	32.69 ± 2.85	Healthy pregnant mother–new-born pairs	Yes	Maternal 25(OH)D levels were positively associated with LTL of new-borns	
Lau, 2017	Malaysia	Cross-sectional	300	NR	68.04 ± 5.56	Elderly individuals divided to 3 aging groups MCI (n= 100) UA (n= 100) SA (n= 100)	Yes	Telomere length was reported to be significantly longer in the SA group which had higher serum Vitamin D levels and higher Vitamin D intake.	
Schöttker, 2019	Germany	Cohort	9,940	4483/5457	62.1 ± 6.6	Healthy adults from the ESTHER cohort study	NO	25(OH)D levels were associated with chronological age but not with LTL.	
Herlin, 2019	Northern Argentina	Cohort	169	0/169	~24	Pregnant mother-child pairs	NO	The studied antioxidant (Vitamin D3) did not modify the associations between multiple toxic metals and relative telomere length (rTL) in the maternal blood, cord blood, and placenta.	

LTL: Leukocyte Telomere Length, HD: Hemodialysis Patient, PBMC: Peripheral Blood Mononuclear Cell, SLE: Systemic Lupus Erythematosus, 25(OH)D: 25-hydroxy Vitamin D, MCI: Mild Cognitive Impairment, UA: Usual Aging, SA: Successful Aging, NR: Not Reported.

statistically significant. These initial findings were confirmed by a subsequent study performed by Liu et al. (34). In this study, analyses were performed on 1424 women of the Nurses' Health Study and the results showed a positive correlation between LTL and serum 25(OH) D3 concentrations. Logistic regression analysis indicated a concentration-dependent relationship. However, calcium intake modified this association significantly. 1,25(OH)₂ D3 was also measured, but did not correlate with LTL. As single nucleotide polymorphisms (SNPs) in genes involved in vitamin D metabolism (like SNPs in VDR, VDBP, CYP2R1 and DHCR7), are reported to affect vitamin D blood concentrations, Liu et al. analyzed vitamin D-related SNPs in their population. They identified a positive association between rs7041 and rs4588 (both SNPs of VDBP), and 25(OH) D3 levels. However, none of the investigated vitamin D-related SNPs were significantly associated with LTL (34). On the other hand, Hoffecker et al. reported a significant correlation between telomere length and serum 25(OH) D3 concentrations in vitamin D deficient (serum 25(OH) D3 <20 ng/mL) patients with systemic lupus erythematosus (SLE) and unaffected controls (35). Moreover, patients with SLE whose serum 25(OH) D3 concentrations remained insufficient/deficient (<30 ng/mL), after 2.8 years of follow-up had shorter telomeres than patients with sufficient concentration (>30 ng/mL). Similar results were observed in two recent cross-sectional studies. Kim et al. demonstrated that maternal 25(OH) D3 levels were positively associated with newborn leukocyte telomere length after adjustment for the maternal age, BMI, leukocytes telomere length, WBC count, glycosylated hemoglobin level, healthy behavior, nutritional intake and sex and birth weight for the newborns (36). The study included 106 healthy pregnant mother–newborn pairs. The percentage of pregnant women with vitamin D deficiency [25(OH) D3 < 50 nmol/L (20 ng/ml)] was 80%. The mean leukocyte telomere lengths differed significantly between mothers and offspring ($p < 0.01$). However, leukocytes telomere length did not differ between male [1.58 (1.23–2.06)] and female [1.69 (1.29–2.38)] newborns ($p = .11$). Leukocytes telomere length in newborns correlated positively with maternal leukocyte telomere lengths ($r = .76$, $p < .01$), maternal 25(OH) D3 concentrations ($r = .72$, $p < .01$), maternal energy intakes ($r = .22$, $p = .03$) and newborn body weight (36). Lau et al. in a comparative cross-sectional study on older adults aged 60 years and above have found that vitamin D intake was significantly higher in successful aging (SA) group compared to mild cognitive impairment (MCI) group. Participants in SA group also showed significantly higher serum vitamin D level than those in MCI group. Telomere length was also reported to be significantly different between SA (97.52 ± 35.49 kb/genome diploid) with usual ageing (80.07 ± 32.78 kb/genome diploid) and MCI (71.84 ± 30.97 kb/genome diploid) ($p < 0.05$) (37). Along with all these studies in two study the results have been inconsistent in terms of the correlation between vitamin D levels and telomere length. In an investigation based on a

population-based, cohort study Schöttker et al. studied Serum 25(OH) D3 levels as an ageing marker (38). In order to test the independence of 25(OH) D3 from other ageing markers, they used leukocyte telomere length (LTL), epigenetic age acceleration and 8-isoprostane levels. 25(OH) D3 levels were associated with chronological age and 8-isoprostane levels but not with LTL and the epigenetic age acceleration. The association of age and 25(OH) D3 levels was linear and could be modelled with a linear regression model, which revealed a mean loss of 2.9 nmol/L 25(OH) D3 per 10 years increase in age. The ageing markers were not associated with each other except for the two estimators of epigenetic age acceleration (Horvath's epigenetic clock and Hannum's epigenetic clock) as well as 8-isoprostane with 25(OH) D3 levels. Additional adjustment for BMI and education did not substantially change the results. The corresponding dose-response curves were not linear. Instead, L-shaped curves for LTL and 25(OH) D3 levels were observed (38). In another mother–child cohort herlin et al. measured multiple toxic metals in the maternal blood or urine collected during late pregnancy, as well as the placenta and cord blood collected in 169 pregnant women to Exploring telomere length in relation to exposure to multiple toxic metals. Additionally, they selected nutritional factors with antioxidant properties (zinc, selenium, folate, and vitamin D3) that might act as modifier of the associations between toxic metals and rTL. In the models with vitamin D3, additionally were adjusted for the season of sampling. Exposure to Boron and Antimony during pregnancy was associated with shorter maternal rTL, and Lithium with longer maternal rTL. Arsenic concentrations in the placenta ($n = 98$), blood and urine were positively associated with placental rTL about 0.2 SD by doubled Arsenic. In the cord blood ($n = 88$), only Lead was associated with rTL (inversely), particularly in boys (p for interaction 0.09). The studied antioxidants did not modify the associations, except that with Antimony (39). There was only one interventional study investigating the effect of vitamin D supplementation on telomere biology while the others were designed as cross-sectional studies. Zhu et al. treated 37 obese Afro-American subjects in a double-blind randomized fashion with either a monthly oral dose of 60,000 IU of vitamin D3 or placebo for a period of 4 months. At the end of the study the serum 25-OHD concentration in vitamin D treated subjects was markedly increased when compared to baseline. The rise in serum 25(OH) D3 was accompanied by a 19.2% increase in peripheral blood mononuclear cell (PBMC) telomerase activity. There were no such changes observed in placebo group. However, LTL was not measured in this study limiting also the strength of their findings (40). Moreover, another study supports these findings. Borrás et al. observed hemodialysis patients treated with calcitriol or analogs for at least 6 months have longer telomeres compared to patients not receiving this treatment (41).

THE RELATIONSHIP BETWEEN VITAMIN D AND TELOMERE/TELOMERASE: A COMPREHENSIVE REVIEW

Animal Studies

So far, only one animal model has studied the role of vitamin D in telomere biology (42). In Siebert et al. study, wistar rats underwent ovariectomy (OVX) surgery received 500IU/kg/day vitamin D3 for 30 days. Although, both OVX and vitamin D per se, were not associated with telomere length, in the OVX+Vitamin D group, VIT D supplementation was able to reverse the telomere shortening process which was observed ($p < 0.005$) in genomic DNA extracted from hippocampus tissue. Unfortunately, this study haven't been able to report the impact of this supplementation on aging and longevity of rats. Otherwise, a limited number of animal studies have investigated the involvement of vitamin D in cellular aging and the results which are reported following, mainly support a significant relationship. VDR knockout mice (VDR $-/-$), have been used to study the function of vitamin D in cellular aging processes (43). Keisala et al. have shown that these mice develop signs of premature aging, such as infertility, muscle atrophy, reduced immune function and osteoporosis. Furthermore, VDR $-/-$ mice have a shorter life span. The phenotype of premature aging in these animals was accompanied by a reduced expression of NF- κ B, FGF-23 and p53 (44).

These pathways are known to play an important role in cellular senescence (5). Valcheva et al. have also shown that in VDR $-/-$ mice, vascular smooth muscle cells produced higher intracellular superoxide anion and thus promoting premature senescence (45).

Besides the VDR $-/-$ mice model, other two mice models (FGF-23 $-/-$ and Klotho $-/-$) have been utilized for studying the relationship of vitamin D pathway and aging (46). In fact, both FGF-23 and Klotho-deficient mice have increased renal expression of 1 α -hydroxylase, accompanied by significantly elevated serum levels of 1,25(OH) $_2$ D $_3$, infertility, atherosclerosis, skin atrophy, muscle wasting, T-cell deregulation and short lifespan (47). A significant rescue of these phenotypes has been achieved providing a vitamin D-deficient diet (48). The hypothesis that hypo- or hyper-vitaminosis D causes accelerated aging is supported by animal studies that show a U-shaped association between serum 25(OH) D $_3$ and the risk of cancer. Based on these results it can also be speculated that there is an optimal serum vitamin D concentration for general health (48).

In Vitro Studies

Further evidence for a role of vitamin D in telomere biology and cellular aging comes from cell culture studies. Treatment of different cell lines with vitamin D drives cell differentiation and has been shown to dramatically reduce telomerase activity (49). For example, a reduction of telomerase activity was observed after treating leukemic HL-60 cells for 5 or 7 days with 100 ng/mL of vitamin D $_3$ (49). However, the strength of this study is limited by the fact that there was no additional test for vitamin D concentration. Therefore, it remains unclear if there is a concentration-dependent relationship between telomerase

activity and vitamin D. A similar phenomenon was observed in human prostate cancer cells (50). Treatment of these cells with a combination of 1,25(OH) $_2$ D $_3$ and 9-cis-retinoic acid significantly reduced telomerase activity. The authors hypothesized a direct interaction of the VDR/RXR heterodimer with the DR3' sequence in the human telomerase reverse transcriptase (hTERT) promoter as the underlying mechanism. The DR3' sequence (5'-AGTTCATGGAGTTCA-3') is a vitamin D response element present in the promoter region of the hTERT gene (50). Jiang et al. have described an alternative mechanism in an ovarian cancer cell line. Vitamin D downregulates hTERT activity in these cells by decreasing the stability of hTERT mRNA (51). Kasiappan et al. proposed another appealing mechanism of vitamin D dependent telomerase regulation through small non-coding RNA molecules. They were able to demonstrate that vitamin D can induce microRNA-498 (miR-498) expression, which in turn decreases the mRNA expression of hTERT (51). A microarray analysis of ovarian cancer cells treated with vitamin D identified miR-498 as the most abundantly induced microRNA. This finding has been subsequently validated in multiple human cancer types (51). Consistent with this observation, a functional VDRE was identified in the 5-prime regulatory region of the miR-498 genome, which is occupied by the VDR and its co-activators. Furthermore, Kasiappan et al. showed that miR-498 targets the 3-prime untranslated region of hTERT and decreases its expression (51). The level of miR-498 expression was decreased in malignant human ovarian tumor cells as well as in human ovarian cancer cell lines. The ability of vitamin D to decrease hTERT and to suppress ovarian cancer growth is reduced when miR-498 is depleted (51). However, all these studies were performed on cancer cell lines that are characterized by genomic instability, chromosome alterations and other features that are associated with transformed cells. Therefore, it is not possible to extrapolate the real role of vitamin D in telomere biology in healthy normal cells. From immunology point of view, Vitamin D has been known to have immunomodulatory effects on a wide range of immune cells including CD4+ and CD8+ lymphocytes, B lymphocytes, macrophages as well as dendritic cells (52), which are the main components of PBMCs. Several in vitro studies revealed that 1,25(OH) $_2$ D $_3$ increases regulatory CD4+ T cell percentages and may reduce CD8+ T cell percentage (53). Moreover, there are differences in telomerase dynamics between CD4+ and CD8+ T cells. Therefore, we cannot exclude the possibility that vitamin D-induced increase in PBMC telomerase activity is due to the increase in certain cell types within the PBMC with higher telomerase activity since we did not sort the subtypes of PBMC at the time of collection and telomerase measurement. Further studies are needed to clarify the role of vitamin D in healthy cell's telomere biology.

Conclusions

In conclusion, it seems that existing evidence can nearly support the concept that vitamin D contributes to cellular aging and telomere biology. In human studies an inverse relationship between serum vitamin D and age-related diseases is shown as well as serum vitamin D and mortality. These studies are predominantly of cross-sectional nature and the included longitudinal and interventional studies have been too small and heterogeneous to draw conclusive findings. Another limitation is the heterogeneity of studied population in age, gender and the type of morbidity. At a cellular level, vitamin D appears to regulate proliferation, senescence and apoptosis through genomic and non-genomic pathways like preservation of telomere biology. Animal and cell culture studies are also heterogeneous remarkably as they have used different animal models and cell types. This hampers the comparison of results and makes general conclusions impossible. Moreover, all studies published so far have significant limitations and results are sometimes conflicting. Further systematic studies are needed to understand the role of vitamin D in telomere biology and cellular aging.

Conflicts of Interest: The authors have no conflict of interest.

References

- Aubert G, Lansdorp PM. Telomeres and Aging. *Physiological Reviews*. 2008;88(2):557-79. 10.1152/physrev.00026.2007
- Pusceddu I, Farrell Christopher-John L, Di Pierro Angela M, Jani E, Herrmann W, Herrmann M. The role of telomeres and vitamin D in cellular aging and age-related diseases. *Clinical Chemistry and Laboratory Medicine (CCLM)*2015. p. 1661.
- Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB. Telomere end-replication problem and cell aging. *Journal of Molecular Biology*. 1992;225(4):951-60. [https://doi.org/10.1016/0022-2836\(92\)90096-3](https://doi.org/10.1016/0022-2836(92)90096-3)
- Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345:458. 10.1038/345458a0
- Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, Lai MK, Kappei D, Kumar AP, Sethi G. Ageing and the telomere connection: An intimate relationship with inflammation. *Ageing Res Rev*. 2016;25:55-69. 10.1016/j.arr.2015.11.006
- Kord-Varkaneh H, Kord-Varkaneh H, Rinaldi G, Hekmatdoost A, Fatahi S, Tan SC, Shadnough M, Khani V, Mousavi SM, Zarezadeh M. The influence of vitamin D supplementation on IGF-1 levels in humans: A systematic review and meta-analysis. 2020.
- Ames BN. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proceedings of the National Academy of Sciences*. 2006;103(47):17589-94. 10.1073/pnas.0608757103
- Cong Y-S, Wright WE, Shay JW. Human Telomerase and Its Regulation. *Microbiology and Molecular Biology Reviews*. 2002;66(3):407-25. 10.1128/mmr.66.3.407-425.2002
- Harley CB, Vaziri H, Counter CM, Allsopp RC. The telomere hypothesis of cellular aging. *Experimental Gerontology*. 1992;27(4):375-82. [https://doi.org/10.1016/0531-5565\(92\)90068-B](https://doi.org/10.1016/0531-5565(92)90068-B)
- Olovnikov AM. A theory of marginotomy: The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *Journal of Theoretical Biology*. 1973;41(1):181-90. [https://doi.org/10.1016/0022-5193\(73\)90198-7](https://doi.org/10.1016/0022-5193(73)90198-7)
- Blackburn EH. Structure and function of telomeres. *Nature*. 1991;350:569. 10.1038/350569a0
- Morin GB. Is Telomerase a Universal Cancer Target? *JNCI: Journal of the National Cancer Institute*. 1995;87(12):859-61. 10.1093/jnci/87.12.859
- Greider CW, Blackburn EH. Telomeres, telomerase and cancer. *Sci Am*. 1996;274(2):92-7.
- Wai LK. Telomeres, telomerase, and tumorigenesis--a review. *MedGenMed*. 2004;6(3):19.
- Gravel S, Wellinger RJ. Maintenance of double-stranded telomeric repeats as the critical determinant for cell viability in yeast cells lacking Ku. *Mol Cell Biol*. 2002;22(7):2182-93. 10.1128/mcb.22.7.2182-2193.2002
- Chan SS, Chang S. Defending the end zone: Studying the players involved in protecting chromosome ends. *FEBS Letters*. 2010;584(17):3773-8. <https://doi.org/10.1016/j.febslet.2010.06.016>
- Zhu H, Shuman S. Gap filling activities of Pseudomonas DNA ligase D (LigD) polymerase and functional interactions of LigD with the DNA end-binding Ku protein. *J Biol Chem*. 2010;285(7):4815-25. 10.1074/jbc.M109.073874
- Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. *J Adv Res*. 2015;6(6):793-804. 10.1016/j.jare.2014.01.011
- Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, Berkowitz R, Martinello S, Holick MF. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? *J Steroid Biochem Mol Biol*. 2007;103(3-5):642-4. 10.1016/j.jsbmb.2006.12.010
- Chatterjea M, Shinde R. *Textbook of Medical Biochemistry: Eighth Edition*: Jaypee Brothers, Medical Publishers Pvt. Limited; 2011.
- Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *The American Journal of Clinical Nutrition*. 2008;88(2):491S-9S. 10.1093/ajcn/88.2.491S
- Cashman KD. Vitamin D in childhood and adolescence. *Postgrad Med J*. 2007;83(978):230-5. 10.1136/pgmj.2006.052787
- Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol*. 2014;21(3):319-29. 10.1016/j.chembiol.2013.12.016
- Matyjaszek-Matuszek B, Lenart-Lipińska M, Woźniakowska E. Clinical implications of vitamin D deficiency. *Prz Menopauzalny*. 2015;14(2):75-81. 10.5114/pm.2015.52149
- Cherniack EP, Troen BR, Florez HJ, Roos BA, Levis S. Some new food for thought: the role of vitamin D in the mental health of older adults. *Curr Psychiatry Rep*. 2009;11(1):12-9.
- Jacobs ET, Kohler LN, Kunihiro AG, Jurutka PW. Vitamin D and Colorectal, Breast, and Prostate Cancers: A Review of the Epidemiological Evidence. *J Cancer*. 2016;7(3):232-40. 10.7150/jca.13403
- Myhr K-M. Vitamin D treatment in multiple sclerosis 2009. 104-8 p.
- McGrath J, Saari K, Hakko H, Jokelainen J, Jones P, Jarvelin MR, Chant D, Isohanni M. Vitamin D supplementation during the first year of life and risk of schizophrenia: a Finnish birth cohort study. *Schizophr Res*. 2004;67(2-3):237-45. 10.1016/j.schres.2003.08.005
- Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis'. *BMC Infectious Diseases*. 2013;13(1):22. 10.1186/1471-2334-13-22
- Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R, Laakso M, Topliss D, Jenkins AJ, Blankenberg S, et al. Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. *Diabetes Care*. 2015;38(3):521-8. 10.2337/dc14-0180
- Gandini S, Boniol M, Haukka J, Byrnes G, Cox B, Sneddy MJ, Mullie P, Autier P. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer*. 2011;128(6):1414-24. 10.1002/ijc.25439
- Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ*. 2003;326(7387):469. 10.1136/bmj.326.7387.469
- Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, Lu X, Surdulescu GL, Swaminathan R, Spector TD, et al. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. *Am J Clin Nutr*. 2007;86(5):1420-5.
- Liu JJ, Prescott J, Giovannucci E, Hankinson SE, Rosner B, Han J, De Vivo I. Plasma vitamin D biomarkers and leukocyte telomere length. *Am J Epidemiol*. 2013;177(12):1411-7.
- Hoffecker BM, Raffield LM, Kamen DL, Nowling TK. Systemic lupus erythematosus and vitamin D deficiency are associated with shorter telomere length among African Americans: a case-control study. *PLoS One*. 2013;8(5).
- Kim JH, Kim GJ, Lee D, Ko JH, Lim I, Bang H, Koes BW, Seong B, Lee DC. Higher maternal vitamin D concentrations are associated with longer leukocyte telomeres in newborns. *Matern Child Nutr*. 2018;14(1). 10.1111/mcn.12475
- Lau H, M.L AF, Ludin M, Rajab N, Shahar S. Identification of Neuroprotective Factors Associated with Successful Ageing and Risk of Cognitive Impairment among Malaysia Older Adults. *Current Gerontology and Geriatrics Research*. 2017;2017:1-7. 10.1155/2017/4218756
- Schottker B, Hagen L, Zhang Y, Gao X, Holleczeck B, Gao X, Brenner H. Serum 25-Hydroxyvitamin D Levels as an Aging Marker: Strong Associations With Age and All-Cause Mortality Independent From Telomere Length, Epigenetic Age Acceleration, and 8-Isoprostane Levels. *J Gerontol A Biol Sci Med Sci*. 2019;74(1):121-8. 10.1093/gerona/gy253
- Herlin M, Broberg K, Igra AM, Li H, Harari F, Vahter M. Exploring telomere length in mother-newborn pairs in relation to exposure to multiple toxic metals and potential modifying effects by nutritional factors. *BMC Med*. 2019;17(1):77. 10.1186/s12916-

THE RELATIONSHIP BETWEEN VITAMIN D AND TELOMERE/TELOMERASE: A COMPREHENSIVE REVIEW

- 019-1309-6
40. Zhu H, Guo D, Li K, Pedersen-White J, Stallmann-Jorgensen IS, Huang Y, Parikh S, Liu K, Dong Y. Increased telomerase activity and vitamin D supplementation in overweight African Americans. *Int J Obes (Lond)*. 2012;36(6):805-9. 10.1038/ijo.2011.197
41. Borrás M, Panizo S, Sarro F, Valdivielso JM, Fernández E. Assessment of the potential role of active vitamin D treatment in telomere length: a case-control study in hemodialysis patients. *Clin Ther*. 2012;34(4):849-56.
42. Siebert C, Dos Santos TM, Berto CG, Parisi MM, Coelho RP, Manfredini V, Barbe-Tuana FM, Wyse ATS. Vitamin D Supplementation Reverses DNA Damage and Telomeres Shortening Caused by Ovariectomy in Hippocampus of Wistar Rats. *Neurotox Res*. 2018;34(3):538-46. 10.1007/s12640-018-9909-z
43. Lanske B, Razzaque MS. Vitamin D and aging: old concepts and new insights. *J Nutr Biochem*. 2007;18(12):771-7. 10.1016/j.jnutbio.2007.02.002
44. Keisala T, Minasyan A, Lou Y-R, Zou J, Kalueff A, Pyykkö I, Tuohimaa P. Premature aging in vitamin D receptor mutant mice 2009. 91-7 p.
45. Valcheva P, Cardus A, Panizo S, Parisi E, Bozic Stanojevic M, Lopez-Novoa J, Dusso A, Fernández E, Valdivielso J. Lack of vitamin D receptor causes stress-induced premature senescence in vascular smooth muscle cells through enhanced local angiotensin-II signals 2014. 247-55 p.
46. Nakatani T, Sarraj B, Ohnishi M, Densmore MJ, Taguchi T, Goetz R, Mohammadi M, Lanske B, Razzaque MS. In vivo genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of systemic phosphate homeostasis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2009;23(2):433-41. 10.1096/fj.08-114397
47. Lanske B, Razzaque MS. Premature aging in klotho mutant mice: cause or consequence? *Ageing research reviews*. 2007;6(1):73-9. 10.1016/j.arr.2007.02.002
48. Davis CD, Milner JA. Nutrigenomics, vitamin D and cancer prevention. *J Nutrigenet Nutrigenomics*. 2011;4(1):1-11. 10.1159/000324175
49. Kang SN, Kim SH, Chung SW, Lee MH, Kim HJ, Kim TS. Enhancement of 1 alpha,25-dihydroxyvitamin D(3)-induced differentiation of human leukaemia HL-60 cells into monocytes by parthenolide via inhibition of NF-kappa B activity. *British journal of pharmacology*. 2002;135(5):1235-44. 10.1038/sj.bjp.0704573
50. Ikeda N, Uemura H, Ishiguro H, Hori M, Hosaka M, Kyo S, Miyamoto K, Takeda E, Kubota Y. Combination treatment with 1alpha,25-dihydroxyvitamin D3 and 9-cis-retinoic acid directly inhibits human telomerase reverse transcriptase transcription in prostate cancer cells. *Mol Cancer Ther*. 2003;2(8):739-46.
51. Kasiappan R, Shen Z, Tse AKW, Jinwal U, Tang J, Lungchukiet P, Sun Y, Kruk P, Nicosia SV, Zhang X, et al. 1,25-Dihydroxyvitamin D3 suppresses telomerase expression and human cancer growth through microRNA-498. *The Journal of biological chemistry*. 2012;287(49):41297-309. 10.1074/jbc.M112.407189
52. Querfeld U. Vitamin D and inflammation. *Pediatric Nephrology*. 2013;28(4):605-10. 10.1007/s00467-012-2377-4
53. Gorman S, Geldenhuys S, Judge M, Weeden C, Waithman J, Hart P. Dietary Vitamin D Increases Percentages and Function of Regulatory T Cells in the Skin-Draining Lymph Nodes and Suppresses Dermal Inflammation. *Journal of Immunology Research*. 2016;2016:1-13. 10.1155/2016/1426503