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Original Contribution

Plasma Vitamin D Biomarkers and Leukocyte Telomere Length

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Vitamin D may reduce telomere shortening through anti-inflammatory and anti-cell proliferation mechanisms. In the present study, we examined the association between vitamin D and relative leukocyte telomere length by using both plasma 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) biomarkers. Vitamin D biomarker levels and leukocyte telomere length were measured using plasma samples collected in 1989–1990 from participants of the Nurses' Health Study, a study of nurses from 11 US states. In total, 1,424 participants had their 25(OH)D levels assessed and 837 had their $1,25(OH)_2D$ levels assessed. Genotyping was performed on 480 participants on 12 single nucleotide polymorphisms in vitamin D–related genes. Linear and logistic regression models were used. Higher 25(OH)D levels were significantly associated with longer telomere length (P for trend = 0.05), and the odds ratio increased from 1.07 ($P = 0.65$) when comparing the second lowest quartile of 25(OH)D with the lowest to 1.59 ($P = 0.01$) when comparing the highest quartile with the lowest. Vitamin D–related single nucleotide polymorphisms and $1,25(OH)_2D$ levels were not significantly associated with telomere length. Total calcium intake significantly modified the association between 25(OH)D and telomere length (P for interaction = 0.05). Higher plasma 25(OH)D levels may be associated with longer telomeres, and this association may be modified by calcium intake.

epidemiology; 25(OH)D; $1,25(OH)_2D$; telomere length

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; SNP, single nucleotide polymorphism.

Both vitamin D and telomere length have been found to be associated with a variety of health conditions, but there have been an insufficient number of studies on the association between the two. Vitamin D is synthesized upon epidermal exposure to solar ultraviolet B radiation, is found naturally in foods such as fish liver oil and fatty fish species, and is added to foods such as milk and cereal. Vitamin D and its metabolites are primarily transported by the vitamin D–binding protein in the circulation. Two hydroxylations allow vitamin D to become biologically active. The first hydroxylation occurs in the liver to convert vitamin D to 25-hydroxyvitamin D (25(OH)D). The second hydroxylation occurs in various organs to form the physiologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which binds to the vitamin D receptor in the cell nucleus. In its hydroxylated active form, vitamin D has effects other than regulation of calcium metabolism, including reducing cell proliferation, promoting cell differentiation, reducing inflammation, and many others (1, 2). Vitamin D deficiency is associated with an increased incidence of cancer, cardiovascular disease, and other chronic illnesses, though it remains unclear which of the associations are causal (3).

Telomeres are repetitive DNA sequences that protect the ends of linear chromosomes. Telomeres shorten over time in cells because DNA polymerases are not able to fully replicate chromosomes during cell division, which is also known as the end-replication problem. Somatic cells lack telomerase activity to restore telomere length, whereas in germ and stem cells, telomerase restores telomere length by adding hexameric repeats to chromosome ends. In addition,

telomere shortening is accelerated by oxidative stress, inflammation, and cell proliferation (4–6). Shorter telomeres in leukocytes have been found to be associated with increased incidence of chronic diseases, such as cancer (6, 7) and cardiovascular disease (8).

Because $1,25(OH)_2D$ receptors have been found in leukocytes, it is possible that vitamin D has an impact on leukocyte telomere length (9). A recent study found that a higher level of plasma 25(OH)D was significantly associated with longer leukocyte telomere length (10). Otherwise, the association between vitamin D and telomere length remains largely unexplored.

In the present cross-sectional analysis of the Nurses' Health Study (NHS) data, we examined the association between vitamin D and telomere length in peripheral blood leukocytes by using plasma biomarkers of both 25(OH)D and $1,25(OH)_2D$. We also looked at whether the association between vitamin D and telomere length was modified by total intakes of dietary calcium, folate, and retinol, which are nutrients that have been shown to potentially interact with vitamin D to affect cancer risk (11–18). Finally, we evaluated the associations between vitamin D–related single nucleotide polymorphisms (SNPs) and telomere length.

MATERIALS AND METHODS

Study population

The NHS is a prospective cohort study that began in 1976, when 121,700 female registered nurses aged 30–55 years and residing in 11 US states completed an initial questionnaire. Personal information, such as data on lifestyle and dietary factors, was subsequently updated every 2 or 4 years through questionnaire responses. From 1989 to 1990, blood was collected from 32,826 participants. In total, 97% of these blood samples arrived within 26 hours of being drawn and were centrifuged and aliquotted into plasma, white blood cell, and red blood cell components. The cryotubes containing the aliquotted samples were stored in liquid nitrogen freezers. The present analysis was restricted to whites, who are the predominant majority of the NHS participants. A total of 4,604 participants from previous nested case-control sets involving various cancer outcomes had leukocyte telomere length measured (19–21); among them, 1,424 participants had their 25(OH)D levels measured and 837 had their $1,25(OH)_2D$ levels measured. Genotyping was performed on 480 participants for whom we had a telomere measurement. The NHS protocol was approved by the Human Research Committee of Brigham and Women's Hospital.

Vitamin D biomarkers

The assays for the measurement of plasma 25(OH)D for breast cancer case-control sets were performed by Dr. Michael F. Holick (Boston University School of Medicine, Boston, Massachusetts), who used a high affinity protein-binding assay after extraction with absolute ethanol (22), and Dr. Bruce W. Hollis (Medical University of South Carolina, Charleston, South Carolina), who used radioimmunoassay with prior acetonitrile extraction (23). For the endometrial cancer case-control set, plasma 25(OH)D was measured using a chemiluminescence immunoassay from Heartland Assays, Inc. (Ames, Iowa) (24). Plasma $1,25(OH)_2D$ was measured using radioimmunoassay in Hollis' lab for breast cancer case-control sets (23). For 25(OH)D, the coefficients of variation were 17.0% for the assay performed by Dr. Holick, 8.7% for the assay performed by Dr. Hollis, and 13.2% for the Heartland assay. For $1,25(OH)_2D$, the coefficient of variation was 7.3%. For 25(OH)D, the interquartile ranges were 15.0 ng/mL for the assay performed by Dr. Holick, 14.9 ng/mL for the assay performed by Dr. Hollis, and 11.8 ng/mL for the Heartland assay.

Telomere length

Relative telomere length in genomic DNA extracted from peripheral blood leukocytes was measured using quantitative real-time polymerase chain reaction, and the ratio of telomere repeat copy number to a single gene copy number (T/S) was determined as previously described (25). Each sample was analyzed in triplicate, and the relative telomere length was the exponentiated T/S corrected for a reference sample. The coefficients of variation for the telomere assay and the single-gene assay were in the ranges of 0.87%–1.03% and 0.56%–1.09%, respectively. The coefficients of variation for the exponentiated T/S of quality control samples ranged from 14% to 16.3%.

Questionnaire information

Questionnaire information was obtained from the 1990 follow-up cycle, except for physical activity level, which was obtained in 1988. Smoking was measured in pack-years, and physical activity level was expressed as metabolic equivalents per week. Total dietary intake variables accounted for both natural food and dietary supplement sources and were adjusted for total energy intake.

SNP selection and genotyping

The 12 SNPs of interest were either selected from the vitamin D receptor gene (*VDR*) and the vitamin D-binding protein gene (*GC*) or from a recent genome-wide association study meta-analysis on genetic predictors of circulating vitamin D levels (26). *VDR* is located on chromosome position 12q13, and *GC* is located on chromosome position 4q11-13. The selected *VDR* SNPs have been studied in relationship to various cancers (27). SNPs on the *VDR* include rs2228570 (Fok1), rs11568820 (Cdx2), rs1989969 (VDR-5132), rs1544410 (Bsm1), rs7975232 (Apa1), rs731236 (Taq1), and rs739837 (Bgl1). The SNPs on the *GC* are rs4588 and rs7041. The remaining 3 SNPs from the genome-wide association study meta-analysis are rs1790349, rs6599638, and rs2060793, which are on the *DHCR7*, *C10orf88*, and *CYP2R1* genes, respectively. The minor allele frequencies ranged from 0.16 to 0.48. Genomic DNA was extracted from blood samples using the QIAmp 96-spin blood protocol (Qiagen, Chatsworth, California). Genotyping was performed at the Dana-Farber/Harvard Cancer Center High-Throughput Genotyping Core (Boston, Massachusetts) using the 5′ nuclease assay (Taqman,

Table 1. Mean and Standard Deviation Values for Characteristics of Key Variables by Quartile of Relative Telomere Length, Nurses' Health Study, 1989–1990

Variable	Quartile of Telomere Length				*P* for Trend[a]
	1 (*n* = 1,164)	2 (*n* = 1,153)	3 (*n* = 1,135)	4 (*n* = 1,152)	
Age, years	59.8 (6.4)	59.3 (6.3)	59.1 (6.6)	58.4 (6.8)	<0.0001
Smoking, pack-years	13.1 (19.2)	12.3 (17.8)	12.7 (19.6)	11.6 (17.7)	0.27
Body mass index[b]	25.8 (5.0)	25.7 (4.9)	25.4 (4.7)	25.4 (4.5)	0.03
Physical activity level, MET/week	16.9 (26.9)	16.9 (25.5)	16.1 (19.2)	16.2 (17.7)	0.47
Total calcium intake, mg/day	1132.7 (571.9)	1115.1 (557.0)	1154.3 (576.2)	1143.5 (559.5)	0.28
Total folate intake, μg/day	472.5 (243.3)	467.1 (230.8)	478.6 (241.3)	482.3 (246.8)	0.23
Total retinol intake, IU/day	4143.6 (4122.4)	4171.6 (4429.6)	4229.7 (4306.9)	4190.8 (4278.1)	0.62

Abbreviation: MET/week, metabolic equivalents per week.
[a] Age-adjusted linear regression *P* value for other variables.
[b] Weight (kg)/height $(m)^2$.

Applied Biosystems, Foster City, California). Laboratory personnel were blinded to case-control status, and 5% of the samples were blinded quality-control samples to validate genotyping procedures; concordance for blinded samples was 100%. The amount of missing genotyping data was less than 4%.

Statistical analyses

After excluding the extreme 1% outlier values of log-normalized relative telomere length, *z* scores were derived to standardize the distribution. Linear regression was used when the telomere length *z*-score outcome was continuous, and unconditional logistic regression was used when the *z* score was dichotomized at the median. We adjusted for potential confounders, including age in years (continuous), smoking status (0, 0.1–20, 20.1–40, or >40 pack-years), body mass index (BMI, measured as weight (kg)/height $(m)^2$) (<25, 25–29.9, 30–34.9, or ≥35), and physical activity levels (nominal quartiles measured in metabolic equivalents per week). To account for differences in means and interquartile ranges across different assays, adjustment was also made for 25(OH)D and $1,25(OH)_2D$ case-control study batches by representing each batch as an indicator variable. Multiplicative interaction terms involving continuous variables were created to test for effect modification using the Wald test. The additive genetic model was used for the SNP analyses; it assumes that the effect of the heterozygous genotype is intermediate between the 2 homozygous genotypes. The homozygous genotype of the reference allele was coded as 0. Analyses were performed using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina). Quartiles for the 25(OH)D, $1,25(OH)_2D$, and relative telomere length variables were created using the rank procedure. All *P* values were 2-sided.

RESULTS

The associations of lifestyle and nutritional variables with relative telomere length in participants for whom we had a telomere length measurement ($n = 4,604$) are shown in Table 1. Age (*P* for trend < 0.0001) and BMI (*P* for trend = 0.03)

Table 2. Mean and Standard Deviation Values for Characteristics of Key Variables by Quartile of 25-Hydroxyvitamin D Level, Nurses' Health Study, 1989–1990

Variable	Quartile of 25-Hydroxyvitamin D Level				*P* for Trend[a]
	1 (*n* = 379)	2 (*n* = 384)	3 (*n* = 334)	4 (*n* = 327)	
Age, years	58.6 (6.6)	59.4 (6.3)	59.7 (6.3)	60.1 (6.0)	<.0001
Smoking, pack-years	13.1 (18.6)	13.2 (19.6)	11.1 (17.7)	11.4 (18.0)	0.04
Body mass index[b]	27.6 (6.1)	25.7 (4.9)	25.2 (4.4)	24.3 (4.0)	<0.0001
Physical activity level, MET/week	15.7 (35.3)	17.1 (31.6)	16.1 (16.3)	20.3 (20.7)	0.05
Total calcium intake, mg/day	1016.7 (505.6)	1172.0 (619.9)	1147.5 (525.9)	1280.4 (601.8)	<0.0001
Total folate intake, μg/day	424.3 (225.0)	478.2 (258.7)	482.9 (234.4)	515.6 (258.1)	<0.0001
Total retinol intake, IU/day	3392.6 (3494.7)	4112.6 (4045.7)	4137.3 (4849.3)	4954.6 (5168.9)	<0.0001

Abbreviation: MET/week, metabolic equivalents per week.
[a] Age-adjusted linear regression *P* value for other variables.
[b] Weight (kg)/height $(m)^2$.

Table 3. Linear Regression Results for the Associations Between Plasma Vitamin D Biomarkers and Relative Telomere Length, Nurses' Health Study, 1989–1990

Biomarker	No. of Samples	Model 1[a]		Model 2[b]	
		β (SE)	*P* for Trend	β (SE)	*P* for Trend
25(OH)D	1,337	0.006 (0.003)	0.02	0.005 (0.003)	0.05
$1,25(OH)_2D$	794	−0.004 (0.005)	0.38	−0.005 (0.005)	0.32

Abbreviation: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; SE, standard error.
[a] Adjusted for study batch and age in years (continuous).
[b] Adjusted for study batch, age in years (continuous), smoking status (0, 0.1–20, 20.1–40, or >40 pack-years), body mass index (weight (kg)/height $(m)^2$) (<25, 25–29.9, 30–34.9, or ≥35), and physical activity level (nominal quartiles measured in metabolic equivalents per week).

were significantly inversely associated with relative telomere length. Table 2 shows the associations between plasma 25(OH)D level and key variables. Age (P for trend < 0.0001), smoking (P for trend = 0.04), BMI (P for trend < 0.0001), physical activity level (P for trend = 0.05), total calcium intake (P for trend < 0.0001), total folate intake (P for trend < 0.0001), and total retinol intake (P for trend < 0.0001) were all significantly associated with plasma 25(OH)D levels.

Table 3 depicts the associations between the vitamin D biomarkers and relative telomere length. After adjusting for study batch and age, a higher 25(OH)D level was significantly associated with longer telomere length (P for trend = 0.02), and the result was similar after further adjustment for smoking status, BMI, and physical activity level (P for trend = 0.05). Logistic regression analysis indicated a dose-response relationship, with the odds of having a relative telomere length above the median increasing with higher 25(OH)D levels (Table 4). After adjustment for study batch, age, smoking status, BMI, and physical activity level, the odds ratio increased from 1.07 (P = 0.65) when comparing the second lowest quartile of 25(OH)D with the lowest quartile to 1.59 (P = 0.01) when comparing the highest quartile with the lowest. The quartile median 25(OH)D values in Table 4 can be compared with previous definitions of vitamin D sufficiency/insufficiency: less than 20 ng/mL is considered vitamin D deficient, 20–29.9 ng/mL is considered vitamin D insufficient, and greater than or equal to 30 ng/mL is considered vitamin D sufficient (3). Level of $1,25(OH)_2D$ was not significantly associated with relative telomere length. In the multivariate categorical analysis of $1,25(OH)_2D$ and relative telomere length that was adjusted for study batch, age, smoking status, BMI, and physical activity level, the odds ratio was 0.89 (95% confidence interval: 0.60, 1.33) for quartile 2 versus quartile 1; for quartile 3 versus quartile 1, it was 0.94 (95% confidence interval: 0.62, 1.42), and for quartile 4 versus quartile 1, it was 0.82 (95% confidence interval: 0.54, 1.22). The Spearman correlation coefficient between 25(OH)D and $1,25(OH)_2D$ was 0.12.

The assessment of whether key nutrients modified the association between 25(OH)D and relative telomere length showed that total calcium intake (P for interaction = 0.05) was a significant effect modifier. Calcium intake was stratified as "high" or "low" using the cut-off of 1,000 mg/day (the recommended dietary allowance for females 19–50 years of age according to the National Academies Press (28)). The median calcium intake in this study population at the time of blood draw was 1,044 mg/day. When setting persons who were in the lowest quartile of 25(OH)D and who had a low calcium intake as the reference group, those in the highest quartiles of

Table 4. Logistic Regression Results for the Association Between 25-Hydroxyvitamin D Level and Relative Telomere Length[a], Nurses' Health Study, 1989–1990

Quartile of 25(OH)D Level	25(OH)D Level, ng/mL[b]	Model 1[c]			Model 2[d]		
		OR	95% CI	*P* Value	OR	95% CI	*P* Value
1 (referent)	15.4	1.00	Referent		1.00	Referent	
2	24.7	1.10	0.82, 1.48	0.53	1.07	0.79, 1.45	0.65
3	32.3	1.38	1.00, 1.91	0.05	1.34	0.96, 1.87	0.08
4	43.0	1.67	1.18, 2.36	0.004	1.59	1.11, 2.29	0.01

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio.
[a] Telomere length was dichotomized at the median.
[b] Median values for each quartile.
[c] Adjusted for study batch and age in years (continuous).
[d] Adjusted for study batch, age in years (continuous), smoking status (0, 0.1–20, 20.1–40, or >40 pack-years), body mass index (weight (kg)/height $(m)^2$) (<25, 25–29.9, 30–34.9, or ≥35), and physical activity level (nominal quartiles measured in metabolic equivalents per week).

Table 5. Associations With Relative Telomere Length[a] Involving Combinations of 25-Hydroxyvitamin D Level and Total Calcium Intake Categories, Nurses' Health Study, 1989–1990

Quartile of 25(OH)D Level	Calcium Intake[b]	No. of Samples	OR[c]	95% CI[c]	*P* Value
1	Low	235	1.00	Referent	
2	Low	191	1.17	0.78, 1.75	0.46
3	Low	150	1.84	1.17, 2.90	0.009
4	Low	122	2.30	1.39, 3.81	0.001
1	High	144	1.33	0.86, 2.05	0.20
2	High	193	1.24	0.83, 1.87	0.30
3	High	184	1.30	0.85, 1.98	0.22
4	High	205	1.56	1.02, 2.41	0.04

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio.

[a] Telomere length was dichotomized at the median.

[b] Low: <1,000 mg/day; high: ≥1,000 mg/day.

[c] Adjusted for study batch, age in years (continuous), smoking status (0, 0.1–20, 20.1–40, or >40 pack-years), body mass index (weight (kg)/height $(m)^2$) (<25, 25–29.9, 30–34.9, or ≥35), and physical activity level (nominal quartiles measured in metabolic equivalents per week).

25(OH)D had a longer telomere length, regardless of whether their calcium intake was low (odds ratio = 2.30; $P = 0.001$) or high (odds ratio = 1.56; $P = 0.04$), although the magnitude of the association appeared stronger for those with a low calcium intake (Table 5). Calcium intake was not associated with telomere length in the multivariate analysis that was adjusted for age, smoking status, BMI, and physical activity level. Total folate and retinol intakes were not significant effect modifiers. In the genotyped sample, rs7041 ($P = 0.04$) and rs4588 ($P = 0.0004$) were significantly associated with 25(OH)D level. However, none of the vitamin D–related SNPs were significantly associated with relative telomere length (Table 6).

DISCUSSION

We found that a higher plasma 25(OH)D level was significantly associated with longer leukocyte telomere length, with evidence of a dose-response relationship. Our finding is consistent with that of Richards et al. (10), whose study was also conducted in a cohort of only women. In that study, 25(OH)D level was found to be negatively correlated with C-reactive protein, suggesting that vitamin D reduces systematic inflammation. In addition to reducing inflammation, vitamin D is also known to reduce cell proliferation (1, 2). Because inflammation from tissue damage and enhanced cell proliferation accelerate telomere shortening (4–6), vitamin D may reduce telomere shortening through both anti-inflammatory and antiproliferative mechanisms.

The following reasons may explain why the association with telomere length is inconsistent for 25(OH)D and $1,25(OH)_2D$. First, because many organs locally convert 25(OH)D to $1,25(OH)_2D$ (3, 29), plasma 25(OH)D levels may provide information on the association between locally activated vitamin D and leukocyte telomere length. Plasma $1,25(OH)_2D$ may be used to assess the association involving non–locally activated vitamin D. Within specific tissues, concentrations of $1,25(OH)_2D$ may depend more on intracellular conversion of 25(OH)D to $1,25(OH)_2D$ than on plasma $1,25(OH)_2D$ level. Second, even if one expects the 2 vitamin D biomarkers to be biologically relevant, their single time-point measurements may not reflect their long-term average levels, especially for $1,25(OH)_2D$, which has a shorter half life.

Table 6. Associations Between Vitamin D–related Single Nucleotide Polymorphisms and Relative Telomere Length[a], Nurses' Health Study, 1989–1990

Single Nucleotide Polymorphism	Alleles	Gene	Minor Allele Frequency	Referent Allele[b]	OR	95% CI	*P* Value
rs6599638	A,G	*C10orf88*	0.48	A	1.07	0.82, 1.38	0.63
rs2060793	A,G	*CYP2R1*	0.38	A	1.00	0.77, 1.31	0.99
rs1790349	C,T	*DHCR7*	0.16	C	1.07	0.75, 1.52	0.72
rs7041	A,C	*GC*	0.41	A	1.15	0.87, 1.51	0.34
rs4588	G,T	*GC*	0.31	G	1.10	0.82, 1.48	0.51
rs7975232 (Apa1)	A,C	*VDR*	0.48	A	1.00	0.78, 1.28	0.99
rs739837 (Bgl1)	G,T	*VDR*	0.47	G	1.02	0.79, 1.30	0.89
rs1544410 (Bsm1)	G,A	*VDR*	0.40	G	1.05	0.80, 1.38	0.74
rs11568820 (Cdx2)	A,G	*VDR*	0.23	A	1.11	0.81, 1.52	0.53
rs2228570 (Fok1)	A,G	*VDR*	0.40	A	0.99	0.76, 1.28	0.92
rs731236 (Taq1)	A,G	*VDR*	0.40	A	1.03	0.80, 1.33	0.80
rs1989969 (VDR-5132)	A,G	*VDR*	0.40	A	0.84	0.65, 1.09	0.19

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Telomere length was dichotomized at the median. The associations in this table were unadjusted.

[b] The homozygous genotype of the reference allele was coded as 0.

The nutrients calcium, folate, and retinol were examined as effect modifiers because of prior biologic and epidemiologic evidence. Studies have shown that vitamin D and calcium may interact to influence cancer risk (13–17). Folate may play a role in the epigenetic regulation of vitamin D hydroxylase expression (11). Retinol intake may compete for retinoid X receptors to antagonize the actions of vitamin D (12), and an epidemiologic study showed that a high retinol intake countered the protective effect of vitamin D on the risk of distal colorectal adenoma (18). We found that total calcium intake was a significant effect modifier and that the association between increased 25(OH)D and longer telomere length was stronger for persons with lower calcium intakes. In a study in rats, Pence and Buddingh (17) found that supplementation with calcium and vitamin D combined led to reduced protection against colon cancer compared with supplementation with either alone. However, other biologic and epidemiologic studies have shown that the anticancer effect of vitamin D increases with higher calcium intakes (13–16). It is unknown whether the interaction between vitamin D and calcium in the present analysis represents a biologic effect. Dietary vitamin D intake is correlated with dietary calcium intake, so for a given 25(OH)D level, persons in the low calcium intake group likely obtained more vitamin D from sun exposure than did those with high calcium intakes. Therefore, this finding could be due to other correlates of sun exposure that are also effect modifiers of the association between vitamin D and telomere length. Nevertheless, total physical activity level was not found to be a significant effect modifier in this study population. It will be interesting to see whether plasma calcium is also a significant effect modifier of the association between 25(OH)D level and telomere length.

To our knowledge, this is the first analysis to examine the association between $1,25(OH)_2D$ level and telomere length, as well as effect modification of the 25(OH)D level and telomere length association by total calcium, folate, and retinol intakes. However, there are several limitations. First, only one plasma measurement was taken per participant for both vitamin D and telomere length, so the vitamin D measurements may not reflect the biologically relevant exposure level, and it was not possible to assess the impact of change in vitamin D level on change in telomere length. Second, because the study was carried out in Caucasian women, the results may not be generalizable to men or women of other ethnicities. Third, the associations in the present analysis are cross-sectional and therefore not necessarily causal.

In conclusion, this study shows that vitamin D may be associated with longer telomere length and that the association may be modified by total calcium intake. Because shorter telomere length has been associated with many chronic illnesses that vitamin D has been shown to protect against, it is possible that a mechanism of vitamin D's protection is through the maintenance of telomere integrity. Pathway analyses involving intermediate variables will be needed to determine how much of vitamin D's protective effect on various diseases is through telomere maintenance. Further studies involving vitamin D and telomere length should examine the association in men or in women with different ethnic backgrounds and study the effect modification of calcium intake in these different populations. Finally, studies involving plasma measurements taken at multiple time points will allow the assessment of the association between change in vitamin D level and change in telomere length.

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