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Early exclusive breastfeeding is associated with longer telomeres in Latino preschool children[1]

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ABSTRACT

Background: Telomere length (TL) is a marker of cellular aging, with the majority of lifetime attrition occurring during the first 4 y. Little is known about risk factors for telomere shortening in childhood.

Objective: We evaluated the relation between early life feeding variables and preschool TL.

Design: We assessed the relation between dietary, feeding, and weight-associated risk factors measured from birth and TL from blood samples taken at 4 y of age (n = 108) and 5 y of age (n = 92) in a cohort of urban, Latino children (n = 121 individual children). Feeding variables were evaluated in children with repeat measurements (n = 77).

Results: Mean TL (in bp) was associated with exclusive breastfeeding at 4–6 wk of age (adjusted coefficient: 353.85; 95% CI: 72.81, 634.89; P = 0.01), maternal TL (adjusted coefficient: 0.32; 95% CI: 0.11, 0.54; $P < 0.01$), and older paternal age (adjusted coefficient: 33.27; 95% CI: 4.10, 62.44; P = 0.03). The introduction of other foods or drinks in addition to breast-milk or replacement-milk substitutes before 4–6 wk of age was associated with mean TL at 4 and 5 y of age (adjusted coefficient: −457.01; 95% CI: −720.50, −193.51; $P < 0.01$). Infant obesity at 6 mo of age and soda consumption at 4 y of age mediated the relation in part between exclusive breastfeeding at 4–6 wk of age and mean TL at 4 and 5 y of age. High soda consumption at 3 y of age was associated with an accelerated attrition from 4 to 5 y of age (adjusted coefficient: −515.14; 95% CI: −986.06, −41.22; P = 0.03).

Conclusion: Exclusive breastfeeding at 4–6 wk of age may have long-term effects on child health as evidenced by longer TL at 4 and 5 y of age. *Am J Clin Nutr* 2016;104:397–405.

Keywords: breastfeeding, infants, obesity, telomere, telomere length, Latino

INTRODUCTION

Telomeres, inflammation, and obesity

Telomeres are the protective nucleoprotein structures that consist of repeated (TTAGGG) DNA sequences and bound proteins that cap the ends of chromosomes. Telomeres shorten as cells divide in vitro and as human beings age in vivo (1, 2). A telomere-sequence loss does not happen at a constant rate throughout life but rather is characterized by a rapid decline from birth through age 4 y (1, 2). Telomeres shorten ∼100 bp for each cell division because of incomplete replication and also likely as a result of exposure to oxidative damage from chronic inflammation (3, 4).

The guanine base triplets of telomere sequences are highly sensitive to hydroxyl radical damage that can cause breakage during DNA replication, which renders telomeres particularly susceptible to reactive oxygen species–induced damage (5). As such, a higher free-radical concentration, as is the case in chronic inflammatory states including obesity and other chronic diseases, can cause more rapid telomere attrition (6). Meta-analyses of 16 adult studies have suggested an inverse association between adult BMI and leukocyte telomere length (LTL) (7).

Studies in children of the relation between obesity and shorter LTL have been inconclusive (8–10). Possibly, the metabolic complications of obesity including inflammatory processes, which are more common in adults than in children, and not obesity per se, are associated with rapid telomere attrition (11).

Telomere length, breastfeeding, and dietary intake

Few studies have examined the relations between telomere attrition and environmental exposures in early childhood when the attrition process is greatest (12, 13). Although breastfeeding has been shown to have a small, consistent, protective effect against obesity in children in meta-analyses (14) and for chronic disease in general, to our knowledge, there have been no previous studies that have examined the relation between early dietary intake, including breastfeeding and other feeding patterns associated with or protective against obesity, and telomere length (TL) attrition in the first 5 y of life.

In adults, dietary factors, including cereal fiber and diets that are high in fruit and vegetables, that are protective against obesity have also been shown to be associated with longer TL, and linoleic acid intake and sugar-sweetened beverage consumption have been inversely associated with TL (15, 16). We examined prospective associations between critical early life factors that are

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known to be important for developmental health, specifically nutritional exposures such as early exclusive breastfeeding and early adiposity, and shorter TL. We hypothesized that a longer duration of breastfeeding would be associated with longer TL at ages 4 and 5 y because of the anti-inflammatory health benefits that are associated with breastfeeding.

METHODS

Study population and procedures

Latina mothers were recruited prenatally at 2 local hospitals in San Francisco during the second and third trimesters of pregnancy (n = 201) in 2006 and 2007. The cohort has been described in previous publications including recruitment and procedures (17–21). The inclusion criterion was being a Latina pregnant woman who was expecting a healthy newborn without any contraindications for breastfeeding, and exclusion criteria included mothers with insulin-treated gestational diabetes mellitus and infant Apgar scores <7 at 5 min. The cohort was relatively socioeconomically homogenous with almost all mothers participating in the Women, Infants, and Children program and the majority of mothers were foreign born and Spanish speaking. Briefly, at follow-up from delivery, at 4–6 wk of age, and subsequently at 6 and 12 mo of age and annually thereafter, child weight and recumbent length or height (to calculate the BMI percentile) and waist circumference were collected. Childhood obesity was defined as having a BMI ≥95th percentile (or weight-for-length ≥95th percentile if the child was <2 y of age), and childhood overweight was defined as having a BMI ≥85th percentile (or weight-for-length ≥85th percentile if the child was <2 y of age including children who were at or above the 95th percentile or obese) with the use of CDC growth curves (22). Children who were underweight had a BMI less than or equal to the fifth percentile (if ≥2 y of age) or weight-for-age less than or equal to the fifth percentile (if <2 y of age). Childhood underweight at birth was defined as having a birth weight <2500 g, and obesity at birth was defined as having a birth weight ≥4000 g. Childhood abdominal obesity was defined as having a waist-circumference measurement ≥90th percentile with the use of NHANES III definitions (23).

Maternal prenatal BMI (kg/m^2) was self-reported and based on CDC cutoffs for underweight or normal weight (<18.5 or 18.5 to <25, respectively), overweight (25 to <30), and obesity (≥30) [http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html (24)]. Maternal age and paternal age were analyzed continuously and also dichotomously with the use of advanced maternal and paternal age as ≥35 y. Maternal ethnicity was categorized as either Mexican or Central American origin on the basis of a self-report with the majority of women originating from Southern Mexico. Maternal self-reported smoking history and second-hand-smoke exposure were also collected prenatally, at delivery, and at the infant ages of 6 and 12 mo. Medical records were also reviewed for self-reported smoking.

Chronic childhood overweight, obesity, and abdominal obesity were defined as having multiple measures of overweight (>1 measure), obesity and abdominal obesity (at 4 and 5 y, 2–5 y, or from 6 mo to 5 y of age) to assess either the impact of chronic obesity during the preschool years (2–5 y) and chronic obesity from infancy through preschool (from 6 mo to 5 y of age).

From 4 to 6 wk of age, dietary recall information was collected by trained research assistants including the initiation of breastfeeding, breastfeeding duration, exclusive breastfeeding, and the age of the introduction of solids with the use of 24-h dietary recalls and food-frequency questionnaires. The consumption of sugar-sweetened beverages, including sodas and fruit drinks, 100% fruit juice (with no added sugars), flavored milks, and water were evaluated in relation to the following frequencies: any, ≥1 time/wk, ≥4 times/wk, and daily. Exclusive breastfeeding was defined as feeding only the infant breast milk with the exception of medicine and vitamins but with the exclusion of water and other non–breast-milk liquids with the use of the WHO's definition of exclusive breastfeeding (14). The introduction of other foods and liquids at 4–6 wk of age was defined as feeding the infant any other liquid or solids at 4–6 wk of age with the exclusion of breast milk and other replacement breast-milk substitutes (infant formulas).

Maternal mental health histories, including depressive symptoms, clinical depression, and child behavior, were collected as described in Wojcicki et al. (25). All procedures were approved by the Institutional Review Board and Committee on Human Research at the University of California, San Francisco, and mothers provided written consent to approve their participation and the participation of their children.

TL measurement

We quantified TL via a quantitative polymerase chain reaction with the use of genomic DNA from dried blood spots obtained from finger-prick specimens. Samples were taken from 108 four-year-old Latino children and their mothers, and 92 five-year-old children during years 4 and 5 of follow-up of our original cohort. Approximately 175 women and children were approached in years 4 and 5 to provide blood spot specimens from children. We collected 108 samples at 4 y of age and 92 samples at 5 y of age as well as from 3 children between 4 and 5 y of age, with 77 children having measures at both 4 or 4.5 and 5 y of age for 121 individual children. Two children had 3 telomere measurements between 4 and 5 y of age. The flowchart for enrollment is shown in **Figure 1**.

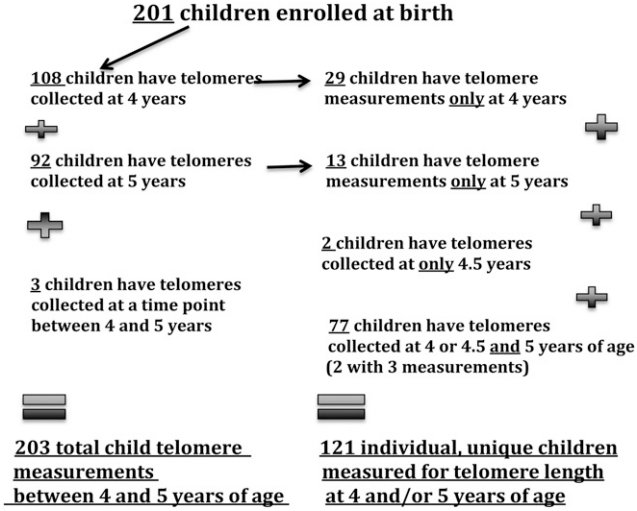

FIGURE 1 Enrollment and telomere-length collection flowchart.

We previously published the within-individual changes in TL in children from 4 to 5 y of age (26).

TL is expressed as the *T*:*S* (i.e., the ratio of a telomeric product to a single-copy gene product). DNA was extracted with the use of the QIAamp DNA Investigator Kit (catalog number 56504; QIAGEN). The TL-measurement assay was adapted from the published original method by Cawthon (27). A detailed description of the method has been presented by Lin et al. (28). The average CV for this study was 4.8% (26).

To convert *T*:*S* ratios to base pairs, the described method was used to determine the *T*:*S* ratios of a set of genomic DNA samples from the human fibroblast primary cell line IMR-90 at a different population doubling as well as with the telomerase protein subunit gene human telomerease reverse transcriptase (*hTERT*) infected on a lentiviral construct. This set of DNA samples represents different *T*:*S* ratios from the same parental cell line. The mean length of a telomeric restriction fragment from these DNA samples was determined with the use of a Southern blot analysis and was compared with the *T*:*S* ratios for these samples to convert *T*:*S* ratios to base pairs according to this formula: base pairs = 3274 + 2413 × (*T*:*S*). All telomere measurements are expressed as bp.

Statistical analysis

Preliminary exploratory analyses were based on graphical comparisons of distributions of TLs between groups that were defined by key categorical predictor variables with the use of density, box plots, and scatter plots to examine marginal relations between TL and continuous predictors.

We used linear regression models to investigate the association of TL with childhood exposures including child obesity and abdominal obesity at different time points in childhood, breastfeeding duration and exclusivity, the timing of the introduction of solids and dietary intake, and sociodemographic factors such as child sex, ethnicity, and socioeconomic status. Because repeated observations of TLs (at both 4 and 5 y of age) were available for a subset of infants, inferences were based on robust SEs to account for the within-individual correlation in outcome measures.

Initial regression analyses focused on assessing marginal associations between selected variables and TL. Variables with associations that were significant at $P \leq 0.01$ and those that were identified as being plausibly related on the basis of previous research findings or for biological reasons were considered in multiple-predictor regression models. Examples of the latter included the reported consumption of soda ≥4 times/wk at age 4 y, breastfeeding, and obesity at 6 mo of age. Because of the role of possible confounding by child age at collection in relation to TL and breastfeeding and other feeding practices, we included child age in all models (29). We also evaluated the evidence for interactions between demographic, weight, and dietary variables and child age in multiple linear regression predictor models.

Last, we evaluated dietary intake in relation to the TL change from 4 to 5 y of age in children who had repeat measurements ($n = 77$) with the use of Student's *t* tests for dichotomous predictors. In addition, we evaluated dietary variables with the use of multiple linear regression predictor models that were adjusted for child baseline TL and sex.

Diagnostic checks of results revealed some evidence for departures from normality of TL measurements, and thus, we conducted parallel analyses with the use of bootstrapped SEs as a sensitivity analysis to assess possible impacts of key findings. We used a lower threshold of inclusion in multivariable models ($P \leq 0.01$) because of the large number of predictors that were tested for significance. Exclusive breastfeeding and the consumption of other non–breast-milk foods and drinks were not included together in models because of collinearity. We ran a separate multivariable model that compared results without paternal age at the child's birth because ∼18% of observations were missing compared with the results that included paternal age. Other predictor variables in the model had <5% of observations missing. We conducted additional regression analyses to investigate the role of early obesity and soda consumption as possible mediators of the effects of exclusive breastfeeding and TL at 4 and 5 y of age. These analyses were based on methods for a causal mediation analysis (30) and are summarized as the estimated percentage of the effect of breastfeeding as explained by the mediating variables. Analyses were conducted with the use of Stata software (version 13; StataCorp LP).

RESULTS

Mean ± SE TL in all children was 7622.0 ± 80.3 bp at age 4 y and 8025.4 ± 80.5 bp at age 5 y (**Table 1**). Mean TL in girls was somewhat higher than in boys (7913.8 ± 93.0 compared with 7669.8 ± 92.1 bp, respectively; $P = 0.07$) (Table 1). Children of fathers who were older (≥35 y of age) also had longer mean TLs than children of younger fathers (8129.8 ± 173.8 compared with 7683.7 ± 74.3 bp, respectively; $P = 0.02$). Paternal age analyzed continuously was also positively associated with child TL ($P < 0.01$). We did not find any differences in mean TL on the basis of ethnicity (Mexican compared with Central American), maternal prenatal BMI category, or maternal age represented as either a continuous or categorical variable (Table 1). We did not evaluate the relation between maternal smoking in the perinatal period or during the first year of life because of the low number of mothers who reported any smoking ($n = 2$ during the perinatal period, and $n = 4$ at 6 mo of age).

Child-weight variables

Children who were underweight at 6 mo, 3 y, and 4 y of age had lower mean TL than children did who were not underweight [7388.2 ± 223.2 compared with 7830.6 ± 70.8 bp ($P = 0.06$); 7466.7 ± 19.8 compared with 7811.9 ± 67.6 bp ($P < 0.01$); and 7805.3 ± 67.6 compared with 8154.1 ± 47.1 bp ($P < 0.01$); respectively] although the number of children underweight at each time point was small ($n = 4$, $n = 2$, and $n = 2$, respectively) (results not shown). Obesity at birth was associated with shorter TL, as was obesity at 6 mo of age compared with children who were not obese [7523.0 ± 130.8 compared with 7829.8 ± 71.8 bp ($P = 0.04$) at birth and 7507.5 ± 169.4 compared with 7878.6 ± 75.1 bp ($P = 0.048$) at 6 mo of age] (**Table 2**), although obesity at later time points were not associated with shorter TLs. When we modeled the possible interaction between obesity and child age in relation to TL, we did show an interaction with age ($P < 0.05$; interaction coefficient: −41.88; 95% CI: −82.58, 1.19;

TABLE 1
Sociodemographic, maternal, and paternal characteristics and telomere length at 4 and 5 y of age (n = 121 children; n = 79 with repeated measurements)[1]

Variable	Telomere length,[2] bp	n/total n (%)	P
4-y visit (age:[3] 53.3 ± 5.8 mo) (n = 108 children)	7622.0 ± 80.3		—
5-y visit (age:[3] 65.7 ± 5.9 mo) (n = 92 children; n = 2 children with 2 measurements)	8025.4 ± 80.5		—
Child sex			
M	7669.8 ± 92.1	95/203 (46.8)	
F	7913.8 ± 93.0	108/203 (53.2)	0.07
Mexican ethnicity			
Yes	7886.5 ± 71.4	119/203 (58.6)	
No (Central American)	7675.7 ± 123.4	84/203 (41.4)	0.14
Parents married or living with partner at time of birth			
Yes	7833.1 ± 68.3	168/201 (83.6)	0.42
No	7785.9 ± 68.5	33/201 (16.4)	
Maternal BMI category (prenatal), kg/m^2			
Underweight or normal weight (<18.5 or 18.5 to <25, respectively)	7842.2 ± 109.4	90/193 (46.6)	0.52
Overweight (25 to <30)	7827.9 ± 131.9	62/193 (32.1)	
Obese (≥30)	7678.8 ± 107.0	41/193 (21.2)	
Maternal age at child's birth, y			
<35	7785.9 ± 68.5	186/203 (91.6)	0.55
≥35	7946.1 ± 257.4	17/203 (8.4)	
Paternal age at child's birth, y			
<35	7683.7 ± 74.3	127/168 (75.6)	0.02
≥35	8129.8 ± 173.8	41/168 (24.4)	

[1] P values reflect a linear regression comparison of mean telomere-length values for each group listed with the chosen reference category for that variable (the reference category is the first category listed under each variable). All comparisons were adjusted for repeated telomere-length measurements within individuals and for child age at collection.
[2] All values are means ± SEs.
[3] Mean ± SD.

results not shown), which suggested that early obesity is associated with shorter child TL, whereas later obesity is not. Abdominal obesity and obesity at other time points were not associated with shorter TL (Table 2). Chronic obesity from 6 mo to 5 y of age was associated with shorter TLs at 4 and 5 y of age (7099.2 ± 302.8 compared with 7830.5 ± 67.2 bp; P = 0.02), whereas obesity from 2 to 5 y of age was not associated with shorter TL nor was obesity at the later time points (4 and 5 y of age) (results not shown). The number of chronically obese children between 6 mo and 5 y of age was small [6 of 200 chronic obesity measurements (3.0%); or 3 of 121 individual children studied (2.4%)].

Breastfeeding

Exclusive breastfeeding at 4–6 wk of age was associated with longer TLs at 4 and 5 y of age compared with nonexclusive breastfeeding (7996.3 ± 113.4 compared with 7678.0 ± 76.3 bp respectively; P = 0.02) (**Table 3**), as was not feeding children other foods or drinks compared with early food and non–breast milk or replacement milk consumption at 4–6 wk of age by ~350 bp (7897.9 ± 82.2 compared with 7538.4 ± 91.3 bp respectively; $P < 0.01$). Any breastfeeding at 4–6 wk of age tended to be associated with longer telomeres at 4 and 5 y of age (P = 0.07) (Table 3). At 6 mo of age, any breastfeeding compared with no breastfeeding was also associated with longer telomeres in the preschool years (7898.9 ± 89.5 compared with 7635.4 ± 96.4 bp; P = 0.048). TLs did not differ at 4 and 5 y of age in relation to breastfeeding at 12 mo of age (Table 3; P = 0.95).

Dietary intake

TLs did not differ on the basis of the timing of the introduction of solid foods, intake of fruit juice, or early consumption of soda at 6 or 12 mo of age (results not shown). Consumption patterns of beverages at 2, 3, and 4 y of age were not associated with shorter TLs at 4 and 5 y of age with the exception that daily high fruit juice consumption at 3 y of age was associated with shorter TLs at 4 and 5 y of age than were lower daily fruit consumption (7667.2 ± 81.6 compared with 7931.4 ± 102.6 bp respectively; P = 0.047) and soda consumption at 4 y of age [≥4 compared with <4 times/wk (7442.7 ± 149.0 compared with 7852.1 ± 71.3 bp; P = 0.015)] (**Table 4**). Soda consumption at 5 y of age (≥4 times/wk) and soda consumption at 2 y of age (>1 time/wk) trended towards significance with shorter TLs at 4 and 5 y of age compared with less soda consumption (P = 0.06 and P = 0.07, respectively) (Table 4). Daily fruit-juice intake from 3 y of age similarly trended toward increased risk of shorter TLs at 4 and 5 y of age compared with lower fruit juice intake (7543.8 ± 144.8 compared with 7844.6 ± 72.3 bp, respectively; P = 0.07; data not shown). Water consumption was not associated with shorter or longer TLs in children at any time point, although almost all children drank water daily in all years (results not shown).

The marginal distribution of TLs in relation to exclusive-breastfeeding status at 4–6 wk of age and the consumption of non–breast milk and other milk liquids and solids are plotted in **Figures 2** and **3**, respectively. These kernel-density plots indicate the marginal distribution of TLs defined by exclusive-breastfeeding

TABLE 2
Child obesity and abdominal obesity in relation to telomere length at 4 and 5 y of age (n = 121; n = 77 with repeated measurements)[1]

Time point (age of child)	Obese (BMI ≥95th percentile)			Abdominal obesity >90th percentile		
	Telomere length, bp	*n*/total *n* (%)	*P*	Telomere length, bp	*n*/total *n* (%)	*P*
Birth						—
Yes	7523.0 ± 130.8[2]	20/203 (9.9)	0.04	—	—	
No	7829.8 ± 71.8	183/203 (90.2)		—	—	
6 mo						—
Yes	7507.5 ± 169.4	29/191 (15.2)	0.048	—	—	
No	7878.9 ± 75.1	162/191 (84.8)		—	—	
12 mo						—
Yes	7685.0 ± 147.9	25/192 (13.0)	0.36	—	—	
No	7837.9 ± 76.0	167/192 (87.0)		—	—	
2 y						—
Yes	7846.3 ± 169.5	49/195 (25.1)	0.81	—	—	
No	7802.0 ± 72.2	146/195 (74.9)		—	—	
3 y						
Yes	7850.6 ± 107.8	73/200 (36.5)	0.63	7601.6 ± 149.1	25/203 (12.3)	0.18
No	7783.7 ± 85.1	127/200 (63.5)		7827.6 ± 72.4	178/203 (87.8)	
4 y						
Yes	7766.4 ± 104.9	63/200 (31.5)	0.65	7653.2 ± 123.6	33/196 (16.8)	0.19
No	7828.5 ± 85.1	137/200 (68.5)		7843.7 ± 77.8	163/196 (83.2)	
5 y						—
Yes	7709.2 ± 76.6	82/200 (41.0)	0.17	—	—	
No	7882.1 ± 99.0	118/200 (59.0)		—	—	

[1] *P* values reflect a linear regression comparison of mean telomere-length values for each group listed with the chosen reference category for that variable (the reference category is the first category listed under each variable). All comparisons were adjusted for repeated telomere-length measurements within individuals and for child age at collection.
[2] Mean ± SE (all such values).

status and the consumption of non–breast milk and other milk liquids and solids at 4–6 wk of age.

Dietary intake and telomere attrition from 4 to 5 y of age

We did not show any differences between changes in TL from 4 to 5 y of age (n = 77) on the basis of early life feeding practices including breastfeeding at 4–6 wk of age (P = 0.65), the consumption of other non–breast milk or replacement milk foods and drinks at 4–6 wk (P = 0.81), exclusive breastfeeding at 4–6 wk of age (P = 0.31), any breastfeeding at 6 mo of age (P = 0.89), or exclusive breastfeeding at 6 mo of age (P = 0.64) (results not shown). Beverage intake, including 100% fruit juices and soda, was also not associated with the rate of telomere attrition from 4 to 5 y of age with the exception of high soda consumption at 3 y of age, which trended toward significance (P = 0.08). Compared with children who drank soda <4 times/wk at 3 y of age, children who drank soda ≥4 times/wk were more likely to have an accelerated loss from 4 to 5 y of age (adjusted coefficient: −515.14; 95% CI: −986.06, −41.22; P = 0.03) (results not shown).

Multivariable regression and mediation

In a multivariable regression model, we entered the child's age at collection in years, the father's age at the child's birth in years, child sex, maternal TL, and exclusive breastfeeding at 4–6 wk of age. Because of the collinearity between exclusive breastfeeding and the consumption of other foods and drinks, we ran separate models (one model with exclusive breastfeeding and one model with the consumption of other foods and drinks).

Independent predictors for longer TLs at 4 and 5 y of age included older paternal age (adjusted coefficient: 33.27; 95% CI: 4.10, 62.44; P = 0.03), having a mother with longer TLs (adjusted coefficient: 0.32; 95% CI: 0.11, 0.54; $P < 0.01$), and exclusive breastfeeding at 4–6 wk of age (adjusted coefficient: 353.85; 95% CI: 72.81, 634.89; P = 0.01) (**Table 5**). These findings suggest that children who were exclusively breastfed at 4–6 wk of age had longer telomeres by 353.85 bp than did children who were not exclusively breastfed. In addition, for each additional maternal base pair in TL, there was a 0.32 increase in child TL, and each additional year of the father's age corresponded to an additional 33.27 bp in child TL. We ran a similar multivariable regression model to the one displayed in Table 5, whereby we replaced exclusive breastfeeding at 4–6 wk of age with the consumption of other nonmilk drinks and foods at 4–6 wk of age. The consumption of other foods and drinks at 4–6 wk of age was similarly associated with reduced TL after controlling for other predictors (adjusted coefficient: −457.01; 95% CI: −720.50, −193.51; $P < 0.01$). We did not show any differences in coefficients or P values with the use of bootstrapped SEs compared with robust SEs in the multivariable analysis.

When we removed paternal age from the multivariable model and had a greater number of observations (n = 192), our independent predictors (exclusive breastfeeding at 4–6 wk of age, other food and liquid consumption at 4–6 wk of age, and adult TL) were similar although the effect size was slightly attenuated. Mediation analyses revealed that an estimated 15% of the effect of early exclusive breastfeeding on TL at 4–6 wk of age was mediated through early obesity at 6 mo of age. Similarly, soda

TABLE 3
Breastfeeding and early feeding in relation to telomere length at 4 and 5 y of age (n = 121 children; n = 77 children with repeated measurements)[1]

Variable	Telomere length,[2] bp	*n*/total *n* (%)	*P*
Any breastfeeding at 4–6 wk of age			
Yes	7836.4 ± 71.2	180/203 (88.7)	0.07
No	7513.6 ± 158.7	23/203 (11.3)	
Exclusive breastfeeding at 4–6 wk of age			
Yes	7996.3 ± 113.4	78/203 (38.4)	0.02
No	7678.0 ± 76.3	125/203 (61.6)	
Other food/non–breast milk or replacement milk drinks at 4–6 wk of age			
Yes	7538.4 ± 91.3	51/198 (25.8)	<0.01
No	7897.9 ± 82.2	147/198 (74.2)	
Any breastfeeding at 6 mo of age			
Yes	7898.9 ± 89.5	130/195 (66.7)	0.048
No	7635.4 ± 96.4	65/195 (33.3)	
Exclusive breastfeeding at 6 mo of age			
Yes	8001.2 ± 130.2	59/182 (32.4)	0.11
No	7744.8 ± 86.9	123/182 (67.6)	
Any breast milk at 12 mo of age			
Yes	7811.3 ± 103.5	74/191 (38.7)	0.95
No	7829.82 ± 93.60	117/191 (61.3)	
Timing of the introduction of solids, mo of age			
<4	7867.4 ± 148.4	59/189 (31.2)	0.51
4–6	7831.6 ± 83.8	102/189 (54.0)	
>6	7618.2 ± 179.1	28/189 (14.8)	

[1] *P* values reflect a linear regression comparison of mean telomere-length values for each group listed with the chosen reference category for that variable (the reference category is the first category listed under each variable). All comparisons were adjusted for repeated telomere-length measurements within individuals and for child age at collection.
[2] All values are means ± SEs.

consumption at 4 y of age (≥4 times/wk) accounted for 13% of the effect. Although the accuracy of these estimates depended on controlling for other important confounders, the results suggest a possible role of these variables as mediating influences. Women who breastfeed are less likely to have children who are obese at 6 mo of age and possibly less likely to have children who consume excessive amounts of soda in preschool. We did not find any role for obesity at 6 mo of age or for soda consumption at 4 y of age in mediating the relation between other food and drink consumption at 4–6 wk of age and TL.

We also evaluated the relation between breastfeeding at 4–6 wk of age and maternal depressive symptoms during the postpartum and preschool time periods as well as child behavioral issues during preschool on the basis of our previous findings (24). Although mothers with depressive symptoms and clinical depression were less likely to breastfeed in the postpartum period, there was no mediation or confounding by maternal depression in the relation between breastfeeding at 4–6 wk of age and TL at 4 and 5 y of age.

DISCUSSION

In this cohort study, we examined prospective detailed measurements of feeding behaviors over time in relation to TL at 4 and 5 y of age to detect whether effects exist in the preschool years and, if so, if there appear to be critical periods. To our knowledge, ours is the first study to find an association in preschool children (4 and 5 y of age) between longer TL and early dietary patterns including exclusive breastfeeding at 4–6 wk of age. We also showed a deleterious impact of introducing other foods and nonmilk liquids to infants at 4–6 wk of age on TL when the children were 4 and 5 y old. Children who were exclusively breastfed and mothers who did not introduce other liquids or solids at 4–6 wk of age had TLs at 4 and 5 y of age that were ∼ 350 and 450 bp longer, respectively, than those of children who were mixed fed, were fed replacement milk, and had solids or nonmilk liquids introduced early. A unique aspect of our cohort is that it was relatively homogenous in terms of possible confounders associated with breastfeeding including socioeconomic status and cultural background. The cohort was almost exclusively of low socioeconomic status, Spanish speaking, and from Southern Mexico and Central America.

These findings, although dramatic, were not surprising because of the strong body of science on the biochemical effects of breastfeeding and anti-inflammatory and immune-system protective effects (31–33), which extend past the weaning period (34).

Exposures to oxidative stress and inflammation are associated with accelerated telomere shortening in adults (4, 35), and the protective effect of breastfeeding against inflammation may explain the association between exclusive breastfeeding at 4–6 wk of age and longer TL in the preschool years.

We showed that the early introduction of nonmilk liquids and solids is associated with shorter TL at 4 and 5 y of age. Early exposure to certain foods may trigger an abnormal immune response to food antigens because the infant gut may be too immature before 4–6 mo of age (36–38). The heightened immune

TABLE 4
Toddler and preschool beverage consumption intake (≥1 time/wk; ≥4 times/wk or daily) in relation to telomere length at 4 and 5 y of age (n = 121; n = 77 with repeated measurements)[1]

	≥1 time/wk			≥4 times/wk or daily		
Variable	Telomere length, bp	*n*/total *n* (%)	*P*	Telomere length, bp	*n*/total *n* (%)	*P*
2 y of age						
Soda consumption						
Yes	7671.6 ± 79.2[2]	89/194 (45.9)	0.07	7987.9 ± 304.2	9/194 (4.6)	0.54
No	7921.2 ± 107.1	105/194 (54.1)		7798.6 ± 70.2	185/194 (95.4)	
High 100% fruit juice consumption (daily)			—			
Yes	—	—		7717.1 ± 84.5	104/194 (53.6)	0.16
No	—	—		7915.2 ± 110.7	90/194 (43.4)	
3 y of age	—	—				
Soda consumption						
Yes	7709.1 ± 83.8	89/199 (44.7)	0.19	7544..9 ± 172.1	22/199 (11.1)	0.13
No	7879.6 ± 99.0	110/199 (55.3)		7835.6 ± 72.1	177/199 (88.9)	
High 100% fruit juice consumption (daily)			—			
Yes	—	—		7667.2 ± 81.6	96/199 (48.2)	0.047
No	—	—		7931.4 ± 102.6	103/199 (51.8)	
4 y of age						
Soda consumption						
Yes	7701.8 ± 79.8	112/200 (56.0)	0.08	7442.7 ± 149.0	21/200 (10.5)	0.015
No	7945.6 ± 110.7	88/200 (44.0)		7852.1 ± 71.3	179/200 (89.5)	
High 100% fruit juice consumption (daily)			—			
Yes	—	—		7732.2 ± 116.4	63/199 (31.7)	0.37
No	—	—		7859.4 ± 80.7	136/199 (68.3)	
5 y of age						
Soda consumption						
Yes	7790.3 ± 86.6	137/199 (68.8)	0.60	7546.1 ± 159.8	41/199 (20.6)	0.06
No	7858.6 ± 97.0	62/199 (31.2)		7880.4 ± 71.9	158/199 (79.4)	
High 100% fruit juice consumption (daily)			—			
Yes	—	—		7673.8 ± 121.3	60/199 (30.2)	0.18
No	—	—		7870.5 ± 79.3	139/199 (69.9)	

[1] *P* values reflect a linear regression comparison of mean telomere-length values for each group listed with the chosen reference category for that variable (the reference category is the first category listed under each variable). All comparisons were adjusted for repeated telomere-length measurements within individuals and for child age at collection.

[2] Mean ± SE (all such values).

response that is associated with early exposures to solid foods may also result in accelerated telomere aging because TL is connected with processes of premature immunosenescence (39).

In the current article, we report that early life obesity (from 6 mo of age) may also affect childhood TL possibly through the mediation of the relation between breastfeeding and shorter TL because breastfed babies are less likely to be obese. We also showed an independent association between shorter TL and early obesity (at 6 mo of age) in the adjusted models with the removal of paternal age to have a larger sample size. Chronic obesity (from 6 mo to 5 y of age) was also associated with shortened telomeres at 4 and 5 y of age in bivariate analyses. Children who are exposed to a more chronic form of obesity rather than episodic obesity are simultaneously exposed to inflammatory processes for more sustained periods and at more severe levels, and the biochemical environment of higher inflammation and insulin is known to be detrimental to telomeres. However, our numbers of children who had exposure to chronic obesity were small, and thus, these results were not tested for independence in a multivariable analysis, and future studies with larger sample sizes are necessary.

The relation between longer preschool TL and early exclusive breastfeeding may also be explained by the association between early exclusive breastfeeding and later dietary habits (40). We showed that high soda consumption at 4 y of age mediated the relation between exclusive breastfeeding and TL, whereas soda consumption has also been independently associated with shorter TL in adults (15).

FIGURE 2 Distribution of telomere length by exclusive breastfeeding status at 4–6 wk of age. bf, breastfeeding; kdensity, kernel density.

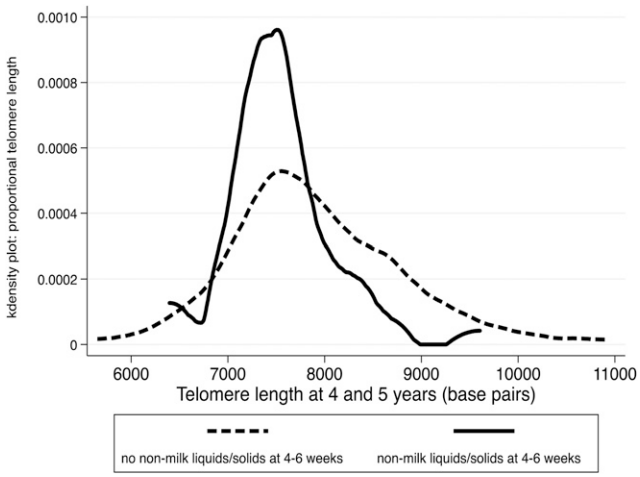

FIGURE 3 Distribution of telomere length by use of non–breast milk or replacement liquids and solids at 4–6 wk of age. kdensity, kernel density.

When we evaluated risk factors for accelerated TL attrition in our cohort, we showed that high intakes of soda were associated with accelerated attrition in children even after controlling for baseline TL, thereby confirming similar findings in adults (15). The mechanisms for accelerated attrition are not clear although we did not show any associations between early life obesity and TL attrition in this cohort (26). It is possible that high soda consumption may increase oxidative stress, inflammation, and subsequently risk of insulin resistance, all of which are processes that also damage TL independent of obesity levels. Meanwhile, larger, more detailed dietary studies are needed to confirm our findings.

Last, our results in Latino children confirmed the results of previous studies on the heritability of TL (41) because we showed a strong correlation between maternal TL and that of children although we could not rule out the commonality of environmental factors. We also showed that paternal age was positively associated with TL as have other studies (42). It has been speculated that TL in sperm may be longer in older men because of a higher amount of exposure to telomerase activity (43).

Limitations of our study include the smaller sample size and the fact that the study was conducted in Latino children only. Therefore, these results need to be replicated in other populations. Also, we had only 2 measures of TL, both of which occurred during preschool years, and a better study design would have had more successive measures of TL, specifically at birth and at 1 y of age, to see the immediate effects of breastfeeding on TL attrition in the first year of life as well as the role of breastfeeding on the TL attrition rate from birth through the preschool years. Future studies should include larger sample sizes, multiethnic populations, and early measurements in childhood. However, as previously discussed, although our sample size was small, and we restricted the cohort to one ethnic group, we were able to minimize the number of possible known and unknown confounders associated with dietary intake by having a relatively homogenous group.

In addition, although our population was, in general, a relatively healthy group of Latino children, it is not clear if the benefits of breastfeeding would be sustained in a subpopulation of children with chronic disease (e.g., asthma or diabetes mellitus) or a sustained exposure to environmental insults. Although we did not see any diminished effect of breastfeeding in regards to the exposure to maternal depressive symptoms or clinical depression, we did not evaluate more severe exposures such as an exposure to trauma or child abuse. Additional studies should evaluate the long-term benefits of breastfeeding in different subpopulations of children.

In conclusion, this study suggests that early breastfeeding in the first month of life, without introducing other foods, may play a critical role in promoting healthy telomere biology, and this effect can be seen 4–5 y later. Other cofactors that could also affect inflammation and reactive oxygen species damage such as maternal smoking were not significant factors in our cohort, and other exposures such as maternal clinical depression or depressive symptoms did not mediate or confound the relation between early breastfeeding and later childhood TL. However, in other populations that have a more extensive exposure to smoking or in which children have other trauma or stress exposures, there may be fewer sustained benefits from breastfeeding; these variables need to be systematically assessed in follow-up studies with repeated TL measurements. In addition, we did not evaluate genetic factors, which could have affected TL and attrition patterns that might have accounted for the differences in our population of children. Also, early obesity, by 6 mo of life, also appears to have an imprint on childhood TL. Together, these novel findings point to the critical role of the early intervention and education of women of childbearing age about nutrition and breastfeeding and infant obesity. It is possible that an understanding of how the pregnancy health of women and feeding practices can affect the telomeres of their babies may offer a new insight and motivation for improved health in the critical perinatal and early years of their children's lives.

The authors' responsibilities were as follows—JMW, MBH, JL, EB, and EE: interpreted the results, wrote the manuscript, and approved the final manuscript; JMW and EE: conceived the manuscript; JMW and DE: collected the data for the manuscript; and JMW and DE: did the analyses for the manuscript. None of the authors reported a conflict of interest related to the study.

TABLE 5
Multivariable linear regression for independent predictors of telomere length at 4–5 y of age (n = 160 total observations; n = 96 individual children)

Demographic	β coefficient (95% CI)	P
Child age at telomere-length collection, y	0.54 (−12.04, 13.12)	0.93
Fathers age at birth, y	33.27 (4.10, 62.44)	0.03
Child sex (M)	−112.36 (−372.79, 148.06)	0.39
Maternal telomere length	0.32 (0.11, 0.54)	<0.01
Exclusive breastfeeding at 4–6 wk of age	353.85 (72.81, 634.89)	0.01

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