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Variants in gene encoding for vitamin D binding protein were associated with leukocyte telomere length: The Pró-Saúde Study

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ABSTRACT

Objective: The aim of this study was to investigate the association between single-nucleotide polymorphisms (SNPs) in vitamin D metabolic pathway genes, serum 25-hydroxyvitamin D [25(OH)D] concentrations, and leukocyte telomere length (LTL) in Brazilian adults.

Methods: The study population comprised 461 participants (33–79 y of age; 51% women) from the Pró-Saúde Study, a cohort of civil servants at a university campus in Rio de Janeiro, Brazil. LTL, genotypes of vitamin D-related SNPs (rs12785878, rs10741657, rs6013897, and rs2282679), and serum 25(OH)D concentrations were determined cross-sectionally. Differences in age- and sex-adjusted LTL means by categories of genotypes and 25(OH)D serum concentrations were evaluated. LTL associations with genotypes and 25(OH)D were investigated using multiple linear regression models adjusted for sociodemographic characteristics and markers of health behavior.

Results: Participants with CC genotype (rs2282679) had shorter age- and sex-adjusted mean LTL than those with AC and AA genotypes (mean ± SE: 0.51 ± 0.03, 0.58 ± 0.01 and 0.5 ± 0.01, respectively, $P < 0.05$). In adjusted analyses, the CC genotype (rs2282679) was inversely associated with LTL ($\beta = -0.061$; 95% confidence interval, −0.120 to −0.001). Other vitamin D-related SNPs and serum 25(OH)D concentrations were not associated with LTL.

Conclusions: Genetic variations in the gene encoding vitamin D binding protein (GC - rs2282679) were associated with LTL, suggesting an influence of vitamin D status on telomere length that may start early in life.

Introduction

Epidemiologic and experimental evidence have shown the importance of vitamin D for the adequate function of many biological systems and for its protective role on a number of extra skeletal complications [1]. Vitamin D can be synthesized in the skin via exposure to ultraviolet B (UVB) radiation or obtained from restricted foods. Regardless of origin, vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] in the liver and subsequently to its active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)2D], which exerts its function mainly through the interaction with nuclear vitamin D receptor (VDR), which is largely distributed in human organisms [2].

Given that 1,25(OH)2D could downregulate the production of reactive oxygen species (ROS) and the expression of several

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proinflammatory cytokines [3], vitamin D can prevent DNA damage, including the telomeric regions [4]. Telomeres are repetitive protein-bound DNA sequences at the end of chromosomes that regulate cellular replicative capacity and protect chromosomes from fusing during mitosis [5]. During the process of cell division, telomeric sequences are gradually lost; therefore, telomere shortening is a continuous process throughout life [6]. Also, the high content of guanine makes telomeres more susceptible to damages caused by ROS and inflammatory processes [7,8]. Because these damages are less efficiently repaired compared with other regions of the genome, both oxidative stress and inflammation can accelerate natural telomere shortening [7,8].

Some authors have explored associations between leukocyte telomere length (LTL) and serum concentrations of 25(OH)D, with inconclusive results [9–16]. A direct association between serum concentrations of 25(OH)D and LTL was observed in women [9,10] but not in men [11]. Larger population studies with adults have also resulted in no association between serum concentrations of 25(OH)D and LTL [12,16], as well as a direct association between them, albeit restricted to middle-aged adults [13]. The mixed findings on the research on vitamin D and LTL may be due to the dynamic fluctuation that serum 25(OH)D is usually subjected to, whereas LTL measurement in a given moment is a result of a continuous process initiated in the beginning of life.

Some single-nucleotide polymorphisms (SNPs) have been identified as genetic determinants of 25(OH)D serum concentrations. Owing to their invariant nature, they may be useful in exploring the relationship between vitamin D and LTL [11]. SNPs in the genes encoding vitamin D binding protein (DBP; *GC* gene), NAD synthetase 1 (*NADSYN1*), vitamin D 25-hydroxylase (*CYP2R1*), and vitamin D 24-hydroxylase (*CYP24A1*) have been identified as strongly associated with 25(OH)D concentrations in genome-wide association studies (GWAS) [17–21]. Therefore, a genetic predisposition to lower vitamin D status may contribute to shorter telomeres.

The aim of the present study was to investigate the association between SNPs in vitamin D metabolic pathway genes (*NADSYN1, CYP2R1, CYP24A1* and *GC*), serum 25(OH)D concentration, and LTL in Brazilian adults.

Material and methods

Overview

The Pró-Saúde Study was a prospective cohort study of civil servants at a university campus in Rio de Janeiro, Brazil. Descriptions of the cohort and design details have been previously published [22]. In brief, detailed self-administered questionnaires were administered to cohort members (N = 3253) during four waves of data collection—1999, 2001–2002, 2006–2007, and 2012-2013—to investigate social determinants of health and health-related behaviors [23]. The Pró-Saúde Study has been periodically approved by the Ethics in Research Committee of the Social Medicine Institute, at the Rio de Janeiro State University. All participants provided written informed consent.

Study population

This was a cross-sectional study conducted with a subsample carried out in parallel with wave 4 of the Pró-Saúde Study (2012–2013) data collection. Participants (n = 520) from the baseline cohort population were randomly selected within strata of sex, age (<50 y versus ≥50 y), and educational level (less than high school versus high school or more), and invited to complete an additional interview that included socioeconomic, nutritional, and body composition data, together with a blood collection for biochemical and genetic analyses. Data collection occurred between July 2012 and October 2013.

Participants who had missing data on socioeconomic or behavioral issues (n = 26) were excluded from the analyses, as well as few participants who self-reported as yellow (n = 2) or indigenous (n = 7). Additionally, participants were also excluded if they did not provide a whole blood sample (n = 8), had inconsistent LTL values based on their age (n = 7, outliers based on confidence intervals), had insufficient serum sample for 25(OH)D analysis (n = 4), or had insufficient DNA sample for genotyping (n = 2). Thus, 464 participants were included in the current analyses.

Outcome assessment

DNA samples were isolated from whole blood using a commercial kit (Puregene Blood Kit – Qiagen, Hilden, Germany). The quality and concentration of DNA samples were evaluated by spectrophotometry (BioDrop DUO, BioDrop, Cambridge, England) and were stored at −80°C, until the determination of LTL and genotyping.

LTL was measured by quantitative real-time polymerase chain reaction (qPCR) based on the method described by Cawthon with modifications [24–26]. Reactions were conducted in triplicate and included genomic DNA (1.6 ng), 2 × Rotor-Gene SYBR Green, PCR Master Mix (Qiagen, Hilden, Germany), primers Tel Forward (300 nM - CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT) and Tel Reverse (300 nM - GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT) or primer single gene Hbg1 Forward (300 nM - GCTTCTGACACAACTGTGTTCACTAGC) and single gene Hbg2 Reverse (700 nM - CACCAACTTCATCCACGTTCACC) (Integrated DNA Technologies, Coralville, IA, USA), in a 24 μL reaction. Amplification was conducted in a Rotor-Gene Q Real-Time PCR cycler (Qiagen, Hilden, Germany) as follows: 95°C by 5 min, 25 cycles (telomere reaction) or 35 cycles (single gene reaction) at 98°C by 7s, and 60°C by 10s. The telomere length for each sample was determined using the telomere-to-single copy gene ratio (T/S ratio) by the ΔCt method (Ct[telomere]/Ct [single gene]). The T/S ratio for each sample (x) was normalized to the mean a T/S ratio of reference sample (r) [2 – (ΔCtx - ΔCtr) = 2-ΔΔCt], which was also used for the standard curve, both as a reference sample and as an interassay validation sample. All sample data included had reference samples with an interassay coefficient of variation (CV) <9%.

For qPCR validation purposes, the LTL was evaluated in a random sample (10%) using the Southern Blot method of Terminal restriction fragment (TRF), as described by Gutierrez-Rodrigues et al. [27]. The analysis was conducted using a commercial kit (Telo TAGGG Telomere Length Assay – Roche Applied Science, Indianapolis, IN, USA).

Exposure assessment

The SNPs rs12785878, rs10741657, rs6013897, and rs2282679 of the *NADSYN1, CYP2R1, CYP24A1,* and *GC* genes, respectively, were analyzed. Real-time PCR allelic discrimination by TaqMan assay (Thermo Scientific Inc., Foster City, CA, USA) was used for genotyping analysis. Probes and primers were designed by Thermo Scientific Inc. Real-time PCR was performed on a Step One Plus Real-Time PCR System (Thermo Scientific Inc). Results were analyzed using the SDS 2.3 software (Thermo Scientific Inc).

Serum 25(OH)D concentration was determined by a semiautomated chemiluminescent enzyme-labeled immunometric assay (Liaison, DiaSorin, Stillwater, MN, USA). Assays were conducted in duplicate, and intra- and interassay CV were 4.9% and 5.8%, respectively. Vitamin D status was categorized according to the Institute of Medicine [28] and the Endocrine Society [29]: <30, 30–50, 51–75, and ≥75 nmol/L.

Covariate assessment

Socioeconomic, demographic, health, and behavioral markers identified from the literature that could affect LTL were included as covariates [8,30–33]. During the interview, participants self-reported their age, sex, marital status (single, married, separated or divorced, or widowed), race/skin color (white, brown, black, yellow, or indigenous, based on official Brazilian criteria) [34], educational attainment (high school or less and college education or higher), and household income. Equivalent per capita household income was calculated using the Organization for Economic Cooperation and Development equivalence scale [35] and categorized into <3, 3–6, and >6 Brazilian minimum wages per capita. Smoking status was categorized as "never-smoker" or "current or former smoker," leisure-time physical activity in the previous 2 wk (classified as "yes" or "no"), and history of medical diagnoses of chronic conditions (diabetes, hypertension, hypercholesterolemia, heart attack, angina, or pulmonary emphysema) were also self-reported. Body mass index (BMI; kg/m^2) was calculated using standing height (m) and body weight (kg) and categorized into three BMI strata based on the World Health Organization's classification [36] as underweight or normal; overweight; and obesity I, II, or III. High-sensitive C-reactive protein (hs-CRP) was measured by immunoturbidimetric latex assay using an automatic analyzer (A25 BioSystems, Barcelona, Spain). Month of blood collection, visceral adipose tissue (VAT) estimated by dual-energy x-ray absorptiometry and sun exposure index (SEI) were previously identified as determinants of 25(OH)D serum concentrations in this population [37] and therefore included as covariates in models of serum vitamin D as the primary exposure.

Statistical analyses

Descriptive statistics were used to characterize the study participants. Continuous variables were expressed as means ± SD and categorical variables as absolute and relative frequencies. Genotype frequencies for each SNP were assessed for Hardy–Weinberg equilibrium using the χ^2 test. All genotypes distribution followed the Hardy–Weinberg equilibrium ($P > 0.05$), except for rs6013897 (CYP24A1, $P = 0.04$), which was subsequently excluded from the analyses. Serum

hs-CRP correlations with 25(OH)D and LTL were evaluated by Pearson's correlation analysis. Mean LTL differences by categories of genotypes and serum 25(OH)D concentrations were evaluated with adjustment for age and sex by analysis of covariance, followed by a least significant difference post hoc test (least significant difference test, LSD). To examine the associations between LTL and genotypes, multiple linear regression models were performed using LTL as the dependent variable and genotypes (mutually adjusted for one another) as independent variables. The first model was adjusted for age, sex, race or skin color, and genotypes. The second model was additionally adjusted for educational attainment, marital status, and equivalent per capita household income. Model 3 was additionally adjusted for smoking status, leisure-time physical activity, BMI, and history of chronic diseases. Linear multiple regression models were used to investigate associations between LTL and serum 25(OH)D concentrations as the independent variable. Model 1 was adjusted for age, sex, educational attainment, marital status, and equivalent per capita household income. Model 2 was additionally adjusted for smoking status, leisure-time physical activity, and history of chronic diseases. Model 3 was additionally adjusted for skin type, month of blood collection, SEI, visceral fat mass (as potential determinants of vitamin D), and hs-CRP. Statistical analyzes were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, U.A), and $P < 0.05$ was considered statistically significant.

Results

The general characteristics of the study population are presented in Table 1. Participants were, on average, 51.4 y of age, predominantly white (49.4%), married (65.7%), and had college education or higher (56.9%), with an equivalent per capita household income <3 minimum wages (45%). Also, the majority of participants did not engage in any physical activity during the previous 2 wk (59.5%), never smoked (63.8%), were overweight (40.9%) or obese (31.9%), and reported history of chronic diseases (58.6%). Serum hs-CRP concentrations were on average 2.0 ± 6.3 mg/L. The average LTL (T/S ratio) was 0.57 ± 0.15 and was significantly higher in women than men (0.59 ± 0.15 and 0.55 ± 0.14, respectively, $P = 0.02$). Additionally, age was inversely correlated with LTL ($r = -0.168$, $P < 0.01$). No statistically significant correlation was observed between LTL and hs-CRP serum concentrations ($r = -0.42$; $P = 0.36$).

Table 1
General characteristics of the study population: Pró-Saúde Study—Rio de Janeiro, Brazil, 2012–2013 (N = 464)

	Frequencies	
	n	%
Age, y		
≤44	90	19.4
45–59	297	64.0
≥60	77	16.6
Sex		
Female	239	51.5
Male	225	48.5
Race/skin color		
Black	83	17.9
Brown	152	32.8
White	229	49.4
Educational attainment		
High school or less	200	43.1
College education or higher	264	56.9
Equivalent household income (per capita, minimum wages)		
<3 minimum wages	209	45.0
3–6 minimum wages	185	39.9
>6 minimum wages	70	15.1
Marital status		
Single	67	14.4
Married	305	65.7
Separated/Divorced	76	16.4
Widower	16	3.4
Physical activity practice in past 2 wk		
Yes	188	40.5
No	276	59.5
Smoking status		
Never-smoker	296	63.8
Current or former smoker	168	36.2
Body mass index, kg/m^2		
Underweight or normal	126	27.2
Overweight	190	40.9
Obesity I, II, or III	148	31.9
Chronic disease		
No	192	41.4
Yes	272	58.6

Age- and sex-adjusted LTL mean (T/S ratio) was significantly lower in individuals carrying CC genotype (0.51 ± 0.03) for rs2282679 (*GC* gene) compared with those carrying AC or AA genotypes (0.58 ± 0.01 and 0.57 ± 0.01, respectively; $P < 0.05$, least significant difference post hoc test; Table 2). No statistically significant differences were observed in LTL means between the three possible genotypes for rs12785878 (*NADSYN1* gene) and rs10741657 (*CYP2 R1* gene; $P > 0.05$). Results from multiple regression analysis showed that LTL of individuals carrying CC genotype (*GC* - rs2282679) was 0.061 T/S ratio units lower (95% confidence interval [CI], −0.120 to −0.001) than those carrying the AA genotype (Table 2). Similar results were observed evaluating the genetic recessive model, in which individuals carrying CC genotype had shorter LTL than those carrying AA combined with AC genotypes (β = −0.065; 95% CI, −0.123 to −0.006). No statistically significant association with LTL was observed for *NADSYN1* - rs12785878 and *CYP2 R1* - rs10741657.

Serum 25(OH)D concentrations were on average 48.3 ± 20.4 nmol/L, and 55.6% of individuals showed vitamin D insufficiency (≤50 nmol/L) (Table 3). No statistically significant differences in age- and sex-adjusted LTL means by categories of serum 25 (OH)D were observed ($P = 0.13$; Table 2). Multiple regression models resulted in no statistically significant associations between categories of 25(OH)D concentrations and LTL (Table 3).

Discussion

The present study investigated the association between LTL and SNPs in genes codifying enzymes or transport proteins involved in vitamin D metabolism, as well as serum 25(OH)D concentrations. The results reported herein suggest that a SNP in vitamin D binding protein gene (*GC*-rs2282679) is associated with telomere length, with individuals carrying CC genotype having shorter LTL.

Biological plausibility of a possible implication of vitamin D on LTL shortening relies on its effects on the inflammatory process or the rate of cell proliferation [3,38,39]. An association between hs-CRP and LTL was not observed in the present study, similar to previous reports [40,41]. Nevertheless, it has been suggested that the process of telomere shortening can result from oxidative stress and inflammation [42], with evidences of an inverse association between LTL and serum CRP [33,43] and inflammatory cytokine [44,45] concentrations. It was demonstrated that active metabolite of vitamin D [1,25(OH)2D] downregulates the expression and production of several proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8 in monocytes [3,46,47]. Moreover, it is also known that vitamin D has a role in the regulation of cell proliferation and differentiation [37,38], which could affect the turnover rate of LTL.

Based on these experimental results, some studies investigated the association between LTL and serum concentrations of 25(OH)D, the major circulating metabolite and the best marker of vitamin D status. However, conflicting results were observed. A direct association between serum 25(OH)D concentrations and LTL has been reported in women [9,10]. Vitamin D deficiency—that is, serum concentrations of 25(OH)D <30 nmol/L—was also significantly associated with shorter LTL in white men and women only, with no association in the other racial groups studied [14]. The same study reported no

Table 2
Association between SNPs in vitamin D pathway genes and LTL: Pró-Saúde Study–Rio de Janeiro, Brazil, 2012–2013

	n (%)	LTL (T/S ratio)*	Model 1† β (95% CI)	Model 2† β (95% CI)	Model 3† β (95% CI)
NADSYN1 - rs12785878 (G>T)					
GG	124 (26.7)	0.57 ± 0.01	Ref	Ref	Ref
GT	249 (53.7)	0.56 ± 0.01	−0.015 (−0.047 to 0.017)	−0.015 (−0.047 to 0.017)	−0.015 (−0.047 to 0.017)
TT	91 (19.6)	0.59 ± 0.02	0.022 (−0.019 to 0.062)	0.019 (−0.022 to 0.060)	0.020 (−0.021 to 0.061)
Dominance					
GG	124 (26.7)	0.57 ± 0.01	Ref	Ref	Ref
GT + TT	340 (73.3)	0.57 ± 0.01	−0.003 (−0.034 to 0.028)	−0.004(−0.034 to 0.027)	−0.003 (−0.034 to 0.028)
Recessive					
GG + GT	373 (80.4)	0.56 ± 0.01	Ref	Ref	Ref
TT	91 (19.6)	0.59 ± 0.02	0.031(−0.003 to 0.065)	0.028(−0.006 to 0.062)	0.029 (−0.006 to 0.063)
Overdominance					
GG + TT	215 (46.3)	0.58 ± 0.01	Ref	Ref	Ref
GT	249 (53.7)	0.56 ± 0.01	−0.024(−0.051 to 0.003)	−0.022(−0.049 to 0.005)	−0.023(−0.050 to 0.005)
CYP2R1 - rs10741657 (A>G)					
AA	41 (8.8)	0.56 ± 0.02	Ref	Ref	Ref
GA	188 (40.5)	0.56 ± 0.01	−0.004 (−0.054 to 0.047)	−0.002 (−0.052 to 0.048)	−0.002 (−0.053 to 0.049)
GG	235 (50.6)	0.58 ± 0.01	0.014 (−0.036 to 0.063)	0.014 (−0.035 to 0.064)	0.015 (−0.035 to 0.064)
Dominance					
GG	235 (50.6)	0.56 ± 0.01	Ref	Ref	Ref
GA + AA	229 (49.4)	0.58 ± 0.01	−0.018(−0.045 to 0.010)	−0.016(−0.044 to 0.011)	−0.017(−0.044 to 0.011)
Recessive					
GG + GA	423 (91.2)	0.57 ± 0.01	Ref	Ref	Ref
AA	41 (8.8)	0.56 ± 0.02	−0.006(−0.053 to 0.042)	−0.007(−0.054 to 0.041)	−0.007(−0.056 to 0.041)
Overdominance					
AA + GG	276 (59.5)	0.58 ± 0.01	Ref	Ref	Ref
GA	188 (40.5)	0.56 ± 0.01	−0.016 (−0.044 to 0.011)	−0.015(−0.043 to 0.012)	−0.016 (−0.043 to 0.012)
GC - rs2282679 (A>C)					
AA	282 (60.8)	0.57 ± 0.01 a	Ref	Ref	Ref
AC	156 (33.6)	0.58 ± 0.01a	0.012 (−0.017 to 0.041)	0.012 (−0.017 to 0.041)	0.012 (−0.017 to 0.041)
CC	26 (5.6)	0.51 ± 0.03b	−0.062 (−0.121 to −0.003)	−0.061 (−0.120 to −0.002)	−0.061 (−0.120 to − 0.001)
Dominance					
AA	282 (60.8)	0.57 ± 0.01	Ref	Ref	Ref
AC + CC	182 (39.2)	0.57 ± 0.01	−0.001 (−0.028 to 0.027)	0.000 (−0.028 to 0.028)	0.000 (−0.028 to 0.028)
Recessive					
AA + AC	438 (94.4)	0.57 ± 0.01a	Ref	Ref	Ref
CC	26 (5.6)	0.50 ± 0.03b	−0.066 (−0.125 to −0.008)	−0.065 (−0.123 to −0.007)	−0.065 (−0.123 to −0.006)
Overdominance					
AA + CC	308 (66.4)	0.56 ± 0.01	Ref	Ref	Ref
AC	156 (33.6)	0.58 ± 0.01	0.017 (−0.011 to 0.046)	0.017 (−0.011 to 0.046)	0.018 (−0.011 to 0.046)

BMI, body mass index; LSD, least significant difference; LTL, leukocyte telomere length; SNP, single-nucleotide polymorphism.
*LTL values are age- and sex-adjusted mean ± SE. For each SNP, different superscript letters (a,b) indicate significant difference on LTL between genotypes by analysis of covariance ($P < 0.05$; LSD post hoc test).
†Multiple regression models used to investigate associations between LTL and SNPs in vitamin D pathway genes: Model 1 adjusted by age, sex, and race/skin color. Model 2 adjusted by variables in model 1 plus marital status, equivalent per capita household income, and educational attainment. Model 3 adjusted by variables in model 2 plus BMI, physical activity practice in past 2 wk, smoking status, and diagnosis of chronic disease.

significant association with LTL when serum 25(OH)D was treated as a continuous variable [14]. In a nationally representative sample of adults in the United States (National Health and Nutrition Examination Survey), a subtle direct association between serum concentrations of 25(OH)D and LTL was observed in middle-aged adults only [13], with no evidence of association without age stratification [15]. Similarly, serum concentrations of 25(OH)D were not associated with LTL after adjustment in adults from the Northern Finland Birth Cohort 1966 [12] or the ESTHER (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung) cohort study [16]. Also, neither 25(OH)D nor 1,25(OH)2D concentrations were associated

Table 3
Association between 25(OH)D serum concentrations and LTL: Pró-Saúde Study–Rio de Janeiro, Brazil, 2012–2013

	N (%)	LTL (T/S ratio)*	Model 1† β (95% CI)	Model 2† β (95% CI)	Model 3† β (95% CI)
Serum 25(OH)D					
<30 nmol/L	86 (18.5)	0.59 ± 0.02	Ref	Ref	Ref
3–50 nmol/L	172 (37.1)	0.58 ± 0.01	−0.004 (−0.041 to 0.034)	−0.004 (−0.042 to 0.034)	0.000 (−0.039 to 0.038)
51–75 nmol/L	168 (36.2)	0.56 ± 0.01	−0.019 (−0.058 to 0.020)	−0.021 (−0.060 to 0.018)	−0.019 (−0.060 to 0.022)
>75 nmol/L	38 (8.2)	0.53 ± 0.02	−0.034 (−0.092 to 0.025)	−0.035 (−0.094 to 0.023)	−0.028 (−0.091 to 0.036)

25(OH)D, 25-hydroxyvitamin D; LSD, least significant difference; LTL, leukocyte telomere length.
*LTL values are age- and sex-adjusted mean ± SE. Differences on LTL by serum 25(OH)D categories were tested by variance analysis ($P < 0.05$; LSD post hoc test).
†Multiple regression models used to investigate associations between LTL and 25(OH)D serum concentrations: Model 1: adjusted by age, sex, marital status, equivalent per capita household income and educational attainment; Model 2: adjusted by variables in model 1 plus physical activity practice in the last two weeks, smoking status, and diagnostic of chronic disease; Model 3: adjusted by variables in model 2 plus skin type, month of blood collection, sun exposure index, visceral fat mass, and hsCRP.

with LTL in US men [11]. In the present study, after adjustment for covariates, no significant association was observed between LTL and serum 25(OH)D concentrations.

At least in part, these mixed findings may be a consequence of evaluating serum concentrations of 25(OH)D cross-sectionally, which may not reflect vitamin D status throughout life. However, the measurement of LTL in a given moment is a result of a continuous shortening process since birth. Therefore, we hypothesized that SNPs in vitamin D metabolic pathway genes that could affect 25(OH)D serum concentrations throughout life would be promising markers to explore long-term effect of vitamin D in LTL. In the present study, one SNP was associated with LTL: *GC*-rs2282679. For this SNP, individuals carrying CC genotype had 12% lower T/S ratio than those carrying AA genotype.

Only two studies evaluated the association of vitamin D-related polymorphisms and LTL [10,11]. Studying women from the US Nurses' Health Study, Liu et al. [10] explored 12 SNPs (other than those explored in the present study) in vitamin D-related genes and found no association with LTL. In the study of Julin et al. [11], one SNP (rs41400444) in the Retinoid X Receptor Alpha gene (*RXRA*) was associated with longer LTL in US men. However, the authors emphasized that despite *RXRA* forms a heterodimer with 1,25(OH)2D-VDR in the cell nucleus before attaching to target genes, *RXRA* also has other roles in the cell that are independent of the vitamin D pathway [11]. It is noteworthy that some of the main SNPs usually associated with vitamin D status were not included in those previous studies on LTL.

Several GWASs have identified SNPs associated with 25(OH)D serum concentrations [17,19,21,48]. Particularly, it has been proposed that SNPs in vitamin D metabolic pathway genes such as *GC, NADSYN1*/*DHCR7, CYP2R1*, and *CYP24A1* could identify individuals genetically predisposed to vitamin D insufficiency [17,19,21,48].

The *GC* gene encodes the DBP that stores and transports both 25(OH)D and 1,25(OH)2D [49]. Thus, it is possible that SNPs in *GC* gene may affect DBP synthesis and, consequently, circulating 25(OH)D concentrations. Studies in different populations observed a strong association between *GC*-rs2282679 and vitamin D status [50], with lower concentrations of 25(OH)D in individuals carrying CC genotype [51-53]. Similarly, *GC*-rs2282679 was also one of the determinants of serum 25(OH)D concentrations in the participants of the present study, with CC genotype associated with lower concentrations of 25(OH)D [54]. The fact that individuals carrying the CC genotype showed lower LTL than individuals carrying the AA genotype supports the hypothesis of a long-lasting effect of vitamin D status on telomere shortening.

Limitations of the present study included its cross-sectional nature. LTL and 25(OH)D were measured once, limiting causal inferences on telomere shortening process by vitamin D status. We believe that, at least in part, using SNPs in vitamin D metabolic pathway as additional markers overcame this limitation. However, the evaluation of genotype combination effect on LTL was limited by the sample size.

Conclusions

To our knowledge, this was the first study reporting a statistically significant association between LTL and a SNP in the DBP gene (*GC* - rs2282679), recognized by GWAS as a determinant of 25(OH)D serum concentrations. The use of 25(OH)D measured at a single time appears to be of little use, when exploring the potential effect of vitamin D on LTL.

Our results suggest that SNPs related to vitamin D status, especially in *GC* gene, could be more relevant, owing to its invariant nature, in explaining the potential continuous influence of vitamin D status on telomere length maintenance throughout life.

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References

[1] Guessous I. Role of vitamin D deficiency in extra skeletal complications: predictor of health outcome or marker of health status? Biomed Res Int 2015;2015:563403.
[2] Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 2008;88:491S–9S.
[3] Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of vitamin d levels on inflammatory status: a systematic review of immune cell studies. PLoS One 2015;10:e0141770.
[4] Nair-Shalliker V, Armstrong BK, Fenech M. Does vitamin D protect against DNA damage? Mutat Res 2012;733:50–7.
[5] Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat Med 2006;12:1133–8.
[6] Blackburn EH, Epel ES, Lin J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. Science 2015;350:1193–8.
[7] Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, et al. Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer 2009;124:1637–43.
[8] Starkweather AR, Alhaeeri AA, Montpetit A, Brumelle J, Filler K, Montpetit M, et al. An integrative review of factors associated with telomere length and implications for biobehavioral research. Nurs Res 2014;63:36–50.
[9] Richards JB, Valdes AM, Gardener JP, Paximadas D, Kimura M, Nessa A, et al. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. Am J Clin Nutr 2007;86:1420–5.
[10] Liu JJ, Prescott J, Giovannucci E, Hankinson SE, Rosner B, Han J, et al. Plasma vitamin D biomarkers and leukocyte telomere length. Am J Epidemiol 2013;177:1411–7.
[11] Julin B, Shui IM, Prescott J, Giovannucci EL, De Vivo I. Plasma vitamin D biomarkers and leukocyte telomere length in men. Eur J Nutr 2015;56:501–8.
[12] Williams DM, Palaniswamy S, Sebert S, Buxton JL, Blakemore AI, Hyppoönen E, et al. 25-Hydroxyvitamin D concentration and leukocyte telomere length in young adults: findings from the Northern Finland Birth Cohort 1966. Am J Epidemiol 2016;183:191–8.
[13] Beilfuss J, Camargo CA Jr, Kamycheva E. Serum 25-hydroxyvitamin D has a modest positive association with leukocyte telomere length in middle-aged US adults. J Nutr 2017;147:514–20.
[14] Liu JJ, Cahaoom EK, Linet MS, Little MP, Dagnall CL, Higson H, et al. Relationship between plasma 25-hydroxymitamin D and leukocyte telomere length by sex and race in a US study. Br J Nutr 2016;116:953–60.
[15] Mazidi M, Michos ED, Banach M. The association of telomere length and serum 25-hydroxyvitamin D levels in US adults: the National Health and Nutrition Examination Survey. Arch Med Sci 2017;13:61–5.
[16] Schöttker B, Hagen L, Zhang Y, Gào X, Holleczek B, Gao X, et al. 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 Biol Sci Med Sci 2019;74:121–8.
[17] Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;19:2739–45.
[18] Engelman CD, Meyers KJ, Ziegler JT, Taylor KD, Palmer ND, Haffner SM, et al. Genome-wide association study of vitamin D concentrations in Hispanic Americans: the IRAS family study. J Steroid Biochem Mol Biol 2010;122. 186–2.
[19] Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180–8.
[20] Lasky-Su J, Lange N, Brehm JM, Damask A, Soto-Quiros M, Avila L, et al. Genome-wide association analysis of circulating vitamin D levels in children with asthma. Hum Genet 2012;131:1495–505.
[21] Jiang X, O'Reilly PF, Achard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nat Commun 2018;9:260.
[22] Nery Guimaraes JM, Chor D, Werneck GL, Carvalho MS, Coeli CM, Lopes CS. Association between self-rated health and mortality: 10 years follow-up to the Pro- Saude cohort study. BMC Public Health 2012;12:676.
[23] Faerstein E, Chor D, Lopes CS, Werneck GL. The Pro-Health Study: general characteristics and methodological aspects. Rev Bras Epidemiol 2005;8:454–66.
[24] Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47.
[25] Lin J, Epel E, Cheon J, Kroenke C, Sinclair E, Bogos M, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B

cells: insights for epidemiology of telomere maintenance. J Immunol Methods 2010;352:71–80.
[26] Calado RT, Cooper JN, Padilla-Nash HM, Sloand EM, Wu CO, Scheinberg P, et al. Short telomeres result in chromosomal instability in hematopoietic cells and precede malignant evolution in human aplastic anemia. Leukemia 2012;26:700–7.
[27] Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PLoS One 2014;9:e113747.
[28] Food and Nutrition Board, Institute of Medicine. Reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.
[29] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Endocrine Society. Evaluation, treatment and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
[30] Kordinas V, Ioannidis A, Chatzipanagioutou S. The telomere/telomerase system in chronic inflammatory diseases. Cause or effect? Genes (Basel) 2016;7. pii: E60.
[31] Ludlow AT, Roth SM. Physical activity and telomere biology: exploring the link with aging-related disease prevention. J Aging Res 2011:790378.
[32] Valdes AM, Andrew T, Gardner JP, Kimura MK, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. Lancet 2005;366:662–74.
[33] Wong JY, De Vivo I, Lin X, Fang SC, Christiani DC. The relationship between inflammatory biomarkers and telomere length in an occupational prospective cohort study. PloS One 2014;9:e87348.
[34] Brazilian Institute of Geography and Statistics (IBGE). Ethnic characteristics – race of people: ratings and identities. Rio de Janeiro, Brazil: Author; 2013.
[35] Organization for Economic Cooperation and Development. The OECD list of social indicators. Paris, France: Author;1982.
[36] World Health Organization. WHO Expert Committee on Physical Status: the use and interpretation of anthropometry. Report of a WHO expert committee. Geneva, Switzerland: Author;1995.
[37] Normando P, Faerstein E, Bezerra FF. Visceral and subcutaneous fat mass association with serum 25(OH)D concentrations in Brazilian adults. FASEB J 2016;30(1 suppl):lb345.
[38] Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 2005;26:662–87.
[39] Pusceddu I, Farrell CJ, Di Pierro AM, Jani E, Herrmann W, Herrmann M. The role of telomeres and vitamin D in cellular aging and age-related diseases. Clin Chem Lab Med 2015;53:1661–78.
[40] Oliveira BS, Pirkle CM, Zunzunegui MV, Batistuzzo de Medeiros SR, Thomasini RL, et al. Leukocyte telomere length and chronic conditions in older women of northeast Brazil: a cross-sectional study. Cells 2018;7. pii: E193.
[41] Hardikar S, Song X, Risques RA, Montine TJ, Duggan C, Blount PL, et al. Obesity and inflammation markers in relation to leukocyte telomere length in a cross-sectional study of persons with Barrett's esophagus. MC Obes 2015;2:32
[42] Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 2010;464:520–8. 25.
[43] Mazidi M, Kengne AP, Sahebkar A, Banach M. Telomere length is associated with cardiometabolic factors in US adults. Angiology 2018;69:164–9.
[44] Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, et al. Ageing and the telomere connection: an intimate relationship with inflammation. Ageing Res Rev 2016;25:55–69.
[45] Lin J, Sun J, Wang S, Milush JM, Baker CAR, Coccia M, et al. In vitro proinflammatory gene expression predicts in vivo telomere shortening: A preliminary study. Psychoneuroendocrinology 2018;96:179–87.
[46] Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract 2007;77:47–57.
[47] Neve A, Corrado A, Cantatore FP. Immunomodulatory effects of vitamin D in peripheral blood monocyte-derived macrophages from patients with rheumatoid arthritis. Clin Exp Med 2014;14:275–83.
[48] O'Brien KM, Sandler DP, Shi M, Harmon QE, Taylor JA, Weinberg CR. Genome-wide association study of serum 25-hydroxyvitamin D in US women. Front Gent 2018;9:67.
[49] Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 2006;372:33–42.
[50] Jolliffe DA, Walton RT, Griffiths CJ, Martineau AR. Single nucleotide polymorphisms in the vitamin D pathway associating with circulating concentrations of vitamin D metabolites and non-skeletal health outcomes: review of genetic association studies. J Steroid Biochem Mol Biol 2016;164:18–29.
[51] Volpio AJ, Pahkala KA, Viikari JS, Mikkilä V, Magnussen CG, Hutri-Kähönen N, et al. Determinants of serum 25(OH)D concentration in young and middle-aged adults. The Cardiovascular Risk in Young Finns Study. Ann Med 2015;47:253–62.
[52] Shao B, Jiang S, Muyiduli X, Wang S, Mo M, Li M, et al. Vitamin D pathway gene polymorphisms influenced vitamin D level among pregnant women. Clin Nutr 2017:S0261–5614. 31397–3.
[53] Slater NA, Rager ML, Havrda DE, Harralson AF. Genetic variation in CYP2R1 and GC genes associated with vitamin D deficiency status. J Pharm Pract 2017;30:31–6.
[54] Bezerra FF, Fonseca ACP, Zembrzuski VM, Campos Junior M, Cabello PH, Faerstein E. Associations between single nucleotide polymorphisms in vitamin D metabolic pathway genes and serum 25(OH)D concentrations in Brazilian adults: the Pro-Saúde Study. FASEB J 2017;31(1 suppl):644.12.