Nutrition 71 (2020) 110645

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Biochemical profile, eating habits, and telomere length among Brazilian children and adolescents

Pâmela Ferreira Todendi Ph.D. [a], J. Alfredo Martínez Ph.D. [b], Cézane Priscila Reuter Ph.D. [c], William Latosinski Matos [d], Silvia Isabel Rech Franke Ph.D. [c], Cristina Razquin Ph.D. [b], Fermín Ignacio Milagro Ph.D. [b], Vivian Francília Silva Kahl Ph.D. [e], Marilu Fiegenbaum Ph.D. [f], Andréia Rosane de Moura Valim Ph.D. [c,*]

[a] Graduate Program in Pathology, Federal University of Health Sciences of Porto Alegre, Porto Alegre/RS, Brazil
[b] Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Madrid, Spain
[c] Graduate Program in Health Promotion, University of Santa Cruz do Sul, Santa Cruz do Sul/RS, Brazil
[d] Undergraduate student, Pharmacy Program, University of Santa Cruz do Sul, Santa Cruz do Sul/RS, Brazil
[e] Telomere Length Regulation Unit, Children's Medical Research Institute—Westmead, Sydney, Australia
[f] Department of Basic Health Sciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre/RS, Brazil

ARTICLE INFO

Article History:
Received 14 May 2019
Received in revised form 3 August 2019
Accepted 1 November 2019

Keywords:
Telomere
Eating habits
Adiposity
Children
Adolescents

ABSTRACT

Objectives: Lifestyle, obesity, and eating habits are emerging as determinants for the instability of telomeres. The increase in childhood and adolescent obesity and the association of biochemical profiles and dietary components with telomere length (TL) makes it an important issue in nutritional research. The aim of the present study was to investigate TL and its association with ethnic background, adiposity, clinical and biochemical parameters, and dietary patterns among Brazilian children and adolescents.
Methods: A cross-sectional study encompassing 981 children and adolescents between 7 and 17 y of age was performed. Dietary intake habits, anthropometry, and clinical data were collected. TL analysis was performed by quantitative polymerase chain reaction.
Results: Children presented significantly longer TL than adolescents ($P = 0.046$). Participants who self-declared as black, mulatto, or brown ($P < 0.001$) also showed longer TL than those who were white. Regarding biochemical parameters, individuals with altered glucose levels had shorter TL than normoglycemic participants in the total sample ($P = 0.014$). Such difference remained statistically significant in adolescents ($P = 0.019$). Participants who reported eating fruits and vegetables regularly had longer TL than those who did not ($P < 0.001$).
Conclusion: The results suggested that both biochemical parameters and the intake of antioxidant-rich food, such as fruits and vegetables, are associated with the stability of telomere biology among young Brazilians.

Introduction

Telomeres are nucleoprotein structures composed primarily of TTAGGG tandem repeats that protect the end of chromosomes maintaining the stability of the genome [1]. They become shorter in each cell division and decline with age [2]. It has been reported that lifestyle factors such as diet or physical activity may play an important role in modulating leukocyte telomere length (TL) [3], and shorter telomeres have been observed in individuals with cardiovascular and neurodegenerative diseases, diabetes, and cancer [4,5]. Oxidative stress, followed by inflammation, seems to be a primary mechanism by which telomere shortening occurs in such diseases [6].

The prevalence of obesity is rapidly increasing worldwide, with high significance for children and adolescents [7], and often is associated with chronic inflammation, altered biochemical indicators, oxidative stress, and higher cardiovascular biomarkers [4,8]. Shorter telomeres are associated with multiple biochemical stressors and obesity in adults and, therefore, may represent a cumulative index of risk factors [4]. Moreover, common unhealthy lifestyle habits such as smoking, sedentarism, and unhealthy eating

This work was financially supported by grants from the Coordination of Improvement of Higher Level Personnel (CAPES, Brazil), National Council for Scientific and Technological Development (CNPq, Brazil), and Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS, Brazil). This study was completed in partnership with the University of Navarra, Center of Biomedical Research in Physiopathology of Obesity and Nutrition (CIBERobn) and IMDEA Food, Madrid.
*Corresponding author: Tel.: +55 51 3717 7341; Fax: +55 51 9335 7393.
E-mail address: avalim@unisc.br (A.R.d.M. Valim).

https://doi.org/10.1016/j.nut.2019.110645
0899-9007/

patterns are emerging as determinants for telomere maintenance instability [9].

Recent studies have shown that high intake of vegetables and fruits [9] and increased intake of ω-3 fatty acids [10] are associated with longer TL. On the other hand, higher intakes of saturated fatty acids [9] or processed meats [11] have been associated with telomere shortening. From this scenario, it has been suggested that an antioxidant-rich diet can help to maintain telomeres and therefore retard biological aging [9].

To our knowledge, few studies have focused on TL in the young population and no Brazilian study has evaluated TL in children and adolescents regarding measures of adiposity, biochemical parameters, and diet. Therefore, the present study aimed to investigate TL and its association with adiposity, clinical and biochemical parameters, and dietary pattern in Brazilian children and adolescents.

Materials and methods

Study population and design

We recruited 981 children and adolescents between 7 and 17 y of age from the School Health study: Phase III (Santa Cruz do Sul, Rio Grande do Sul, Brazil). The School Health study is a lifestyle education program supported by a multidisciplinary team of dietitians, nurses, pharmacists, physiotherapists, and physical educators from the University of Santa Cruz do Sul (UNISC). Those between 7 and 9 y of age were classified as children, and adolescents were those between 10 and 17 y. Study protocols were carried out in accordance with the ethical standards established by the Declaration of Helsinki. This cross-sectional research study was approved by the Research Ethics Committee of the UNISC and by the Federal University of Health Sciences of Porto Alegre (UFCSPA).

A written consent was obtained from parents or guardians before the beginning of the study. Students or parents were interviewed face-to-face and a questionnaire regarding students' demographic data (age, ethnicity, sex, etc.), lifestyle habits (practice of physical activity, hours of sleep per night, etc.), and eating patterns was administered. Questions related to eating habits were adapted from Nahas et al. [12]. The following questions were investigated:

1. How often do you eat red meat? (*never or once, 2 to 3 times,* or *4 to 5 times a week*)
2. How often do you eat fish? (*never or once, 2 to 3 times,* or *4 to 5 times a week*)
3. Your daily diet includes at least 5 servings of fruits and vegetables (*never/occasionally* or *very frequently/always*)
4. Do you avoid eating fatty foods (fats, fried foods) and sweets? (*never/occasionally* or *very frequently/always*)

Family income and parents' educational level were classified according to the criteria of the Brazil Economic Classification [13].

Brazil has a mixed ethnic population [14], thus, participants' ethnicity determination was done according to Parra et al. [15], based on an evaluation of the following phenotypic characteristics: skin color in the medial part of the arm, color and texture of hair, and the shape of the nose and lips. Blood samples were collected via venipuncture by an authorized professional after 12 h of fasting. The collections were carried out from 2014 to 2015.

Anthropometric and clinical measurements

Height (m) and body weight (kg) were measured using a Welmy scale with a coupled stadiometer (Welmy, Santa Bárbara D'Oeste-SP, Brazil). Body mass index (BMI) was obtained for each participant according to the formula BMI = weight/height^2 (kg/m^2). Criteria established by the World Health Organization were followed for the variable BMI Z-score [16], where ≤+1 SD, normal; >+1 SD, overweight; and >+2 SD obesity.

Waist circumference (WC) was obtained using a measuring tape. The parameters established by Taylor et al. [17] were followed for classification as normal percentile circumference ≤80 cm and altered percentile >80 cm. Body fat percentage (BF%) was measured using a Lange (Beta Technology Incorporated, Atlanta, Georgia, USA) compass to measure triceps and subscapular cutaneous folds. After, the equation of Slaughter et al. [18] was applied and the values were classified according to the criteria established by Lohman [19]: normal (boys <20%, girls <25%) and high (boys ≥20%, girls ≥25%).

The systolic and diastolic blood pressure (SBP and DBP, respectively) measurements were obtained using the auscultatory method, with previously calibrated instruments, respecting the width-to-length ratio of 1:2 for each arm circumference. Values in the <90 percentile were classified as normotensive; 90 to 95 percentiles, as borderline; and ≥95 percentile as arterial hypertension, according to the VI Brazilian Guidelines for Hypertension [20].

Biochemical profile considered total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TG), and glucose. Commercial kits from Kovalent (Kovalent do Brasil, Ltd.a São Gonçalo, Rio de Janeiro) were used to assess TC, HDL-C, TG, and glucose. LDL-C values were obtained using Friedewald equation [21]. TC, LDL-C, and TG were classified as normal and high (TC >199.9 mg/dL, LDL-C >129.9 mg/dL, TG >99.9 mg/dL for those <9 y and >129.9 mg/dL for those >10 y), whereas HDL-C was classified as normal and low (<39.9 mg/dL) [22]. The lipid change variable (TC, HDL-C, LDL-C, and TG) was classified into two categories: a group presenting one to two alterations or three to four lipid alterations. Glucose was considered normal at <99 mg/dL [23].

Telomere length assessment

Ethylenediaminetetraacetic acid-anticoagulated whole blood was used for DNA extraction by salting out [24], using a standardized protocol that helps preserve its stability. DNA was then quantified using a NanoDrop 2000 c spectrophotometer unit (Thermo Fisher Scientific, Wilmington, DE, USA). TL was measured on genomic DNA using real-time polymerase chain reaction as described by Cawthon [25]. This method measures (T) and a single-copy gene (Ribosomal Protein Large PO - RPLPO) as reference (S), for each sample.

For T and S reactions, quantitative PCRs were performed separately on 384-well paired plates in the 7900 HT system (Applied Biosystems, Foster City, CA, USA). Total reaction volume was 10 μL, containing 10 ng of genomic DNA and Sybr Green PCR Kit (Qiagen, Valencia, CA, USA) was used as master mix. Telomere-to S-ratio (T/S) was normalized as follows:

$$\left[2^{CT(\text{telomeres})}/2^{CT(\text{single copy gene})}\right] = 2^{-\Delta CT}$$

A calibration curve of a DNA reference sample (64-0.25 ng in twofold dilutions) was included for each measurement as standard, to control day-to-day variability. A standard curve with linearity R^2 >0.98 was accepted. For quality control, all samples were analyzed in triplicate and verified for agreement among the values in triplicate. Samples that presented high variation (>10%) were reanalyzed. Intra-assay coefficient of variation (CV) among the triplicates was 1.5% and inter-assay CV between the plates was 3.7%, which supports the power of this procedure. To avoid thawing cycles, qPCR plates containing telomere and normalizing gene primers were run on the same day.

Statistical analyses

A descriptive analysis stratified by sex was performed. Subsequently, clinical, sociodemographic, anthropometric, and biochemical means were analyzed in relation to TL, stratifying the children and adolescents in the total sample and by age group. Pearson correlation and *t* test were used to analyze relative TL (T/S ratio) among children and adolescents and age, respectively. General linear model test was used to test the association of TL and anthropometric and biochemical data and eating behavior. Analyses were performed adjusting TL for age, sex, ethnicity, and family income in total sample and for sex, family income, and ethnicity when children and adolescents were separately evaluated. Statistical analyses were performed using SPSS version 23 for Windows (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the main characteristics of the 981 participants stratified by sex. Of the participants, 42% were overweight or obese according to the categorization performed through BMI Z-score. Among the anthropometric characteristics, girls presented a higher BF% mean (23.1 ± 6.5; mean ± SD) when compared with boys (17.4 ± 8.1, $P < 0.001$). Both SBP and DBP, in addition to TL, did not differ between boys and girls. Among the biochemical parameters analyzed, girls showed significantly higher levels of LDL-C and TG (86.7 ± 28.3 versus 82.4 ± 26.8 mg/dL, $P = 0.017$; 77.4 ± 35.9 versus 68.6 ± 34 mg/dL, $P < 0.001$, respectively) than boys. On the other hand, when compared with girls, boys had higher levels of HDL-C (65.9 ± 11.5 versus 63.6 ± 11 mg/dL, $P = 0.001$) and glucose (90 ± 7.5 versus 87.7 ± 12.6 mg/dL, $P = 0.001$).

Figure 1 shows that children presented slightly longer TL than adolescents ($P = 0.046$). Additionally, when both groups were analyzed together, there was an inverse correlation between age and TL ($r = -0.083$, $P = 0.009$; Fig. 2). However, when stratified, such correlation remained significant only for children ($P = 0.04$; data

Table 1
Characteristics of the sample stratified by sex

	n	Girls (n = 549)	n	Boys (n = 432)	*P*-value
Children, 7–9 y; n (%)		111 (20.2)		108 (25)	
Adolescents, 10–17 y; n (%)		438 (79.8)		324 (75)	
BMI Z-score	550	0.75 ± 1.25	431	0.90 ± 1.26	0.061
WC, cm	550	66.5 ± 10.1	431	67.7 ± 11	0.088
BF%	550	23.1 ± 6.5	431	17.4 ± 8.1	**<0.001**
SBP, mm Hg	550	107 ± 15.6	431	107 ± 16.3	0.935
DBP, mm Hg	550	66.4 ± 11	431	65.4 ± 11	0.165
TC, mg/dL	546	165.8 ± 31.9	427	161.9 ± 29.5	0.052
LDL-C, mg/dL	546	86.7 ± 28.3	427	82.4 ± 26.8	**0.017**
HDL-C, mg/dL	546	63.6 ± 11	427	65.9 ± 11.5	**0.001**
TG, mg/dL	546	77.4 ± 35.9	427	68.6 ± 34	**<0.001**
Glucose, mg/dL	546	87.7 ± 12.6	427	90 ± 7.5	**0.001**

BF%, body fat percentage; BMI, body mass index; DBP diastolic blood pressure; HDL-C, high- density lipoprotein cholesterol; LDL-C low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglyceride; WC, waist circumference.
All values mean ± SD unless otherwise noted.
n, number of participants.
Values in bold, $P < 0.05$.

not shown). Association analysis of TL with ethnicity, anthropometric, clinical and biochemical parameters for the total sample and according to age groups is described in Table 2. Participants who self-declared as black/mulatto/brown showed longer TL than those who identified as white (1.18 ± 0.57 versus 1.05 ± 0.42, $P < 0.001$). Regarding biochemical parameters, participants who presented altered glucose levels had shorter TL than normoglycemic individuals (0.93 ± 0.38 versus 1.10 ± 0.47, $P = 0.014$). Such difference remained statistically significant in adolescents (0.93 ± 0.40 versus 1.08 ± 0.44, $P = 0.019$). There was a trend of shorter telomeres in the total sample ($P = 0.059$) and in adolescents ($P = 0.056$) with three to four lipid alterations.

Results of the association of TL with eating habits are shown in Table 3. Children who reported that they *always* or *very frequently* eat fruits and vegetables had longer TL than those who did not (1.17 ± 0.52 versus 1.06 ± 0.45, $P < 0.001$). This result was also seen among adolescents (1.19 ± 0.53 versus 1.04 ± 0.41, $P < 0.001$). Although not statistically significant, there was a trend of longer TL in those individuals who consumed fish four to five times per week or more.

Discussion

Although shorter telomeres had already been associated with adiposity measurements [26], cardiovascular risk [4], and diet [27], only a few studies have associated clinical and biochemical profiles

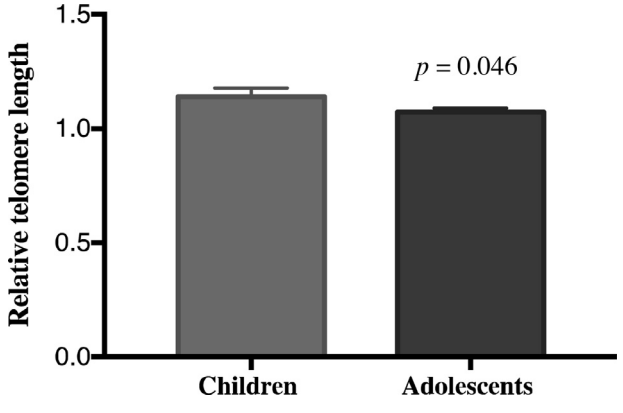

Fig. 1. Relative telomere length (T/S ratio) for children and adolescents. Unpaired *t* test.

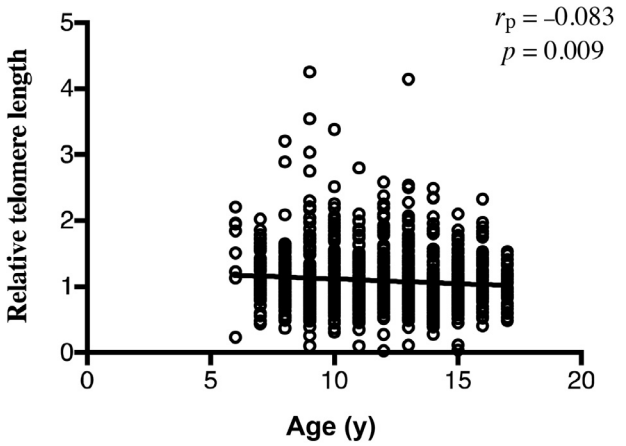

Fig. 2. Pearson correlation between relative telomere length (T/S ratio) and age for the entire population of children and adolescents.

with TL in children and adolescents. In the present study, we observed that adiposity parameters and blood pressure measurements were not associated with TL, whereas glucose levels were. There was a trend toward association between higher lipid score and shorter TL in the sample studied. Age was inversely associated with TL, and those who self-declared as black/mulatto/brown showed longer TL than white children and adolescents.

Telomeres are major markers of cellular and biological aging. Its shortening depends on exposure related to physiologic, pathophysiologic, and epigenetic effects [28]. In the present study, children had longer telomeres than adolescents. Previous data have demonstrated that TL inversely correlates with age. In a cohort study of >100 000 participants, TL was shown to decrease with increasing age [29]. Although the present investigation studied this correlation in children and adolescents, it is already possible to observe the influence of age on telomere shortening. This result shows the importance of knowing the external factors that accelerate telomere shortening from an early age and subsequently, to identify preventive strategies that are directly associated with the development of chronic diseases and early aging.

Similar to our results, Dong et al. [30] also observed longer TL in black compared with white adolescents. Interestingly, in an observational study, London school children between 8 and 9 y of age were evaluated regarding air pollution, ethnicity, and TL. Regardless of other parameters, children reported as black had longer telomeres [31]. Polygenetic adaptation is suggested to influence the longer telomeres observed in Africans compared with Europeans, which may also explain, at least in part, ethnic differences in risks for human diseases that have been associated with TL [32].

A study performed in U.S. children and adults showed that telomere shortening was associated with BMI, WC, age, lipid profile, smoking, and metabolic risk factors [33]. A recent research in a U.S. adult population revealed that leukocyte TL was associated with adiposity and was a strong predictor of cardiovascular disease [4]. Telomere maintenance was also associated with HDL-C, TGs, insulin resistance, blood pressure measurements, proinflammatory marker (C-reactive protein), and adiposity measurements (BMI, WC, and BF%) [4]. In the present study, biochemical parameters showed an association with TL, particularly in adolescents. The present study demonstrated that those with altered glucose levels had lower TL. The same was observed in a study with non-diabetic adults from Sweden [34]. A biological explanation for this association suggests that telomere wear may play an important role in the pathogenesis of type 2 diabetes, increasing the likelihood of β-cell

Table 2
Telomere length results associated with ethnicity and clinical, anthropometric, and biochemical parameters

	n	All	*P*-value*	n	Children (7–9 y)	*P*-value†	n	Adolescents (10–17 y)	*P*-value†
Skin color			**<0.001**‡			**0.008**‡			**0.009**‡
White	736	1.05 ± 0.42		174	1.09 ± 0.45		562	1.04 ± 0.42	
Black/mulatto/brown	245	1.18 ± 0.57		45	1.33 ± 0.80		200	1.14 ± 0.50	
SBP, mm Hg§			0.570			0.541			0.431
Normal	780	1.08 ± 0.48		187	1.15 ± 0.54		593	1.07 ± 0.45	
High	200	1.09 ± 0.42		31	1.09 ± 0.57		169	1.09 ± 0.40	
DBP, mm Hg§			0.210			0.116			0.465
Normal	803	1.10 ± 0.48		199	1.16 ± 0.55		608	1.08 ± 0.46	
High	171	1.03 ± 0.38		99	0.94 ± 0.47		154	1.04 ± 0.37	
BMI Z-score‖			0.795			0.425			0.992
Low/Normal weight	619	1.08 ± 0.45		100	1.17 ± 0.54		465	1.07 ± 0.44	
Overweight/Obesity	362	1.09 ± 0.50		119	1.11 ± 0.55		297	1.07 ± 0.45	
WC, cm¶			0.897			0.404			0.672
Normal	777	1.09 ± 0.46		165	1.16 ± 0.56		612	1.07 ± 0.44	
Altered	204	1.08 ± 0.48		54	1.08 ± 0.51		150	1.07 ± 0.47	
BF%			0.866			0.652			0.824
Normal	638	1.09 ± 0.47		163	1.15 ± 0.55		475	1.07 ± 0.44	
High	343	1.07 ± 0.46		56	1.11 ± 0.55		287	1.07 ± 0.44	
TC, mg/dL#			0.303			0.388			0.453
Normal	548	1.10 ± 0.48		128	1.17 ± 0.61		420	1.08 ± 0.42	
High	424	1.06 ± 0.46		89	1.09 ± 0.44		335	1.06 ± 0.46	
LDL-C, mg/dL**			0.547			0.542			0.708
Normal	808	1.09 ± 0.47		182	1.15 ± 0.58		626	1.07 ± 0.43	
High	164	1.06 ± 0.47		35	1.08 ± 0.35		129	1.05 ± 0.50	
HDL-C, mg/dL††			0.196			0.868			0.144
Normal	962	1.08 ± 0.47		215	1.14 ± 0.55		747	1.07 ± 0.44	
Low	10	0.92 ± 0.24		2	1.14 ± 0.12		8	0.86 ± 0.24	
TG, mg/dL‡‡			0.345			0.953			0.365
Normal	692	1.07 ± 0.45		142	1.14 ± 0.55		550	1.06 ± 0.42	
High	280	1.10 ± 0.51		75	1.14 ± 0.54		205	1.09 ± 0.49	
Glucose (mg/dL)§§			**0.014**			0.313			**0.019**
Normal	906	1.10 ± 0.47		211	1.14 ± 0.55		701	1.08 ± 0.44	
High	60	0.93 ± 0.38		6	0.95 ± 0.26		54	0.93 ± 0.40	
Lipid changes‖‖			0.059			0.538			0.056
0–2 changes	891	1.10 ± 0.47		201	1.14 ± 0.56		696	1.08 ± 0.44	
3–4	75	0.98 ± 0.46		16	1.06 ± 0.37		59	0.96 ± 0.48	

BF%, body fat percentage; BMI, body mass index; DBP diastolic blood pressure; HDL-C, high- density lipoprotein cholesterol; LDL-C low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglyceride; WC, waist circumference; n, number of participants.
Values in bold, $P < 0.05$.
*Adjusted by sex, ethnicity, family income, and age.
†Adjusted by sex, family income, and ethnicity.
‡Not adjusted.
§SBP and DBP: percentile normal 90–95 and high ≥95.
‖BMI Z-score: Low/normal weight <+1 SD, Overweight/obesity >+1 SD.
¶WC (according to sex and age): percentile normal ≤80 and percentile high >80.
#High TC >199.9 mg/dL.
**High LDL-C >129.9 mg/dL.
††Low HDL-C <39.9 mg/dL.
‡‡High TG >99.9 mg/dL (<9 y of age) and >129.9 mg/dL (>10 y of age).
§§High glucose: ≥100 mg/dL.
‖‖Lipid changes: 0–2 and 3–4 changes in lipids dosages.

senescence, leading to reduced cell mass and decreased insulin secretion [35]. This association reflects a direct detrimental effect of early and minimal changes in glucose metabolism and senescence at the cellular level, which is important for the development of new studies.

Eating habits are also associated with TL [9] and, in our study, children and adolescents who rarely eat fruits and vegetables presented shorter telomeres. The increasing number of overweight and obese individuals and the association of dietary components with TL make the rise of such a question especially important in nutritional research. There is evidence that adherence to the Mediterranean diet, which is rich in plant-derived foods, is associated with longer telomeres in adults [36]. A study with Spanish children and adolescents showed that longer telomeres were associated with a higher antioxidant capacity of the diet [37], and inversely associated with plasma levels of micronutrients in Australian children [38]. Also, another study carried out with Finnish adults found that foods with high antioxidant concentration, such as fruits and vegetables, were associated with longer telomeres [9]. In vivo studies have shown that folate, vitamin D, ω-3 fatty acids, multivitamin use, weight loss by caloric restriction, and cereal fiber intake are associated with longer telomeres, whereas intake of processed and/or red meat and linoleic acid, are associated with shorter TL as well as obesity [36]. Diet-related telomere shortening is highly linked to chromosome instability [39] because enzymes and proteins involved in DNA damage and repair and in chromosome segregation require micronutrients as a substrate, a cofactor, or an integral part of the enzyme [36].

Shortening of telomeres in blood cells may serve as a biomarker of cumulative oxidative stress [6], and this may be the explanation for their association in adolescence, as shown in the results of this study. In fact, telomeres have been shown to be highly sensitive to oxidative stress damage due to their high guanine content [40]. It has been suggested that the mechanism involved could be attributed to the damaging action of the hydroxyl radicals, which can cause DNA damage in telomere length with each replication of hematopoietic stem cells that

Table 3
Association of eating habits with telomere length

Groups of foods*	n	All	*P*-value†	n	Children (7–9 y)	*P*-value‡	n	Adolescents (10–17 y)	*P*-value‡
Red meat			0.839			0.577			0.936
0–1	192	1.10 ± 0.51		59	1.16 ± 0.62		133	1.06 ± 0.46	
2–3 times	268	1.07 ± 0.44		65	1.06 ± 0.45		203	1.08 ± 0.43	
4–5 times	520	1.09 ± 0.46		95	1.18 ± 0.55		426	1.07 ± 0.44	
Fish			0.279			0.893			0.258
0–1	897	1.08 ± 0.47		203	1.14 ± 0.55		694	1.07 ± 0.44	
2–3 times	46	1.01 ± 0.45		12	1.07 ± 0.54		35	1.00 ± 0.42	
4–5 times	37	1.15 ± 0.50		4	1.10 ± 0.21		33	1.16 ± 0.53	
Fruits and vegetables			**0.001**			0.883			**<0.001**
Never/Occasionally	768	1.06 ± 0.45		167	1.15 ± 0.56		602	1.04 ± 0.41	
Very frequently/Always	212	1.17 ± 0.52		52	1.11 ± 0.50		160	1.19 ± 0.53	
Greasy and sweet foods			0.731			0.479			0.410
Never/Occasionally	734	1.09 ± 0.47		165	1.13 ± 0.52		569	1.08 ± 0.45	
Very frequently/Always	246	1.07 ± 0.47		54	1.17 ± 0.62		193	1.04 ± 0.41	

n, number of participants.
Values in bold, $P < 0.05$.
*Per week.
†Adjusted by sex, family income, ethnicity, and age.
‡Adjusted by sex, family income, and ethnicity.

is expressed in shortening of TL. As telomere shortening is induced by a chronic increase in the systemic load of oxidative stress, it is suggested that antioxidants can prevent telomeres shortening [41].

In the light of these results, further assessments are needed to investigate the relationship between TL, cardiovascular biomarkers, and dietary patterns, including longitudinal and epidemiologic studies. To minimize small differences between research participants and potential confounders, adjustments were used in the statistical models for the current analyses. An advantage of this study was that the disorders associated with chronic obesity did not interfere in the sample, as the population was composed of children and adolescents with no major complications. To our knowledge, this was the first study to show the association of biochemical parameters and dietary habits with TL in a young Brazilian population. On the other hand, the study had some limitations. Because participants or their parents self-reported their dietary intake, there is a possibility of measurement error. However, a dietitian and nutrition students carefully collected dietary information through individual sessions and used a previously validated questionnaire [12]. Therefore, we carefully controlled the experimental conditions to avoid possible errors, as described.

Conclusions

The present results suggested that both biochemical parameters and intake of antioxidants from fruits and vegetables are associated with telomere biology maintenance in young Brazilians. We were able to corroborate TL as a biomarker of aging and its association with African ethnicity. These findings hold the importance of telomere investigation as biomarkers for diseases related to diet and lifestyle habits and the importance of an adequate intake of antioxidants in the diet. However, further research is needed to confirm those results.

Acknowledgments

The authors acknowledge the volunteers who participated in this study, and the Biotechnology and Genetics Laboratory of UNISC.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Blackburn EH. Telomeres and telomerase: the means to the end (Nobel lecture). Angew Chem Int Ed Engl 2010;49:7405–21.
[2] Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, et al. A natural product telomerase activator as part of a health maintenance program. Rejuvenation Res 2011;14:45–56.
[3] Sun Q, Shi L, Prescott J, Chiuve SE, Hu FB, De Vivo I, et al. Healthy lifestyle and leukocyte telomere length in U.S. women. PLoS One 2012;7:e38374.
[4] Rehkopf DH, Needham BL, Lin J, Blackburn EH, Zota AR, Wojcicki JM, et al. Leukocyte telomere length in relation to 17 biomarkers of cardiovascular disease risk: a cross-sectional study of US Adults. PLoS Med 2016;13:e1002188.
[5] Barrett JH, Iles MM, Dunning AM, Pooley KA. Telomere length and common disease: study design and analytical challenges. Hum Genet 2015;134:679–89.
[6] Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther 2011;18:e209–26.
[7] NCD Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet 2017;390:2627–42.
[8] Todendi PF, Possuelo LG, Klinger EI, Reuter CP, Burgos MS, Moura DJ, et al. Low-grade inflammation markers in children and adolescents: Influence of anthropometric characteristics and CRP and IL6 polymorphisms. Cytokine 2016;88:177–83.
[9] Tiainen AM, Männistö S, Blomstedt PA, Moltchanova E, Perälä MM, Kartinen NE, et al. Leukocyte telomere length and its relation to food and nutrient intake in an elderly population. Eur J Clin Nutr 2012;66:1290–4.
[10] Kiecolt-Glaser JK, Epel ES, Belury MA, Andridge R, Lin J, Glaser R, et al. Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: a randomized controlled trial. Brain Behav Immun 2013;28:16–24.
[11] Fretts AM, Howard BV, Siscovick DS, Best LG, Beresford SA, Mete M, et al. Processed meat, but not unprocessed red meat, is inversely associated with leukocyte telomere length in the Strong Heart Family Study. J Nutr 2016;146:2013–8.
[12] Nahas MV, Barros MVG, Francalacci VL. O The pentacle of well-being: conceptual basis for assessing the lifestyle of individuals or groups. Rev Bras Ativ Fís Saúde 2000;5:48–59.
[13] Brazilian Association of Research Companies (ABEP). Brazil economic classification criteria. Available at: http://www.abep.org/criterioBr/01_cceb_2018.pdf. Accessed November 13, 2019.
[14] Santos SA. Historical roots of the "whitening" of Brazil. Lat Am Perspect 2002;122:61–82.
[15] Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003;100:177–82.
[16] de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 2007;85:660–7.
[17] Taylor RW, Jones IE, Williams SM, Goulding A. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3-19 y. Am J Clin Nutr 2000;72:490–5.
[18] Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988;60:709–23.
[19] Lohman TG. The use of skinfold to estimate body fatness on children and youth. J Phys Health Recreat Dance 2013;58:98–103.

[20] Brazilian Society of Cardiology; Brazilian Society of Hypertension; and Brazilian Society of Nephrology. VI Brazilian guidelines on hypertension. Arq Bras Cardiol 2010;95:553.
[21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[22] National Heart, Lung, and Blood Institute. NHLBI Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents. Available at: nhlbi.nih.gov/files/docs/peds–guidelines–sum.pdf. Accessed November 13, 2019.
[23] American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes–2018. Diabetes Care 2018;41:S13–27.
[24] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
[25] Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 2009;37:e21.
[26] Batsis JA, Mackenzie TA, Vasquez E, Germain CM, Emeny RT, Rippberger P, et al. Association of adiposity, telomere length and mortality: data from the NHANES 1999-2002. Int J Obes (Lond) 2018;42:198–204.
[27] García-Calzón S, Martínez-González MA, Razquin C, Arós F, Lapetra J, Martinez JA, et al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the PREDIMED-NAVARRA study. Clin Nutr 2016;35:1399–405.
[28] Epel ES. Psychological and metabolic stress: a recipe for accelerated cellular aging? Hormones (Athens) 2009;8:7–22.
[29] Lapham K, Kvale MN, Lin J, Connell S, Croen LA, Dispensa BP, et al. Automated assay of telomere length measurement and informatics for 100,000 subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort. Genetics 2015;200:1061–72.
[30] Dong Y, Huang Y, Gutin B, Raed A, Dong Y, Zhu H. Associations between global DNA methylation and telomere length in healthy adolescents. Sci Rep 2017;7:4210.
[31] Walton RT, Mudway IS, Dundas I, Marlin N, Koh L, Aitlhadj L, et al. Air pollution, ethnicity and telomere length in east London schoolchildren: an observational study. Environ Int 2016;96:41–7.
[32] Hansen ME, Hunt SC, Stone RC, Horvath K, Herbig U, Ranciaro A, et al. Shorter telomere length in Europeans than in Africans due to polygenetic adaptation. Hum Mol Genet 2016;25:2324–30.
[33] Lee M, Martin H, Firpo MA, Demerath EW. Inverse association between adiposity and telomere length: the Fels Longitudinal Study. Am J Hum Biol 2011;23:100–6.
[34] Ahmad S, Heraclides A, Sun Q, Elgzyri T, Rönn T, Ling C, et al. Telomere length in blood and skeletal muscle in relation to measures of glycaemia and insulinaemia. Diabet Med 2012;29:e377–81.
[35] Canudas S, Hernández-Alonso P, Galié S, Muralidharan J, Morell-Azanza L, Zalba G, et al. Pistachio consumption modulates DNA oxidation and genes related to telomere maintenance: a crossover randomized clinical trial. Am J Clin Nutr 2019;109:1738–45.
[36] Fenech MF. Nutriomes and personalised nutrition for DNA damage prevention, telomere integrity maintenance and cancer growth control. Cancer Treat Res 2014;159:427–41.
[37] García-Calzón S, Gea A, Razquin C, Corella D, Lamuela-Raventós RM, Martínez JA, et al. Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: the PREDIMED-NAVARRA trial. Int J Obes (Lond) 2014;38:177–82.
[38] Milne E, O'Callaghan N, Ramankutty P, de Klerk NH, Greenop KR, Armstrong BK, et al. Plasma micronutrient levels and telomere length in children. Nutrition 2015;31:331–6.
[39] Marcon F, Siniscalchi E, Crebelli R, Saieva C, Sera F, Fortini P, et al. Diet-related telomere shortening and chromosome stability. Mutagenesis 2012;27:49–57.
[40] Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. Telomere length assessment: biomarker of chronic oxidative stress? Free Radic Biol Med 2008;44:235–46.
[41] Tarry-Adkins JL, Ozanne SE, Norden A, Cherif H, Hales CN. Lower antioxidant capacity and elevated p53 and p21 may be a link between gender disparity in renal telomere shortening, albuminuria, and longevity. Am J Physiol Renal Physiol 2006;290:F509–16.