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BRIEF COMMUNICATION

Vitamin D status and leukocyte telomere length in middle childhood

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Short telomere length is associated with chronic diseases and decreased lifespan. Vitamin D and its binding protein (DBP) may maintain telomeres through anti-inflammatory actions, yet the role of vitamin D on telomere length is uncertain, especially in children. We assessed the cross-sectional associations of plasma 25-hydroxy vitamin D (25(OH)D) and DBP with leukocyte telomere length (LTL) in a group of 447 children ages 5–12 years from the Bogotá School Children Cohort. We compared the distribution of age-standardized LTL (*z*-score) between 25(OH)D categories and between DBP quartiles overall and by sex. Overall, 25(OH)D was not significantly associated with LTL. Nonetheless, among boys, 25(OH)D < 50 nmol/L was related to an adjusted 0.36 shorter LTL *z*-score (95% CI: −0.71, −0.01; $P = 0.046$) compared with 25(OH)D ≥ 75 nmol/L. There was no association among girls. DBP was not significantly related to LTL. Intervention studies are warranted to determine whether increasing vitamin D status enhances telomere length.

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INTRODUCTION

Telomeres are repeated DNA sequences that protect the ends of chromosomes from degradation during cell division. Short telomere length is related to increased incidence of chronic illness including coronary heart disease and cancer, and to decreased lifespan [1].

Inflammation is related to shortened telomeres [2]. Vitamin D may maintain telomere length by reducing inflammation and enhancing telomerase activity [3, 4]. Nonetheless, epidemiologic evidence on the association between vitamin D status and leukocyte telomere length (LTL) is equivocal. Some studies in apparently healthy adults found a positive association between vitamin D serostatus and LTL as discussed elsewhere [3, 4], whereas others found no association [5–7]. Childhood vitamin D deficiency has been related to early risk factors for chronic disease [8]; thus, examining the role of vitamin D on childhood LTL, a potential mediator in this etiologic chain, is of significant interest. Yet only three studies have been conducted among presumably healthy children; newborn's LTL was positively correlated with maternal 25-hydroxy vitamin D (25(OH)D) concentrations in one investigation [9], but it was unrelated to cord blood 25(OH)D in another [10]. Among Australian children, 25(OH)D was not associated with LTL [11].

Vitamin D binding protein (DBP), the main carrier of 25(OH)D in blood, has anti-inflammatory functions, and variants in genes coding for DBP have been related to LTL [7]. However, the association of DBP concentrations with LTL has not been examined.

This study aimed to assess the relations of plasma total 25(OH)D and DBP concentrations with LTL in middle childhood.

METHODS

We conducted a cross-sectional study using baseline data from the Bogotá School Children Cohort, a longitudinal investigation of 3202 low- and middle-income children aged 5–12 years at enrollment [12]. At baseline, we administered a parental questionnaire inquiring on sociodemographic characteristics, performed height and weight measurements on the children, and collected fasting blood samples. All participants provided informed consent prior to enrollment. The study was approved by the Ethics Committee at the National University of Colombia Medical School; the University of Michigan Institutional Review Board approved the use of data and samples.

Whole blood collected in EDTA tubes was separated into its components by centrifugation. Total plasma 25(OH)D was quantified in a random subset ($n = 544$) by a validated enzyme immunoassay (Immunodiagnostic Systems Inc.) with a competitive binding technique. Plasma DBP was measured with a Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN) that uses a specific monoclonal antibody. DNA was extracted from the buffy coat and cryopreserved. LTL was quantified in the DNA samples of a random subgroup of participants ($n = 723$) as the telomere-to-single-copy-gene (T/S) ratio, using a multiplex qPCR method. LTL measurements had high internal and external validity [13] (Appendix 1).

The primary exposure, 25(OH)D, was categorized (nmol/L) as <50, 50–<75, or ≥75 [14]. No participants had 25(OH)D < 30 nmol/L, a cut point indicative of deficiency. A secondary exposure, DBP was categorized into quartiles of the sex-specific distribution since there are no conventional cut points. Both exposures were also considered as continuous variables. The outcome, LTL, was transformed into *z*-scores using the study population as the standard.

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Table 1. Leukocyte telomere length *z*-score according to plasma total 25-hydroxy vitamin D [25(OH)D] and vitamin D binding protein (DBP) concentrations in school-age children from Bogotá, Colombia.

	Overall			Boys			Girls		
	n	Mean ± SD	Adjusted difference (95% CI)[a]	*n*	Mean ± SD	Adjusted difference (95% CI)[a]	*n*	Mean ± SD	Adjusted difference (95% CI)[a]
Total 25(OH)D (nmol/L)									
<50	43	0.03 ± 1.42	0.10 (−0.34, 0.53)	14	−0.56 ± 0.68	−0.36 (−0.71, −0.01)	29	0.32 ± 1.60	0.29 (−0.32, 0.91)
50–<75	216	0.08 ± 1.02	0.12 (−0.06, 0.31)	95	−0.01 ± 1.06	0.10 (−0.16, 0.35)	121	0.15 ± 0.99	0.12 (−0.17, 0.40)
≥75	188	−0.03 ± 0.91	Reference	103	−0.12 ± 0.81	Reference	85	0.07 ± 1.01	Reference
Per 25 nmol/L			−0.05 (−0.14, 0.04)			−0.01 (−0.11, 0.09)			−0.08 (−0.26, 0.09)
DBP quartile (median overall/boys/girls, nmol/L)									
Q1 (1501/1439/1519)	111	−0.09 ± 0.83	−0.26 (−0.55, 0.03)	52	−0.22 ± 0.72	−0.09 (−0.41, 0.23)	59	0.03 ± 0.90	−0.42 (−0.87, 0.02)
Q2 (2159/2131/2173)	111	0.05 ± 0.98	−0.09 (−0.40, 0.21)	53	−0.02 ± 1.06	0.11 (−0.26, 0.48)	58	0.11 ± 0.90	−0.32 (−0.77, 0.13)
Q3 (2820/2845/2820)	111	−0.01 ± 0.90	−0.18 (−0.47, 0.12)	53	−0.03 ± 0.96	0.06 (−0.34, 0.40)	58	0.00 ± 0.88	−0.46 (−0.89, −0.02)
Q4 (4007/4046/4007)	112	0.15 ± 1.31	Reference	53	−0.13 ± 0.97	Reference	59	0.42 ± 1.50	Reference
Per 1 SD			0.08 (−0.03, 0.18)			0.03 (−0.11, 0.16)			0.12 (−0.04, 0.29)
<Q4 vs. Q4			−0.16 (−0.43, 0.10)			0.03 (−0.27, 0.32)			−0.39 (−0.79, 0.01)

[a]From multivariable linear regression with leukocyte telomere length *z*-score as the continuous outcome and indicator variables for 25(OH)D or DBP quartiles as predictors, adjusted for child's age (log-transformed), height-for-age *z*-score < −1, body mass index-for-age *z*-score > 1, and socioeconomic status (3 indicators). Empirical variances were specified in all models.

The analytic sample comprised 447 participants with both 25(OH)D and LTL measurements. Analyses were conducted overall and separately for boys and girls since both vitamin D [15] and LTL [16] vary by sex. We compared the distribution of LTL *z*-score by categories of exposures using means ± SD and estimated mean differences with 95% confidence intervals (CI) with multivariable linear regression. Models were adjusted for known independent predictors of LTL [13] that have also been related to vitamin D status [15] but are not its consequence; these included age, height- and body mass index-for-age *z*-scores per the World Health Organization Growth Reference, and socioeconomic status according to the city government's classification. In supplemental analyses, we explored non-linear associations between 25(OH)D and LTL *z*-score using restricted cubic spline models. Analyses were performed with Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC).

RESULTS

Age at enrollment was 8.8 ± 1.7 years; 47.4% of children were male. Mean ± SD LTL *z*-score and plasma 25(OH)D were, respectively, 0.03 ± 1.02 and 73.6 ± 22.8 nmol/L. 25(OH)D was unrelated to LTL overall (Table 1). However, among boys, 25(OH)D < 50 nmol/L was associated with an adjusted 0.36 units lower LTL *z*-score (95% CI: −0.71, −0.01; $P = 0.046$) compared with 25(OH)D ≥ 75 nmol/L. The association was non-linear (Fig. 1). 25(OH)D was not significantly related to LTL *z*-score in girls.

Mean ± SD plasma DBP was 2677 ± 1169 nmol/L. Among girls, LTL *z*-score was higher in the fourth DBP quartile compared with the lowest three, but the association was not statistically significant (Table 1).

DISCUSSION

In this cross-sectional study, total 25(OH)D < 50 nmol/L was associated with decreased LTL *z*-score in school-age boys. This finding is novel; only one previous study had examined this question in a comparable age group of Australian children [11] and found no association, possibly due to lack of stratification by sex or low statistical power. Previous studies in adults, discussed elsewhere [3, 4], had reported positive associations between vitamin D and LTL in both males and females, although results

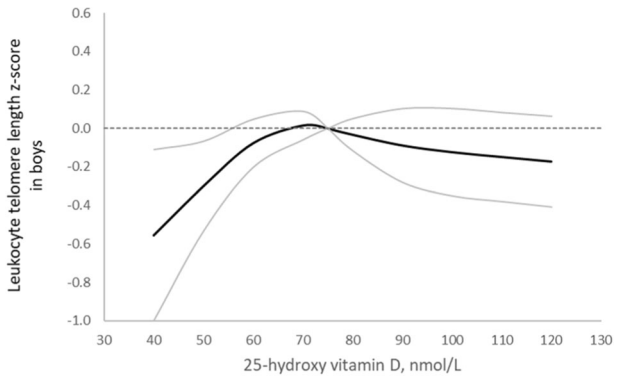

Fig. 1 Leukocyte telomere length (LTL) *z*-score according to plasma total 25-hydroxy vitamin D [25(OH)D] in Colombian boys ($n = 212$). The black line represents estimated mean adjusted differences in LTL between given 25(OH)D concentration values and 75 nmol/L (reference). Gray lines represent 95% confidence intervals for the differences. Estimates are from linear regression models with LTL *z*-score as the continuous outcome and predictors that included linear and spline terms for 25(OH)D, child's age (log-transformed), height-for-age *z*-score < −1, body mass index-for-age *z*-score > 1, and socioeconomic status. Empirical variances were specified in the model.

from a Mendelian randomization study [6] did not lend support to causation. It is possible that an association between vitamin D and LTL changes throughout the life cycle. Potential mechanisms that could explain an effect of vitamin D on LTL are a vitamin-induced enhancement of telomerase activity [4] and anti-inflammatory actions [17]. Proinflammatory cell activity, a result of infection or stress, can increase release of reactive oxygen and nitrogen species which damage the guanine-rich telomeric ends of DNA and promote cell replication, further shortening telomeres.

DBP at the highest quartile was related to longer LTL in girls, but statistical power may have been limited to examine this question. Specific genotypes coding for DBP have been related to LTL [7]; thus, an association could be due to genetic confounding.

Despite its cross-sectional design, this study provides evidence of an association between low vitamin D serostatus and LTL in school-age boys. Assessing sex-specific associations is warranted

in future longitudinal and intervention investigations to elucidate a potential causal effect of vitamin D on LTL.

DATA AVAILABILITY

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

EV designed the research. MM-P and CM conducted the research and provided critical revisions to the manuscript. RMB performed the statistical analysis. RMB and EV wrote the paper and had primary responsibility for the final content. All authors have read and approved the final version of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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