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Relationship between plasma 25-hydroxyvitamin D and leucocyte telomere length by sex and race in a US study‡

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Abstract

A few studies have examined the association between vitamin D and telomere length, and fewer still have examined the relationship in black or male populations. We investigated the cross-sectional association between the vitamin D metabolite 25-hydroxyvitamin D (25(OH)D) concentration in plasma and relative leucocyte telomere length (LTL) in 1154 US radiologic technologists who were 48–93 years old (373 white females, 278 white males, 338 black females, 165 black males). Plasma 25(OH)D concentration was measured by the chemiluminescence immunoassay, and relative LTL was measured by quantitative PCR. Logistic regression was used to obtain OR and 95 % CI for long *v.* short (based on median) LTL in relation to continuous 25(OH)D, quartiles of 25(OH)D and 25(OH)D deficiency. We found no significant association between continuous 25(OH)D and long LTL in all participants ($P_{trend}=0{\cdot}440$), nor in white females ($P_{trend}=0{\cdot}845$), white males ($P_{trend}=0{\cdot}636$), black females ($P_{trend}=0{\cdot}967$) or black males ($P_{trend}=0{\cdot}484$). Vitamin D deficiency (defined as 25(OH)D <30 nmol/l), however, was significantly associated with short LTL in whites ($P=0{\cdot}024$), but not in other groups. In this population, we found little evidence to support associations between 25(OH)D and long LTL over the entire range of 25(OH)D in the overall study population or by sex and race.

Key words: Vitamin D: 25-Hydroxymitamin D: Leucocyte telomere length: Sexes: Race

Vitamin D and telomere length have both been associated with various health outcomes, but there have been only a few studies on the relationship between them[1–4]. Vitamin D is a hormone synthesised photochemically in the skin or ingested from foods and supplements that is subsequently hydroxylated in the liver to its major circulating form 25-hydroxyvitamin D (25(OH)D). Vitamin D deficiency has been associated with cancer and other chronic diseases, although whether those relationships are causal remains uncertain[5–7]. Vitamin D may also play a role in telomere biology by reducing cell proliferation[6,7] and inflammation[1,8]. Telomeres consist of nucleotide repeats and a protein complex at the ends of linear chromosomes that are essential for chromosomal integrity[9]. Telomeres naturally shorten with each cell division, and the shortening process can be accelerated by oxidative stress[10]. Thus, telomere length is considered to be a biomarker of cellular ageing and has been associated with disease risk and mortality[11,12].

Previous studies on plasma 25(OH)D concentration (commonly regarded as a biomarker of vitamin D status) and leucocyte telomere length (LTL) have been largely limited to women and whites[1–3]; two studies of predominantly or exclusively white women found associations between lower 25(OH)D concentration and shorter leucocyte telomeres[1,2]. Another small study on women with systemic lupus erythematosus found no correlation between 25(OH)D baseline levels and LTL[3]. Recently, a study of white men found no association between vitamin D and LTL[4], and another study on young individuals (all age 31 years), predominantly white men and women, also detected no relationship between them[13]. It is important to investigate whether the associations previously observed between 25(OH)D and LTL in white women can be replicated and are generalisable to other groups. To our knowledge, no study thus far has evaluated the relationship between 25(OH)D and LTL in a population with considerable proportions of men, women, blacks and whites, and none have examined effect modification of the relationship between 25(OH)D and LTL by sex and race.

The objective of this study was to assess the relationship between 25(OH)D and LTL using data from the US Radiologic

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; LRT, likelihood ratio test; LTL, leucocyte telomere length; USRT, US Radiologic Technologists.

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Technologists (USRT) study. This study includes 1154 participants with substantial proportions of women, men, blacks and whites with measurements of plasma 25(OH)D concentration, LTL and information on an extensive number of lifestyle factors.

Methods

Overview

The USRT is an occupational cohort, which comprises radiologic technologists who were certified by the American Registry of Radiological Technologists for at least 2 years from 1926 through 1982[14]. Descriptions of the cohort and methods have been previously published[14,15]. In brief, detailed self-administered questionnaires were mailed to cohort members during three time periods – 1983–1989, 1994–1998 and 2003–2005 – to assess work history, lifestyle factors, reproductive, medical and family cancer history and a broad range of serious disease outcomes. The USRT study has been annually approved by human subjects review boards at the University of Minnesota and the National Cancer Institute. All participants of this study provided written informed consent.

Study population

The target population for this study included a sample of 10 752 technologists chosen to represent the overall cohort. To this group, we added all black participants (*n* 2593) of the USRT study who were not part of the representative sample because black participants constitute only a small subset of the entire cohort (Fig. 1). Of these, only 9141 technologists from the representative sample and 2374 from the additional sample of black participants were eligible for the present study because they were alive and had not previously refused blood sample collections. We requested a blood sample and sent a mailed questionnaire between August 2008 and December 2009. Each month, random samples from the representative and black group were chosen within strata defined by sex, age (<60; 60+ years) and ambient UV radiations (based on National Aeronautics and Space Museum (NASA) satellite data). A total of 4117 participants provided blood samples as requested. Owing to limited resources, 25(OH)D assays were performed on a subset of 1500 individuals (selected on the basis of a random stratified sample), including 842 white and 646 black participants and twelve participants identified as not white or black[16]. Of the 1488 samples from white and black participants, 1154 were successfully assayed for relative LTL (711 women, 443 men, 651 whites and 503 blacks), with an age range of 48–93 years.

Outcome assessment

Relative telomere length determination by quantitative PCR measures the ratio of telomere (T) signals, specific to the telomere hexamer repeat sequence TTAGGG, to autosomal single-copy gene (S) signals. This ratio is normalised by control DNA samples to yield relative standardised T:S ratios proportional to the average telomere length. In this technique, reactions are performed independently, so a standard curve of pooled genomic DNA samples is utilised to assess the amount of each signal, while compensating for inter-plate variations in PCR efficiency. This telomere length measurement assay was adapted from the published method by Cawthon[17,18].

Sample DNA of 4 ng, according to Quant-iT PicoGreen dsDNA quantitation (Life Technologies), was transferred into LightCycler-compatible 384-well plates (Roche) and dried down. An internal standard curve (six concentrations of pooled reference DNA samples spanning a ninety-seven·6-fold range in concentration, prepared by serial dilution) and randomly located internal quality control samples utilised as calibrator samples were applied to the assay plates to guide analysis and indicate the overall quality of assay performance. In addition, a no-template control was added to random wells of the 384-well plate to provide a unique fingerprint for each plate. All experimental and control samples were assayed in triplicate on each plate. PCR was performed using 5-ul reaction volumes consisting of 2·5 ul of 2X Rotor-Gene SYBR Green PCR Master Mix (QIAGEN), 2·0 ul of molecular biology-grade water and 0·5 ul of 1 μM-assay-specific mix of primers. Oligonucleotides (Integrated DNA Technologies) were manufactured by LabReady by Integrated DNA Technologies (normalised to 100 μM in 1X Tris EDTA, pH 8.0 and HPLC purified). Primers for the telomeric PCR were Telo_FP (5'-CGGTTT(GTTTGG)$_5$GTT-3') and Telo_RP (5'-GGCTTG(CCTTAC)$_5$CCT-3')[19]. Primers for the single-copy gene (*36B4*) PCR were *36B4*_FP (5'-CAGCAAGTG GGAAGGTGTAATCC-3') and *36B4*_RP (5'-CCCATTCTATC ATCAACGGGTACAA-3')[17]; 1 μM-assay mixes were generated by combining 990 ul of 1X Tris-EDTA buffer with 5 ul of forward oligo and 5 ul of reverse oligo.

Thermal cycling was performed on a LightCycler 480 (Roche) where PCR conditions consisted of the following: cycling for T (telomeric) PCR – 95°C hold for 5 min, denature at 98°C for 15 s, anneal at 54°C for 2 min, with fluorescence data collection, thirty-five cycles;cycling for S (single-copy gene, *36B4*) PCR – 98°C hold for 5 min, denature at 98°C for 15 s, anneal at 58°C for 1 min, with fluorescence data collection, forty-three cycles. LightCycler software (Release 1.5.0) was used for initial analysis of raw data. Utilising absolute quantification analysis with the second derivative maximum method and high-sensitivity detection algorithm, single target sequences were quantified and expressed as an absolute value (ng/ul) based on the internal standard curve of known concentrations. The concentration of telomere (T) signal was divided by the concentration of *36B4* (S) signal to yield a T:S ratio. This raw T:S ratio was then divided by the average T:S ratio of the internal quality control (QC) calibrator samples, within the same plate, to yield the final standardised T:S ratio for the unknown sample. The inter-assay CV, calculated using the internal QC calibrator samples (*n* 80), was 8·4 % for the relative LTL assay.

Exposure assessment

Plasma 25(OH)D level was measured by Heartland Assays, Inc. using the LIAISON chemiluminescence immunoassay analyzer of DiaSorin. Heartland Assays, Inc. participated in the Vitamin D External Quality Assessment Scheme (DEQAS) proficiency testing scheme for the Liaison instrument; five quality control samples per batch were selected in a run-in to represent low, medium and

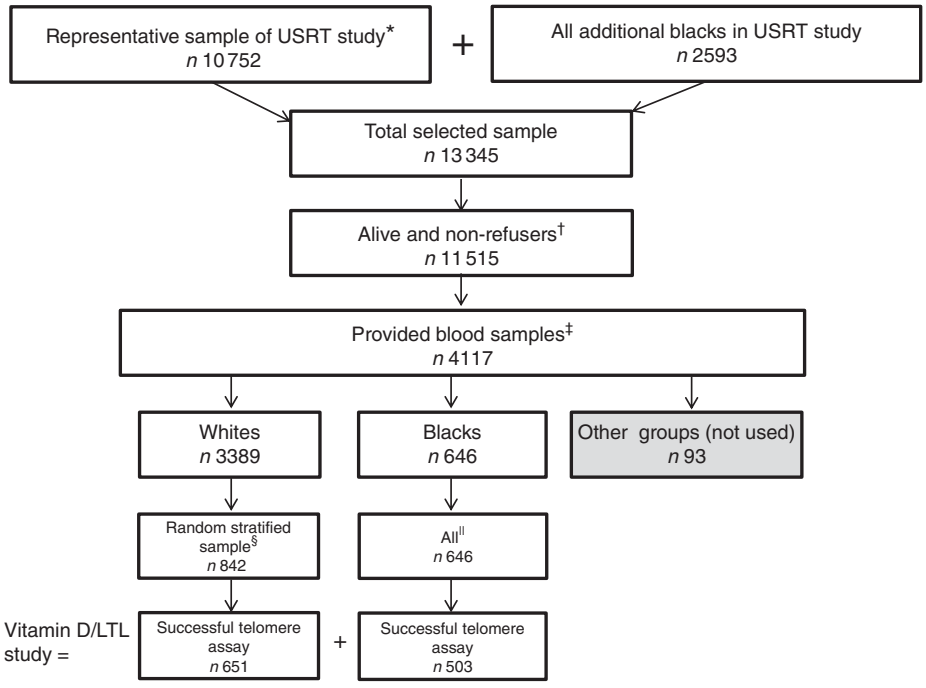

Fig. 1. Selection of 1154 subjects in the US Radiologic Technologists' (USRT) study of plasma 25-hydroxyvitamin D (25(OH)D) and relative leucocyte telomere length (LTL). * Derived from all racial/ethnic groups. † Had not previously refused blood sample collection. ‡ ≤9 d between blood collection and questionnaire administration. § Randomly stratified sample based on sex, ambient UV radiations, age (<60; 60+ years) and season, with 25(OH)D measurements. ‖ With 25(OH)D measurements.

high plasma 25(OH)D concentrations and these averaged 42·1, 69·8 and 101·2 nmol/l, respectively. Quality control samples were randomly distributed across and within batches. For low, medium and high plasma 25(OH)D, the total CV were 8·3, 7·2 and 5·8 %, respectively, with an overall average of 7·1 %.

Covariate assessment

Information on potential confounders in this study population was ascertained from self-administered questionnaires, which were mailed to participants at the time of blood sample collection (2008–2009). These questionnaires collected information on current smoking status (yes/no), current weight, indoor physical activity levels in last 30 d, use of vitamin D supplementation in the past 30 d, menopausal status and use of menopausal hormone therapy (never, former, current). Estimated cumulative occupational ionising radiation dose to the red bone marrow was based on badge readings, work history and literature-based badge dose survey data and has been previously described in detail[20]. For individuals who reported not being a current smoker, we used information from previously completed questionnaires to determine whether they were former smokers. For participants with missing information on weight (*n* 69), we used weight reported on the third USRT survey (2003–2005) to calculate BMI for fifty-eight individuals with available information.

Statistical analyses

To evaluate the relationship between plasma 25(OH)D concentration and LTL, logistic regression was used to compute OR and 95 % CI of long LTL in relation to plasma 25(OH)D concentration. Long LTL was defined as normalised T:S LTL values higher than the median in the total population (with the reference group representing participants with LTL values below the median). We examined the relationship of long LTL with continuous plasma 25(OH)D concentration, plasma 25(OH)D quartiles based on the total study population (<45, 45–65, 66–85 and >85 nmol/l) and the clinical definition of vitamin D deficiency (above and below a blood serum 25(OH)D concentration of 30 nmol/l)[21]. The likelihood ratio test (LRT) was used to evaluate the statistical significance of continuous plasma 25(OH)D and 25(OH)D quartiles. Owing to the small number of observations in some cells, exact methods were used for estimating CI and *P* values for the relationships of long LTL and vitamin D deficiency.

The following factors were considered as potential confounders because they could be associated with both LTL and plasma 25(OH)D concentrations, but were not believed to be on the causal pathway: age, sex, race, BMI, smoking history, indoor physical activity, season of blood collection, cumulative occupational radiation dose to the red bone marrow, menopausal status and use of menopausal hormone therapy. We also examined vitamin D supplementation because, although it may contribute to plasma 25(OH)D concentrations, it may serve as a surrogate for other general health-related behaviours that may impact LTL as well. These factors were first examined in relation to long LTL using log-LRT and plasma 25(OH)D using *F*-tests from linear regression models; the square root transformation of 25(OH)D suggested normally distributed residuals.

Final regression models included age at blood sample collection, sex, race and season of blood sample collection for *a priori* reasons because they were considered in previous

publications or were strong risk factors for LTL in this cohort. Forward selection was used to assess the impact of additional potential confounders on model fit using log-LRT; however, none of them provided an improvement in fit. Non-linearity of the relationship between plasma 25(OH)D and long LTL was assessed in regression models by including a plasma 25(OH)D squared term. Differences in the relationship between plasma 25 (OH)D and long LTL across the entire range of plasma 25(OH)D were assessed by testing for heterogeneity across plasma 25(OH) D quartiles. Effect modification of the relationship between continuous plasma 25(OH)D, vitamin D deficiency and long LTL was assessed across race, sex and race–sex subgroups. Missing values were coded as separate categories and were included as indicator variables in the models unless otherwise noted.

Statistical tests were two-sided, and p values were considered significant at the 0·05 α level. Analyses were performed using SAS version 9.3 (SAS Institute Inc.). For certain analyses involving small numbers of events (Table 4), exact logistic regression analyses were conducted using LogXact-11 version 11.0.0 (Cytel Inc., 1989–2015).

Results

The study population included 1154 participants with a mean age at blood sample collection of 63·2 years (data not shown). The odds of long LTL were higher for participants of younger age, black race, BMI of 35 kg/m^2 or higher and blood sample collection during the summer (Table 1). They were also higher for females, but the relationship was of borderline statistical significance. Smoking history, indoor physical activity, cumulative estimated ionising radiation dose to the red bone marrow, menopausal status and use of menopausal hormone therapy were not associated with odds of long LTL. Plasma 25(OH)D concentrations were significantly higher in participants of older age, whites (*v.* blacks), those reporting longer time being physically active, participants who reported using vitamin D supplements and menopausal hormone therapy users (Table 2).

We found no significant relationship between continuous 25 (OH)D concentration and long LTL in the total population (P_{trend} = 0·440), nor in white females (P_{trend} = 0·845), white males (P_{trend} = 0·636), black females (P_{trend} = 0·967) or black males (P_{trend} = 0·484), after adjusting for age at blood sample collection (years), race, sex and season (Table 3). We found no 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 most quartiles of plasma 25(OH)D after adjusting for age at blood sample collection (years), race, sex and season (Table 3). The exception was a significantly increased odds of long LTL among men with plasma 25(OH)D of quartile 2 *v.* quartile 1 (OR 1·90; 95 % CI 1·10, 3·32). A test of heterogeneity for long LTL among males did not indicate a significant difference across plasma 25(OH)D quartiles ($P_{heterogeneity}$ = 0·084);however, tests of non-linearity with the inclusion of a plasma 25(OH)D squared term in the final models did indicate significant non-linearity among all males (LRT P value = 0·020) and among black males (LRT P value = 0·049). Multiplicative interaction tests of continuous 25(OH)D and long LTL were NS for sex, race or race–sex categories (Table 3).

Similarly, we found no significant relationship between vitamin D deficiency (based on the Institute of Medicine's cut-off value of 30 nmol/l for plasma 25(OH)D concentration) in relation to long LTL in the total population and in most race and sex subgroups (Table 4). An exception was among whites, for whom we found that vitamin D non-deficiency (≥30 nmol/l) was associated with significantly increased odds of long LTL (OR 2·67; 95 % CI 1·16, 6·61; P value = 0·024), after adjustment for age (5-year categories), race and season. In addition, we also found a significantly increased odds of long LTL associated with vitamin D non-deficiency among white males (P value = 0·005). We found no significant interaction on the multiplicative scale between vitamin D deficiency and long LTL for race, sex or race–sex subgroups (Table 4).

Discussion

To our knowledge, this is the first study to examine the vitamin D and telomere length associations across sex and race subgroups. We found no significant associations between continuous 25(OH)Dconcentration and long LTL for our overall analysis population or in whites, blacks, females, males or race–sex subgroups. However, we found that non-deficient concentrations of vitamin D (≥30 nmol/l) were significantly associated with longer LTL in whites.

We also examined how sex and race were associated with telomere length after adjusting for age. Our finding that women had borderline significantly higher odds of long LTL than men was consistent with a recent meta-analysis of thirty-six cohorts[22]. We also found a significant age-adjusted association between white *v.* black race and long LTL, with blacks having higher odds for long LTL. Previous studies of adults reported whites having either significantly longer[23] or shorter[24] telomeres than blacks.

Although we did not find significant linear associations between 25(OH)D and long LTL, we found that vitamin D non-deficiency (25(OH)D concentration ≥30 nmol/l) was significantly associated with increased odds of long LTL in whites. These findings should be cautiously interpreted given the small number of vitamin D-deficient participants. Moreover, as we did not identify a statistically significant interaction by race, our data do not support racial differences in the vitamin D–telomere length relationship.

In contrast to the significantly positive linear association between 25(OH)D concentration and telomere length that was found in two previous studies of white women[1,2], we did not find a significant association among the white women in our study population. Compared with these studies, we had a smaller number of white women (n 373), and therefore lower statistical power for examining associations within this subgroup. Age differences may have contributed to the different findings, because the age range for white women in our population was 48–93 years (mean age = 62·8), whereas Richards *et al.*[1] studied younger women aged 18–79 years (mean age = 49·4 years) and Liu *et al.*[2] studied women under 69 years of age (mean age = 59·4). However, we note that Williams *et al.*[13] found no relationship in predominantly white women who were young adults. Given the

British Journal of Nutrition

Table 1. Distribution of leucocyte telomere length (LTL) quartiles (Q) of long *v.* short LTL in relation to demographic and other characteristics of 1154 US radiologic technologists, 2008–2009
(Numbers and percentages; odds ratios and 95 % confidence intervals)

		LTL quartile*										
		Q1 (lowest)		Q2		Q3		Q4 (highest)		Long LTL†		
Characteristics	No.	*n*	%	*n*	%	*n*	%	*n*	%	OR	95 % CI	*P* (df)‡
Total	1154	288		289		288		289				
Age at blood sample collection (years)												
48–64	678	126	44	153	53	185	64	214	74		Ref.	
65–93	476	162	56	136	47	103	36	75	26	0·42	0·33, 0·53	<0·001 (1)
Race												
Whites	651	163	57	184	64	162	56	142	49		Ref.	
Blacks	503	125	43	105	36	126	44	147	51	1·33	1·04, 1·69	0·021 (1)
Sex												
Females	711	157	55	175	61	180	63	199	69		Ref.	
Males	443	131	45	114	39	108	38	90	31	0·79	0·62,1·01	0·060 (1)
Race and sex												
White females	373	82	28	103	36	91	32	97	34		Ref.	
White males	278	81	28	81	28	71	25	45	16	0·75	0·54, 1·04	
Black females	338	75	26	72	25	89	31	102	35	1·22	0·90, 1·65	
Black males	165	50	17	33	11	37	13	45	16	1·10	0·75, 1·61	0·035 (3)
Smoking status												
Never	565	135	47	134	46	141	49	155	54		Ref.	
Former	459	116	40	126	44	115	40	102	35	0·96	0·74, 1·24	
Current	105	30	10	25	9	26	9	24	8	0·71	0·46, 1·10	
Unknown	25	7	2	4	1	6	2	8	3	1·27	0·56, 2·98	0·412 (3)
BMI (kg/m^2)												
<25	283	85	30	70	24	66	23	62	21		Ref.	
25–29	473	112	39	126	44	123	43	112	39	1·20	0·89, 1·63	
30–34	222	59	20	51	18	57	20	55	19	1·22	0·85, 1·75	
35+	165	29	10	41	14	40	14	55	19	1·50	1·00, 2·23	
Unknown	11	3	1	1	0	2	1	5	2	2·61	0·73, 10·6	0·059 (1)
Time spent being physically active indoors during the last 30 d												
<1 h/week	335	92	32	78	27	92	32	73	25		Ref.	
1–2 h/week	293	61	21	80	28	63	22	89	31	1·01	0·73, 1·40	
3+ h/week	444	107	37	112	39	118	41	107	37	0·97	0·72, 1·30	
Unknown	82	28	10	19	7	15	5	20	7	1·06	0·63, 1·76	0·879 (1)
Took vitamin D supplements in the last 30 d												
Never	610	152	53	149	52	148	51	161	56		Ref.	
Ever	466	111	39	125	43	122	42	108	37	1·11	0·86, 1·42	
Unknown	78	25	9	15	5	18	6	20	7	1·13	0·69, 1·85	0·701 (2)
Season of blood collection												
Winter	398	103	36	110	38	90	31	95	33		Ref.	
Spring	331	78	27	94	33	90	31	69	24	1·17	0·86, 1·58	
Summer	178	45	16	32	11	51	18	50	17	1·69	1·17, 2·46	
Fall	247	62	22	53	18	57	20	75	26	1·34	0·94, 1·86	0·034 (3)
Occupational radiation dose to the red bone marrow (mGy)												
0–9	873	194	67	207	72	233	81	239	83		Ref.	
10–19	219	76	26	63	22	40	14	40	14	0·81	0·57, 1·13	
20–135	62	18	6	19	7	15	5	10	3	1·25	0·70, 2·20	0·366 (1)
Menopausal status												
Premenopausal or perimenopausal	28	6	4	4	2	6	3	12	6		Ref.	
Postmenopausal	635	139	89	164	94	161	89	171	86	1·15	0·49, 2·57	
Unknown	48	12	8	7	4	13	7	16	8	1·39	0·50, 3·72	0·783 (2)
Menopausal hormone therapy use, among females												
Never	311	66	42	73	42	77	43	95	48		Ref.	
Former	267	63	40	68	39	69	38	67	34	1·01	0·72, 1·42	
Current	97	21	13	26	15	24	13	26	13	0·82	0·51, 1·31	
Unknown	36	7	4	8	5	10	6	11	6	1·28	0·63, 2·66	0·710 (3)

Ref., referent values; mGy, milligrey.
* Normalised LTL quartiles: Q1 is 0·1113319 to <0·2988862, Q2 is 0·2988862 to <0·3395012, Q3 is 0·3395012 to <0·3909937 and Q4 is 0·3909937 to <1·0467953.
† OR based on logistic regression of long (above median 0·3395012) *v.* short normalised LTL with all models adjusted for continuous age at blood sample collection except for age at sample collection.
‡ *P* values based on likelihood ratio test from logistic regression of long *v.* short LTL using categorical variables as presented in the table except for age at blood sample collection (years), occupational radiation dose (mGy), continuous BMI (coded 1 through 4) and continuous physical activity (coded 1 through 3) with corresponding missing observations dropped from these analyses. All models adjusted for continuous age at blood sample collection except for age at blood sample collection.

Table 2. Distribution of plasma 25-hydroxyvitamin D (25(OH)D) quartiles (Q) in relation to demographic and other characteristics of 1154 US radiologic technologists, 2008–2009
(Numbers and percentages)

	Plasma 25(OH)D quartile									
	Q1 (<45 nmol/l)		Q2 (45–65 nmol/l)		Q3 (66–85 nmol/l)		Q4 (>85 nmol/l)		Plasma 25(OH)D	
Characteristics	*n*	%	*n*	%	*n*	%	*n*	%	(median, nmol/l)	*P* (df)*†
Total	287		290		289		288			
Age at blood sample collection (years)										
48–64	196	68	180	62	168	58	134	47	62·1	
65–93	91	32	110	38	121	42	154	53	70·7	<0·001 (1)
Race										
Whites	106	37	163	56	191	66	191	66	71·3	
Blacks	181	63	127	44	98	34	97	34	57·6	<0·001 (1)
Sex										
Females	190	66	148	51	187	65	186	65	67·8	
Males	97	34	142	49	102	35	102	35	64·1	0·615 (1)
Race and sex										
White females	62	22	77	27	119	41	115	40	73·4	
White males	44	15	86	30	72	25	76	26	68·2	
Black females	128	45	71	24	68	24	71	25	57·0	
Black males	53	18	56	19	30	10	26	9	58·6	<0·001 (3)
Smoking status										
Never	141	49	138	48	138	48	148	51	66·5	
Former	99	34	127	44	118	41	115	40	66·4	
Current	39	14	18	6	25	9	23	8	62·9	
Unknown	8	3	7	2	8	3	2	1	59·2	0·199 (3)
BMI (kg/m^2)										
<25	47	16	62	21	71	25	103	36	75·7	
25–29	100	35	120	41	140	48	113	39	68·0	
30–34	67	23	65	22	47	16	43	15	59·1	
35+	69	24	40	14	30	10	26	9	53·3	
Unknown	4	1	3	1	1	0	3	1	56·0	<0·001(1)
Time spent being physically active indoors during the last 30 d										
<1 h/week	95	33	92	32	80	28	68	24	61·4	
1–2 h/week	70	24	79	27	67	23	77	27	66·0	
3+ h/week	101	35	104	36	119	41	120	42	68·4	
Unknown	21	7	15	5	23	8	23	8	69·5	0·020 (1)
Took vitamin D supplements in the last 30 d										
Never	217	76	187	64	120	42	86	30	54·1	
Ever	51	18	83	29	147	51	185	64	78·0	
Unknown	19	7	20	7	22	8	17	6	65·5	<0·001 (2)
Season of blood sample collection										
Winter	116	40	100	34	88	30	94	33	62·6	
Spring	78	27	86	30	89	31	78	27	66·3	
Summer	39	14	44	15	46	16	49	17	68·1	
Fall	54	19	60	21	66	23	67	23	69·3	0·123 (3)
Occupational radiation dose to the red bone marrow(mGy)										
0–9	226	79	217	75	226	78	204	71	65·6	
10–19	45	16	57	20	55	19	62	22	68·4	
20–135	16	6	16	6	8	3	22	8	64·5	0·890 (1)
Menopausal status, among females										
Premenopausal or perimenopausal	10	5	8	5	8	4	2	1	57·0	
Postmenopausal	164	86	131	89	166	89	174	94	68·3	
Unknown	16	8	9	6	13	7	10	5	63·5	0·430 (2)
Menopausal hormone therapy use, among females										
Never	104	55	67	45	74	40	66	35	61·8	
Former	53	28	58	39	72	39	84	45	71·4	
Current	21	11	13	9	35	19	28	15	72·5	
Unknown	12	6	10	7	6	3	8	4	55·3	<0·001 (3)

mGy, milligrey.
* *P* values based on *F*-test from linear regression of square root of plasma 25(OH)D as the outcome with independent factors coded categorically as shown in table, except for continuous age at blood sample collection (years), continuous BMI (coded 1 through 4), continuous physical activity (coded 1 through 3) and occupational radiation dose (mGy).
† All *P* values adjusted for continuous age at blood sample collection except for age at blood sample collection. Observations with unknowns for continuous variables removed from the analyses.

Table 3. Long (above median) *v.* short leucocyte telomere length (LTL) in relation to plasma 25-hydroxyvitamin D (25(OH)D) quartile (Q) in 1154 US radiologic technologists by race and sex
(Odds ratios and 95 % confidence intervals)

		Odds ratios* of long LTL and 95 % CI by plasma 25(OH)D quartile†										
		Q1 (<45 nmol/l)		Q2 (45–65 nmol/l)		Q3 (66–85 nmol/l)		Q4 (>85 nmol/l)				
	No.	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	$P_{for\ trend}$‡	$P_{for\ heterogeneity}$§	$P_{for\ interaction}$ (df)‖
Total	1154		Ref.	1·22	0·86, 1·72	1·13	0·80, 1·61	0·98	0·69, 1·40	0·440	0·552	
Race												
White	651		Ref.	1·44	0·86, 2·41	1·25	0·76, 2·06	1·05	0·63, 1·73	0·643	0·410	P_{race} = 0·872 (3)
Black	503		Ref.	1·05	0·65, 1·70	1·08	0·64, 1·81	0·99	0·59, 1·68	0·555	0·990	
Sex												
Females	711		Ref.	0·87	0·56, 1·37	1·04	0·68, 1·61	0·92	0·60, 1·43	0·789	0·861	P_{sex} = 0·159 (3)
Males	443		Ref.	1·90	1·10, 3·32	1·31	0·72, 2·41	1·11	0·59, 2·07	0·348	0·084	
Race and sex												
White females	373		Ref.	1·12	0·56, 2·24	1·29	0·68, 2·47	1·02	0·53, 1·94	0·845	0·798	$P_{race,sex}$ = 0·654 (9)
White males	278		Ref.	1·87	0·87, 4·12	1·17	0·53, 2·66	1·10	0·49, 2·50	0·636	0·265	
Black females	338		Ref.	0·74	0·40, 1·35	0·90	0·48, 1·68	0·98	0·52, 1·83	0·967	0·783	
Black males	165		Ref.	1·96	0·88, 4·48	1·76	0·66, 4·84	1·18	0·40, 3·43	0·484	0·359	

Ref., referent values.
* Adjusted for age at blood sample collection (continuous), season (categorical), race (in total population and sex-specific results) and sex (total population and race-specific results).
† Plasma 25(OH)D cut-off values based on total study population.
‡ Trend *P* values from likelihood ratio test calculated using continuous plasma 25(OH)D, df = 1.
§ Heterogeneity *P* values from likelihood ratio test of differences in OR between four quartiles, df = 3.
‖ Multiplicative interaction *P* values from likelihood ratio test using continuous plasma 25(OH)D.

Table 4. Long *v.* short leucocyte telomere length (LTL) for plasma 25-hydroxyvitamin D (25(OH)D) ≥30 *v.* <30 nmol/l in 1154 US radiologic technologists by race and sex
(Odds ratios and 95 % confidence intervals)

	No. long LTL/no. short LTL*		Long LTL†			
	≥30 nmol/l	<30 nmol/l	OR	95 % CI	*P*†	$P_{for\ interaction}$ (df)‡
Total	518/519	59/58	1·38	0·89, 2·14	0·161	
Race						
Whites	295/323	9/24	2·67	1·16, 6·61	0·024	P_{race} = 0·083 (1)
Blacks	223/196	50/34	1·04	0·61, 1·77	0·931	
Sex						
Females	331/294	48/38	1·23	0·74, 2·04	0·466	P_{sex} = 0·463 (1)
Males	187/225	11/20	1·94	0·81, 4·97	0·168	
Race and sex						
White females	179/173	9/12	1·50	0·57, 4·10	0·489	$P_{race,sex}$ = 0·261 (3)
White males	116/150	0/12	INF	2·28, INF	0·005	
Black females	152/121	39/26	1·14	0·62, 2·07	0·732	
Black males	71/75	11/8	0·78	0·25, 2·41	0·765	

INF, positive infinity.
* Categorisation of long and short LTL on the basis of median normalised LTL in total population.
† OR based on maximum likelihood estimate from logistic regression with exact mid-*P* CI; adjusted by stratification for 5-year age categories (48–49, 50–54, 54–59, 60–64, 65–69, 70–74, 75–79, 80–84, 85–89, 90–93 years), season (categorical), race (white, black) and sex.
‡ *P* values for multiplicative interaction use exact score tests, with adjustment by stratification as described above.

limited number of studies on the vitamin D–telomere length relationship in women, it remains unclear whether there is a relationship between these two factors. With regard to men, our null findings were consistent with those of Julin *et al.*[4] as well as those of Williams *et al.*[13].

Although our results were null, the rationale for studying the relationship between vitamin D and LTL remains plausible, given the potential anti-inflammatory role of vitamin D and the sensitivity of telomeres to inflammation. Vitamin D has been inversely related to several inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis[25]. In experimental studies, vitamin D metabolites inhibited production by human monocytes of inflammatory cytokines including IL-6 and TNF-α[26]. In cross-sectional study populations, the predominant circulating metabolite (25(OH)D) was inversely linked to the inflammatory marker C-reactive protein in persons with low 25(OH)D levels and those with elevated inflammatory states[8]. Likewise, shortened LTL has been observed in groups with medical conditions related to inflammation such as vascular disease and arthritis[27,28], and has been related to behaviours/conditions that are themselves associated with inflammation, such as cigarette smoking and obesity[29].

Our findings should be interpreted in the context of several limitations. Despite having a reasonably large overall sample size, this analysis still had limited statistical power for formal tests of effect modification by sex and race. Similar to previous studies

of this association[1–3], the analysis was cross-sectional and could not establish temporality. As we only measured 25(OH)D concentration at a single timepoint, those concentrations may not reflect the average long-term vitamin D status, which may be more relevant to vitamin D's potential effects on telomere length.

Overall, our study aimed to expand the generalisability of the vitamin D and telomere length associations previously reported, because most assessments have been limited to white females. Larger studies will be needed to better test for effect modification by sex or race for associations involving vitamin D. Finally, longitudinal studies examining multiple measurements of 25(OH)D and LTL in individual participants can help establish temporality, control for person-level differences and examine associations involving changes in telomere length.

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References

1. Richards JB, Valdes AM, Gardner JP, *et al.* (2007) Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. *Am J ClinNutr* **86**, 1420–1425.
2. Liu JJ, Prescott J, Giovannucci E, *et al.* (2013) Plasma vitamin D biomarkers and leukocyte telomere length. *Am J Epidemiol* **177**, 1411–1417.
3. Hoffecker BM, Raffield LM, Kamen DL, *et al.* (2013) Systemic lupus erythematosus and vitamin D deficiency are associated with shorter telomere length among African Americans: a case-control study. *PLOS ONE* **8**, e63725.
4. Julin B, Shui IM, Prescott J, *et al.* (2015) Plasma vitamin D biomarkers and leukocyte telomere length in men. *Eur J Nutr* (epublication ahead of print version 11 November 2015).
5. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
6. Reichel H, Koeffler HP & Norman AW (1989) The role of the vitamin D endocrine system in health and disease. *N Engl J Med* **320**, 980–991.
7. Deeb KK, Trump DL & Johnson CS (2007) Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* **7**, 684–700.
8. Zanetti M, Harris SS & Dawson-Hughes B (2014) Ability of vitamin D to reduce inflammation in adults without acute illness. *Nutr Rev* **72**, 95–98.
9. Blackburn EH (1991) Structure and function of telomeres. *Nature* **350**, 569–573.
10. Houben JM, Moonen HJ, van Schooten FJ, *et al.* (2008) Telomere length assessment: biomarker of chronic oxidative stress? *Free RadicBiol Med* **44**, 235–246.
11. Pooley KA, Sandhu MS, Tyrer J, *et al.* (2010) Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res* **70**, 3170–3176.
12. Bojesen SE (2013) Telomeres and human health. *J Intern Med* **274**, 399–413.
13. Williams DM, Palaniswamy S, Sebert S, *et al.* (2016) 25-Hydroxyvitamin D concentration and leukocyte telomere length in young adults: findings from the Northern Finland birth cohort 1966. *Am J Epidemiol* **183**, 191–198.
14. Boice JD Jr, Mandel JS, Doody MM, *et al.* (1992) A health survey of radiologic technologists. *Cancer* **69**, 586–598.
15. Doody MM, Mandel JS, Lubin JH, *et al.* (1998) Mortality among United States radiologic technologists, 1926–90. *Cancer Causes Control* **9**, 67–75.
16. Freedman DM, Cahoon EK, Rajaraman P, *et al.* (2013) Sunlight and other determinants of circulating 25-hydroxyvitamin D levels in black and white participants in a nationwide U.S. study. *Am J Epidemiol* **177**, 180–192.
17. Cawthon RM (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res* **30**, e47.
18. Cawthon RM (2009) Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* **37**, e21.
19. Callicott RJ & Womack JE (2006) Real-time PCR assay for measurement of mouse telomeres. *Comp Med* **56**, 17–22.
20. Simon SL, Preston DL, Linet MS, *et al.* (2014) Radiation organ doses received in a nationwide cohort of U.S. radiologic technologists: methods and findings. *Radiat Res* **182**, 507–528.
21. Institute of Medicine (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: The National Academies Press.
22. Gardner M, Bann D, Wiley L, *et al.* (2014) Gender and telomere length: systematic review and meta-analysis. *ExpGerontol* **51**, 15–27.
23. Diez Roux AV, Ranjit N, Jenny NS, *et al.* (2009) Race/ethnicity and telomere length in the multi-ethnic study of atherosclerosis. *Aging Cell* **8**, 251–257.
24. Hunt SC, Chen W, Gardner JP, *et al.* (2008) Leukocyte telomeres are longer in African Americans than in whites: the national heart, lung, and blood institute family heart study and the Bogalusa Heart Study. *Aging cell* **7**, 451–458.
25. Srikanth P, Chun RF, Hewison M, *et al.* (2016) Associations of total and free 25OHD and 1,25(OH)D with serum markers of inflammation in older men. *Osteoporos Int* (epublication ahead of print version 23 February 2016).
26. Zhang Y, Leung DY, Richers BN, *et al.* (2012) Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol* **188**, 2127–2135.
27. Fitzpatrick AL, Kronmal RA, Gardner JP, *et al.* (2007) Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* **165**, 14–21.
28. Steer SE, Williams FM, Kato B, *et al.* (2007) Reduced telomere length in rheumatoid arthritis is independent of disease activity and duration. *Ann Rheum Dis* **66**, 476–480.
29. Valdes AM, Andrew T, Gardner JP, *et al.* (2005) Obesity, cigarette smoking, and telomere length in women. *Lancet* **366**, 662–664.