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Obesity at Age Six Months Is Associated with Shorter Preschool Leukocyte Telomere Length Independent of Parental Telomere Length

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Abstract

Objective: To assess whether early modifiable dietary factors and obesity measures are associated with leukocyte telomere length at 3–5 years after controlling for heritability of telomere length in a prospective cohort of low-income Latina mothers and their children in San Francisco.

Study design: We analyzed data from the Latinx, Eating and Diabetes (LEAD) cohort, a prospective study of 97 woman-infant dyads. We used linear regression models to evaluate associations between early dietary factors and obesity measures and child leukocyte telomere length at 3–5 years. Multivariable models included child age at the time of telomere collection, breastfeeding at 6 months (yes/no), obesity at 6 months, maternal education, child sex and maternal and paternal leukocyte telomere length.

Results: Data for 73 of the 97 children at 3–5 years of age were analyzed. Any breastfeeding at 6 months was positively associated ($\beta = 0.14$, $P = .02$) and obesity at 6 months was negatively associated ($\beta = -0.21$, $p<0.001$) with leukocyte telomere length in bivariate analyses. In multivariable models including parental leukocyte telomere length, obesity at 6 months was associated with shorter leukocyte telomere length at 3–5 years of age ($\beta = -0.15$, $p=0.02$). Analyses of dietary factors showed high flavored milk consumption at 3 years of age was associated with shorter leukocyte telomere length after adjustment for possible confounders.

Conclusions: In a low-income Latinx population, obesity at 6 months is negatively associated with cellular health at 3–5 years of age after controlling for genetic factors (parent leukocyte

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telomere length) associated with leukocyte telomere length. Early life obesity may be more deleterious for cellular health than obesity later in childhood.

Keywords

telomere length; childhood obesity; breastfeeding; sugar-sweetened beverages

Telomeres are the terminal pieces of chromosomes that serve to protect DNA from degenerating and fusing together. Telomeres are composed of a variable number of a repeating nucleotide sequence (TTAGGG) and must maintain a minimal length to maintain cellular stability. Telomeres shorten with cellular division as a result of the “end replication problem,” but are also sensitive to oxidative damage.(1) Once telomeres become too short, internal signaling forces the cell into a state of arrest (senescence) or cellular death (apoptosis).(2) Senescent cells produce inflammatory mediators that impact other cells in their vicinity, and an accumulation of senescent cells results in overall decreased tissue function, leading to the many manifestations of biologic aging.(3) Leukocyte telomere length is a cumulative marker of cellular health.

Although the majority of research to date has focused on leukocyte telomere length attrition in adults, research has highlighted the importance of leukocyte telomere length early in life,(4–6) demonstrating that telomeres undergo the greatest attrition during the first few years.(7–9) There is a degree of telomere heritability(10, 11) and likely telomere developmental programming that takes place in the intrauterine period,(12, 13) but early life modifiable factors that influence leukocyte telomere length are most attractive as potential intervention points. Estimates of leukocyte telomere length heritability range from 36–80%,(14) with some research showing a more dominant paternal influence(14) and others a more dominant maternal influence.(5, 11)

Obesity is associated with shorter leukocyte telomere length through proposed inflammatory mechanisms.(15, 16) However, as obesity has a significant genetic component, it remains unclear the extent to which obesity and shorter leukocyte telomere length risk is explained by genetic versus environmental (including dietary) factors. The association between obesity and shorter leukocyte telomere length in younger children has been mixed, with some studies reporting an association,(17, 18) and others finding no link.(19, 20) Previous studies have suggested that leukocyte telomere length attrition may be influenced by fat mass and obesity associated gene (FTO) pathways.(21) Studies of newborns have found that maternal pre-pregnancy body mass index (BMI) impacts newborn telomere length independent of birthweight.(22) Genetic studies of leukocyte telomere length have found heritability associations decline with a child’s age.(23) Thus for young children, in contrast with adults and adolescents, heritability of leukocyte telomere length and obesity may play a more central role than environmental factors.

In a longitudinal cohort of low-income Latina mothers and their infants, a group that is disproportionately affected by obesity and chronic disease, we studied the impact of environmental and genetic factors on child leukocyte telomere length between 3 and 5 years of age. Specifically we evaluated how leukocyte telomere length in preschool-aged children is affected by modifiable dietary factors such as shorter breastfeeding duration and sugar

sweetened beverage (SSB) intake,(24, 25) as well as obesity at different time points in early childhood, maternal pre-pregnancy BMI and parental leukocyte telomere length. Clarification of when obesity during childhood may be most damaging to leukocyte telomere length is important for the timing of intervention efforts to mitigate long term chronic health risks.

Methods:

Data were collected from the Latino, Eating and Diabetes (LEAD) cohort, a prospective study of low-income Latina pregnant women and their children described in detail elsewhere.(26) We assessed relationships between child and maternal factors and leukocyte telomere length at 3–5 years of age. All study participants were recruited at the prenatal clinics of Zuckerberg San Francisco General Hospital (ZSFG) by study staff prior to delivery during the second and third trimesters in 2012 and 2013. The primary objective of LEAD was to assess the relationship between perinatal depression and infant weight gain in Latinx children. Exclusion criteria for this cohort included mothers who anticipated having or had infants with special care needs or chronic disease, pregnant mothers who reported drug or alcohol abuse on a screening questionnaire, mothers with endocrine disorders such as polycystic ovarian syndrome, pre-existing type 1 or type 2 diabetes mellitus or insulin treated gestational diabetes, mothers with eating disorders, mothers with any contraindications to breastfeeding, mothers of babies who required additional care in the Intensive Care Nursery, and infants with Apgar scores <7 at 1 and 5 minutes. All mothers provided written consent for themselves and their children. Study subjects participated in an initial interview, with a follow-up interview at the time of delivery. Subsequent interviews were conducted by phone at 4–6 weeks postpartum and in person at 6 months, 12 months and then yearly until age 6. Cord blood samples were taken at the time of birth, venous samples at 24 months, and finger stick collection from 3–5 years of age for leukocyte telomere length analysis. The study was approved by the Committee on Human Research (CHR), the Institutional Review Board (IRB) of the University of California, San Francisco.

Variables

Our primary outcome of interest was leukocyte telomere length at 3–5 years of age. Leukocyte telomere length is often used as a proxy for overall telomere length; although telomere length does vary by tissue type, there is a strong correlation between tissue types and the rate of attrition is similar.(4) Leukocyte telomere length is reported as a T/S ratio, or the ratio of telomeric product to a single-copy gene product, as determined by quantitative PCR. The exact methodology used has been described.(7, 24, 27, 28) Blood spots were collected via finger stick and genomic DNA was extracted from six 3mm diameter cutouts from dried blood spot samples using QIAamp DNA Investigator Kits according to manufacturer's instructions. Although the samples were taken at different time points, they all were collected by the same procedures and analyzed in batch. The leukocyte telomere length assay was run immediately after the DNA extraction and there were no differences between samples in terms of timing of DNA extraction and leukocyte telomere length measurement. We also adjusted for length of storage of samples.(29) Mean storage for samples was 2.14 +/−0.91 years. We did not find any association between length of storage

and child leukocyte telomere length (p=0.75), nor was there an association when adjusted for child age at time of sample collection (p=0.59). There was also no association for mothers' (p=0.14) or for fathers' (p=0.12) leukocyte telomere length and length of storage and the effect estimates for these associations were in the opposite direction. As such we did not adjust for sample length of storage in multivariable models. To control for batch variance, the T/S ratio for each sample were measured twice. T/S values were then adjusted to account for systematic differences between the two runs. When the duplicate T/S values varied by >7% after adjustments, the sample was analyzed for a third or fourth time and the two closest T/S values were reported. Using this method, the average coefficient of variation for this study was 2.47%.

Our primary predictors of interest were early childhood obesity and breastfeeding status. Obesity was defined as weight for length z-score ≥95th percentile for age for children under 2 years, and BMI z-score ≥95th percentile for age for children 2 years and older using the Center of Disease Control growth curves.(30) Rapid infant weight gain was defined as >0.67 change in weight for age z-score from birth to 6 months of age.(31) Breastfeeding status was determined via parent self-report at the scheduled interview time points, and exclusive breastfeeding at 4–6 weeks and 6 months was defined, as per the World Health Organization, as only breast milk intake with no other liquids or solids given, with the exception of vitamins, minerals or medicines as medically recommended.(32)

Secondary predictors of interest were exploratory analyses of sugar intake and fast food consumption, as indicated via parent report through a food frequency questionnaire (FFQ) given at 3 years of age assessing weekly consumption patterns.

To assess SSB intake we combined soda (defined as colas/sodas, Kool-Aid, non-diet Hi-C, juices like Capri Sun, Sunny D, and Tampico), fruit juice (defined as 100% fruit juice - no added sugar), and flavored milk (defined as milk flavorings - chocolate, strawberry, etc.) consumption. Sweets/dessert intake included "cakes, brownies, muffins, donuts, cookies", "candy or chocolate", and ice cream consumption. We then combined SSB and sweets intake into one sugar intake category. Fast food consumption was also measured on the FFQ as "Fast food: Wendy's, McDonald's, Burger King." For all of the above, we analyzed the data from each category of interest and determined a cutoff point between high and low intake, with the top 15–32% deemed to be "high."

Other parental predictors of interest included maternal pre-pregnancy BMI collected via medical chart review and self-report and parental leukocyte telomere length collected at the same time as child leukocyte telomere length at 3–5 years of age, also via finger stick using the same procedures as described above.

Statistical Analyses:

STATA 15 was used to perform all statistical analyses. As our outcome of interest, leukocyte telomere length at ages 3–5 years, is a continuous variable, we first looked at its graphical distribution and found that there were departures from normality. We subsequently confirmed non-normality using the Shapiro-Wilk and Shapiro-Francia tests. Similarly, for our regression analyses, the residuals were not normally distributed; however, when we

increased the sample size using bootstrapping, the results did not alter conclusions from those assuming normality.(33) We assume these departures from normality are related to our small sample size and with increasing numbers would expect a normal distribution. As such, we proceeded using the untransformed data for multivariable regression. We ran linear regressions to assess the relationships between predictors and leukocyte telomere length at ages 3–5 years. To increase our power to detect differences, we used the cluster command in STATA with regressions, which allowed for multiple leukocyte telomere length data points for a given child to be used as separate observations. In our cohort, 11 children had one sample, 41 children had two samples, and 21 children had three samples between ages 3 and 5.

Variables included in the multivariable models were those that had a p value <0.1 in bivariate regressions, as well as other variables that had previously been associated with leukocyte telomere length in previous research studies (age, maternal education). Child leukocyte telomere length at birth was not included in the multivariable models due to its high correlation with the outcome of interest. We analyzed multivariable models with and without paternal leukocyte telomere length, given the small sample size for that variable (n=48). Although there are other plausible variables that may be associated with leukocyte telomere length at age 3–5, including annual household income and maternal smoking, we limited the number of variables in our model due to small sample size as well as small cell size for some of these predictors in our population (e.g. absence of a high prevalence of maternal smoking). Graphical distributions of obesity status at 6 months in relationship to leukocyte telomere length were plotted using kernel (K) density estimates, which show the density of observations similar in function to a histogram (Figure).(34)

Due to the potential relationship between breastfeeding and weight status, we included interaction terms in our multivariable models but did not find a statistically significant interaction between breastfeeding and obesity at 6 months so did not include any interaction terms in final models. We also assessed for potential mediation of breastfeeding at 12 months between obesity at 6 months and leukocyte telomere length at 3–5 years, but found that breastfeeding only mediated 3.1% of that relationship so we did not pursue further mediation analyses.

Finally, due to our interest in sugar consumption and previous associations between leukocyte telomere length and sugar, particularly the relationship between SSBs and shorter leukocyte telomere length,(35) we analyzed sugar-associated variables in multivariable models as a sensitivity analysis.

Results:

Of the 97 children in the cohort, 73 had at least one leukocyte telomere length recorded between 3 and 5 years. The mean leukocyte telomere length (T/S Ratio) for children ages 3–5 was 1.67 (standard deviation (SD) 0.28), and the mean leukocyte telomere length (T/S Ratio) for mothers was 1.325 (SD 0.27) and for fathers 1.327 (SD 0.18). In this subcohort of 73 mother-child pairs, 89% had a household annual income <$25,000 and 82% of mothers had a high school education level or less (Table I).

Mean birthweight was 3354 grams (SD 406). Fifteen percent of the children were obese at 6 months of age, 33% had rapid infant weight gain by 6 months of age, and 17% were obese at age 5 years (Table 2). Obesity at 6 months was predictive of obesity at both 3 years of age (p=0.006, data not shown) and 5 years of age (p=0.014, data not shown); 31% were exclusively breastfed at 4–6 weeks of age, and 71% were breastfed to some extent at 6 months of age (Table 2). By 3 years, parents reported little fast food consumption, but a significant amount of sugar intake, with 81% drinking any soda (data not shown) and nearly one in five children drinking soda 4 or more times a week (Table 2).

Predictors of Telomere Length at 3–5 Years

A genetic association of child leukocyte telomere length with maternal leukocyte telomere length was demonstrated, showing a slightly stronger association with child leukocyte telomere length at 3–5 years (β= 0.31, Confidence Interval (CI) 0.05, 0.56) than paternal leukocyte telomere length (β= 0.29, CI −0.01, 0.59). Leukocyte telomere length at birth (β= 0.62, CI 0.35, 0.90) was strongly associated with leukocyte telomere length at 3–5 years, and no significant association was seen with either child sex or age at time of collection (Table 1).

Children who were obese at 6 months had shorter leukocyte telomere length at 3–5 years compared with children who were normal weight (β= −0.21, CI −0.32, −0.10). There was no association between rapid infant weight gain in the first six months and child leukocyte telomere length at 3–5 years (β= 0.004, CI −0.12, 0.13). Continuous measures of weight status including weight for length z-score for those <2 years, and BMI z-score for those ≥2 years old did not show an association with shorter leukocyte telomere length. Obesity at 2, 3 and 5 years similarly did not show any association with leukocyte telomere length at 3–5 years. Any breastfeeding at 6 months of age was associated with longer leukocyte telomere length at 3–5 years (β= 0.14, CI 0.02, 0.27), and any breastfeeding at 12 months was not associated with leukocyte telomere length at 3–5 years (β= 0.08, CI −0.04, 0.20). Exclusive breastfeeding at 4–6 weeks of age was also not associated with longer leukocyte telomere length age 3–5 (β= 0.05, CI −0.07, 0.16) (Table 2).

Measures of sugar intake at 3 years were not associated with leukocyte telomere length at 3–5 years, though all beta (β) values for the linear regressions were negative, the direction we would expect. The combined categories of sugar intake, such as the high SSB intake group, which combined frequency of soda and soda-like drinks, juice, and flavored milk consumption (β= −0.07, CI −0.20, 0.06), were similar to associations seen in each individual consumption group (Table 2). The combination category that included both liquid and solid sources of sugar intake, or “high combined sugar intake”, showed a similar non-significant relationship to leukocyte telomere length at 3–5 years (β= −0.08, CI −0.22, 0.05). High fast food consumption was defined as consumption greater than once per week and did not show any association with leukocyte telomere length at 3–5 years (β= −0.06, CI −0.20, 0.08) (Table 2).

Multivariable regression

Independent predictors for shorter leukocyte telomere length at 3–5 years included obesity at 6 months (β= −0.18, CI −0.27, −0.08) and male sex (β= −0.12, CI −0.24, −0.01). Any breastfeeding at 6 months (β= 0.11, CI −0.01, 0.23) and longer maternal leukocyte telomere length (β= 0.26, CI −0.03, 0.56) approached statistical significance (p=0.08 and p=0.08, respectively) for association with longer leukocyte telomere length at 3–5 years (Table 3).

In multivariable models including paternal leukocyte telomere length at a smaller sample size than the previous model (n=48), obesity at 6 months (β= −0.15, CI −0.27, −0.02) was the only predictor for shorter leukocyte telomere length at 3–5 years. Similarly, any breastfeeding at 6 months (β= 0.13, CI −0.004, 0.26) and longer maternal leukocyte telomere length (β= 0.23, CI −0.008, 0.47) approached statistical significance (p=0.06 and p=0.06 respectively).

In our sensitivity analyses that included sugar-associated variables in multivariable models, the only variable that was significant was high flavored milk consumption (≥3x/week) (β = −0.14, CI −0.29, −0.004) adjusting for child age at the time of telomere collection, breastfeeding at 6 months, child sex, maternal education, maternal leukocyte telomere length, and obesity status at 6 months of age. Similar to the other models, breastfeeding at 6 months remained associated with longer leukocyte telomere length (p<0.01), whereas obesity at 6 months (p<0.01) and male sex (p=0.04) were associated with shorter leukocyte telomere length (results not shown).

Discussion:

In this longitudinal cohort study, infant obesity at 6 months was associated with shorter preschool leukocyte telomere length independent of both maternal and paternal leukocyte telomere length, but there was no association between preschool leukocyte telomere length and obesity at later timepoints in early childhood. Breastfeeding at 6 months may be associated with longer leukocyte telomere length and flavored milk consumption with shorter leukocyte telomere length in multivariable models.

In a similar Latinx birth cohort, the Hispanic Eating and Nutrition (HEN) cohort, we also found obesity at 6 months was associated with shorter leukocyte telomere length in the preschool years.(24) In that study and this, we did not find an association between leukocyte telomere length at ages 3–5 and obesity at age points beyond 6 months of age, from age 2 through age 5.

Obesity in adults and adolescents has repeatedly been found to be associated with shorter leukocyte telomere length.(15, 16, 18) Obesity as a systemic inflammatory state with higher levels of oxidative stress may impact infants and young children through a similar mechanism. Weight and obesity in this younger age group have distinct features from weight and obesity in adolescents and adults. Studies of leukocyte telomere length in this early period show a general trend toward greater birthweight being associated with longer leukocyte telomere length, and large for gestational age babies having longer leukocyte telomere length than appropriate for gestational age or small for gestational age babies.(36–

38) However, there are limits to this early growth and weight benefit, as rapid infant weight gain has been linked to metabolic concerns later in life(39). This study suggests that obesity as early as 6 months has a negative impact on later cellular health, although we did not find any association between rapid infant weight gain by 6 months of age and leukocyte telomere length at 3–5 years in our cohort. Although obesity at 6 months was associated with obesity at age 3 and age 5, our study suggests that earlier obesity may be more damaging to cellular health than later obesity. Leukocyte telomere length at 3–5 years was not associated with age at time of collection, which indicates that the majority of accelerated leukocyte telomere length attrition may be complete by this age(7) and further emphasizes the importance of this early time window for intervention.

Obesity is highly heritable, with estimates in some studies as high as 85%.(40–42) The FTO gene, which regulates fat mass and obesity risk, has been proposed to be involved with leukocyte telomere length, but other obesity susceptibility loci have been found in the genome and may play a role.(21) Du et al. examined genetic risk scores for BMI and associations with leukocyte telomere length and found no significant relationship after adjustment for age and case-control status, though this study was in adults and was underpowered to detect weak or modest associations.(43) Our study analyzed the relationship between obesity and leukocyte telomere length genetic heritability in a young patient population and suggests that obesity at 6 months is associated with later child leukocyte telomere length separate from parental genetic influence.

Maternal leukocyte telomere length was strongly associated with child leukocyte telomere length in 3–5 year olds in bivariate models, and neared statistical significance in multivariable models. Paternal leukocyte telomere length neared statistical significance in bivariate models, but given the small sample size it is possible that we were underpowered to assess any association with paternal leukocyte telomere length.

Maternal pre-pregnancy weight and diabetes status have been shown to be associated with neonatal leukocyte telomere length.(22, 44, 45) We found no studies showing such an association at later time points or any association between weight gain in pregnancy or maternal pre-pregnancy BMI and preschool leukocyte telomere length. Additional larger studies are needed to assess whether shared pathways explain a relationship of genetic determinants of leukocyte telomere length heritability and early life obesity, which appear independently associated with leukocyte telomere length in our study.

Our findings suggest that breastfeeding at 6 months of age may be associated with longer telomere length, similar to our previous findings in the HEN cohort.(24) This may be explained in part by the protective effect of breastfeeding against obesity, though interaction terms were not significant.

Compared with formula, breastmilk has beneficial immunologic properties through the direct transfer of maternal IgA antibodies and other bioactive substances.(46) Infants who are breastfed have lower risk of a host of infections, including diarrheal illness, respiratory tract infections, otitis media, bacteremia and necrotizing enterocolitis.(47) The presence of

antioxidants in breastmilk may be directly beneficial to telomere health, counteracting deleterious effects that reactive oxygen species have on telomere length.(48)

In our previous study with this Latinx cohort, breastfeeding at 6 months was not associated longer leukocyte telomere length at age 2–3 years,(25) but by 3–5 years, we did see an association similar to our finding with the HEN cohort.(24) The mechanisms for this apparent delayed impact are not clear, but might be explained by a type of cellular imprinting that takes place very early in life, when variables such as breastfeeding or obesity may differentially affect factors involved in establishing and maintaining leukocyte telomere length. Telomerase, the enzyme that can add back basepairs and lengthen telomeres, could be one of these mediators. Thus, the first 6 months of life may be a critical period where cellular vulnerability is created. Later insults, such as exposure to familial stress, environmental toxins or a poor diet, could then result in the differences in leukocyte telomere length that become discernable in the preschool years. Until a certain threshold of these insults build up, a difference in leukocyte telomere length may not become apparent.

We found no association between SSB consumption as a composite variable at age 3 and shorter leukocyte telomere length at 3–5 years, in contrast to our previous findings in this cohort of soda intake at 2 years and shorter leukocyte telomere length at 2–3 years.(25) Possibly, earlier exposure to SSB is more deleterious for telomere health and some of these exposures become less significant by the preschool years. Of note, our previous analysis assessed quantity of intake in ounces, whereas we had only frequency of intake at 3 years, which could account for differences. However, we did find that higher consumption of flavored milks at 3 years was associated with shorter leukocyte telomere length in multivariable models, which is in the setting of an increase in consumption from 12 months to 3 years (12.1% consumption at 12 months vs 32.7% at 3 years). It is not clear why only higher flavored milk intake, and not other forms of sugar intake, would be associated with shorter leukocyte telomere length at 3–5 years of age, as soda and 100% fruit juice consumption similarly increases over the same time period. Other confounders may include feeding practices that were not assessed, such as maternal depression, which in a similar cohort was associated with feeding behaviors associated with later obesity.(49) We investigated potential relationships between other forms of sugar intake, including cookies, sweets and ice cream, and leukocyte telomere length in this early age group. No association was found between any of the individual categories examined, nor for combined categories that aimed to capture a more cumulative measure of sugar intake. Future studies should use 48 hour recalls combined with food frequencies diaries to more precisely capture total sugar intake.

Limitations of this study include its small sample size and that we assessed leukocyte telomere length only in Latinx children. Some studies have reported longer leukocyte telomere length in Black newborns,(50) raising the possibility that unmeasured genetic factors are associated with leukocyte telomere length at birth or alternatively, in utero environmental factors need to be better characterized to understand leukocyte telomere length dynamics.(12) Other studies suggest that genetic loci associated with longer telomere length in adults may signal greater environmental susceptibility to telomere length attrition in early childhood versus determining newborn leukocyte telomere length.(51) Our study

showed an association between early obesity and greater leukocyte telomere length attrition in Latinx children, independent of parental leukocyte telomere length. However, it is possible that shared genetic pathways could impact leukocyte telomere length and risk for early obesity in Latinx populations. Latinxs are at a higher risk for non-alcoholic fatty liver disease (NAFLD) and other diseases associated with obesity via increased genetic risk and environmental factors.

Additional limitations include that all survey questions were self-reported, which introduced the potential for response bias, particularly related to the FFQ. Exposure to air pollution and second hand smoke are two additional factors that have been shown to be associated with shorter child leukocyte telomere length.(52–54) We did not assess environmental impacts of air pollution by neighborhood and our population had a very low level of smoking and exposure to second hand smoke (approximately 7%). As a result, we did not have sufficient power to assess these associations. We determined leukocyte telomere length via quantitative PCR, which is widely used in population-based studies such as our own, but has been shown to have larger measurement error than Southern Blot.(55)

Most public health interventions for obesity begin at earliest in the preschool years with few focused on the period of infancy. Our data suggests that a public health shift may be warranted to focus on earlier timepoints in childhood for obesity prevention, mirroring the data suggesting that telomeres undergo the greatest attrition during the first few years of life(7–9). Decreasing obesity at 6 months of age may have a positive impact on later cellular health and interventions that target reduced weight gain during this critical age period should be specifically supported. Encouraging and supporting breastfeeding is one potential intervention target, as breastfeeding may independently increase leukocyte telomere length and has been shown to be associated with a reduced risk for obesity.(56, 57) Additionally, as a means to prevent and reduce obesity risk in toddlers and preschool children, all sugar-sweetened beverages should be avoided including flavored milks.

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Abbreviations:

BMI	body mass index
SSB	Sugar sweetened beverages
CI	Confidence Interval
FFQ	Food frequency questionnaire
HEN	Hispanic Eating and Nutrition
LEAD	Latinx, Eating and Diabetes

SD	Standard deviation

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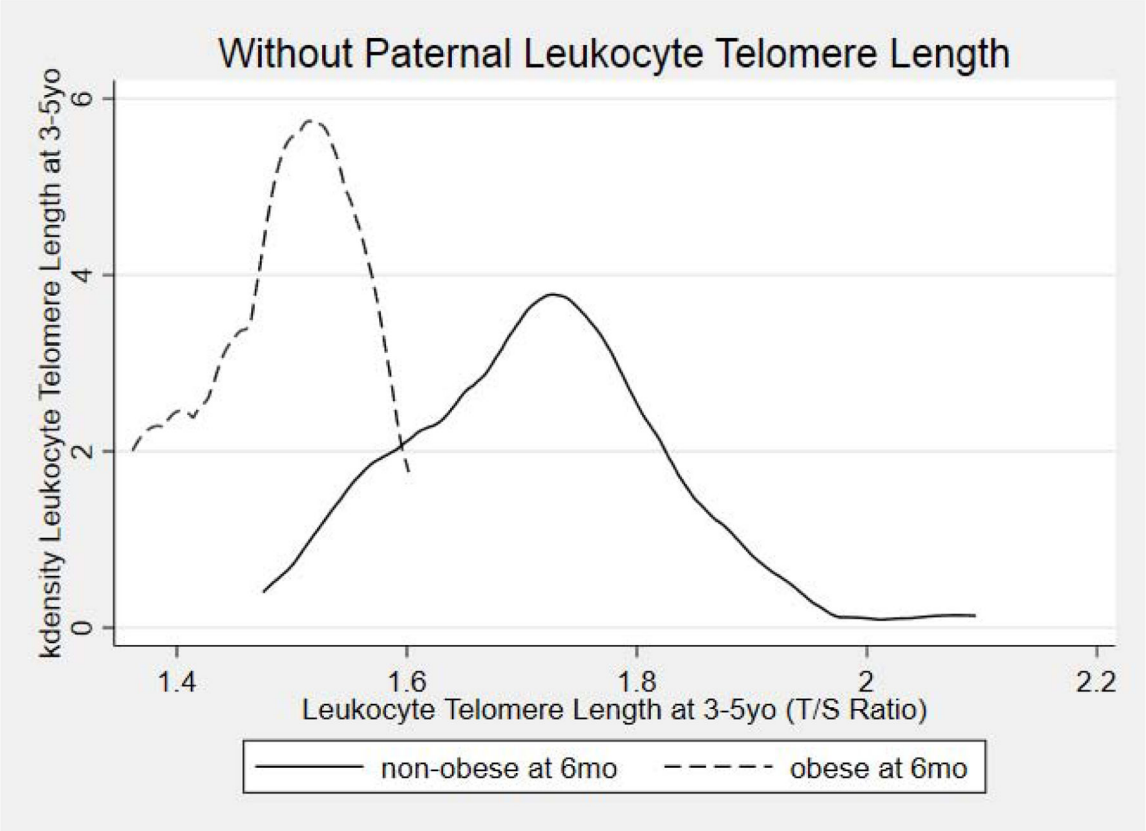

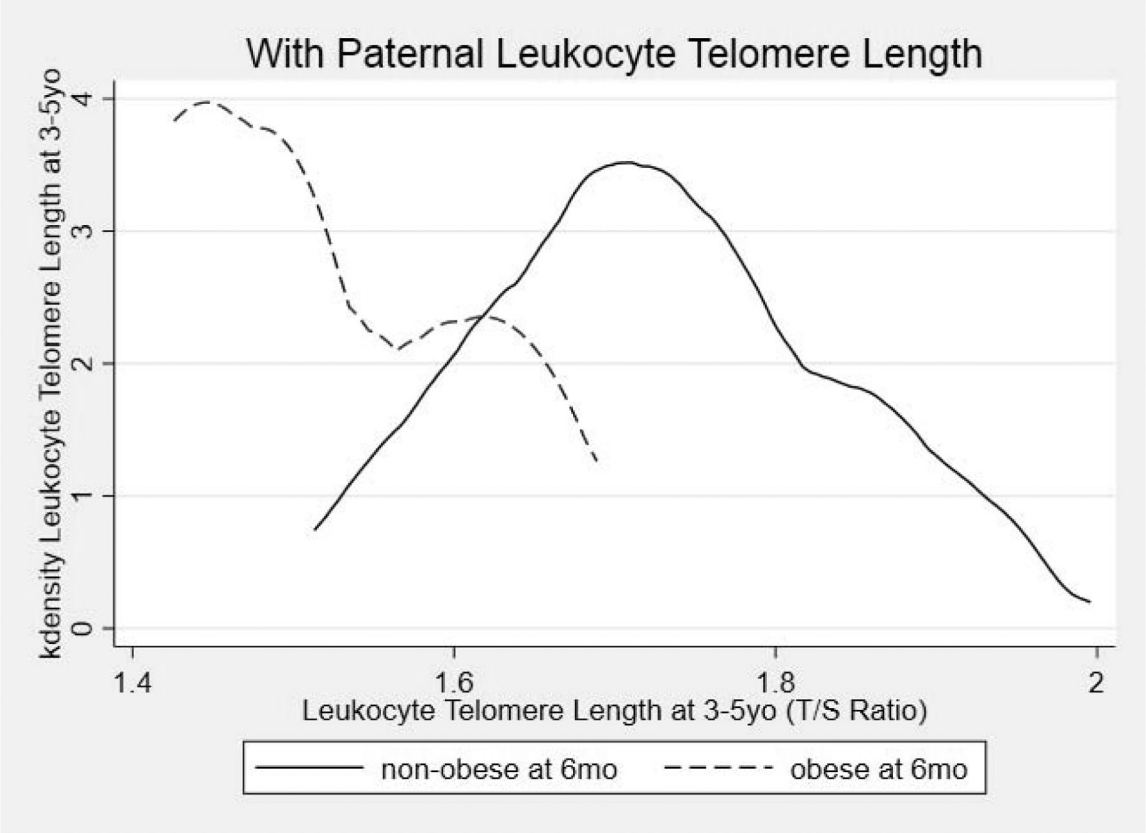

Figure: Distribution of Leukocyte Telomere Length by Obesity Status at 6 Months in Multivariable Models of Predictors of Child Telomere Length at 3–5 y of Age, With and Without Paternal Leukocyte Telomere Length

In multivariable models with and without paternal leukocyte telomere length, this Figure shows that children who were obese at 6 months (dashed line) had shorter leukocyte telomere length at 3–5 years of age compared with their peers who were not obese at 6 months (solid line). This is demonstrated by the kdensity estimates of leukocyte telomere length at age 3–5 years peaking, indicating a higher number of observations, at a smaller leukocyte telomere length value for children who were obese at 6 months.

Table 1:

Characteristics of Study Population, with Relationship to Child Leukocyte Telomere Length Ages 3–5 Years (N=73)

		Child TL 3–5yo	
Demographics	**% or Mean (SD)**	**β (95% CI)**	**p-value**
Ethnicity/Country of Origin (n=73)		0.04 (−0.08, 0.16)	0.52
Mexico/Mexico American (n=38)	52%		
Central American (n=35)	48%		
Maternal age (years) (n=73)	28.7 (5.5)	0.004 (−0.005, 0.01)	0.42
Annual income (n=73)		0.06 (−0.11, 0.24)	0.46
<$25,000 (n=65)	89%		
$25,000-$50,000 (n=8)	11%		
Maternal education (n=72)		−0.004 (−0.17, 0.16)	0.96
High school or less (n=59)	82%		
Some college or more (n=13)	18%		
Maternal/Paternal Health			
Maternal Leukocyte Telomere Length (T/S Ratio) (n=70)	1.325 (0.27)	**0.31 (0.05, 0.56)**	**0.02**
Paternal Leukocyte Telomere Length (T/S Ratio) (n=48)	1.327 (0.18)	0.29 (−0.01, 0.59)	0.06
Maternal BMI pre-pregnancy (kg/m^2) (n=68)	27.8 (4.11)	0.002 (−0.01, 0.02)	0.79
Maternal weight change >35lbs during pregnancy (n=65)		−0.005 (−0.15, 0.14)	0.95
No (n=45)	69%		
Yes (n=20)	31%		
Any household smokers 6mo post-partum (n=70)		−0.13 (−0.33, 0.07)	0.21
No (n=65)	93%		
Yes (n=5)	7%		
Any symptoms of maternal depression during pregnancy (n=73)		0.11 (−0.04, 0.26)	0.14
No (n=58)	79%		
Yes (n=15)	21%		
Infant/Child health			
Leukocyte Telomere Length at birth (T/S Ratio) (n=38)	1.8008 (0.29)	**0.62 (0.35, 0.90)**	**<0.001**
Leukocyte Telomere Length age 3–5y (T/S Ratio) (n=73)	1.676 (0.28)	-	-
Age at time of Leukocyte Telomere Length collection, age 3–5y (years) (n=156)	3.95 (0.75)	−0.025 (−0.10, 0.05)	0.52
Child Sex (n=73)		−0.1 (−0.21, 0.02)	0.09
Male (n=33)	45%		
Female (n=40)	55%		
Gestational age (weeks) (n=72)	39.2 (1.18)	−0.006 (−0.06, 0.04)	0.81
Birthweight (g) (n=72)	3354 (406)	−0.00003 (−0.0002, 0.0001)	0.7

Table 2:

Linear Regression Models of Child Leukocyte Telomere Length and Weight and Dietary Factors

		Child Leukocute Telomere Length 3–5yo	
	% or Mean (SD)	**β (95% CI)**	**p-value**
Weight for length z-score age 6mo (n=73)	0.41 (1.3)	−0.02 (−0.06, 0.03)	0.39
Obese age 6mo (n=73)		**−0.21 (−0.32, −0.10)**	**<0.001**
No (n=62)	85%		
Yes (n=11)	15%		
Weight for length z-score age 1y (n=71)	0.66 (1.03)	0.02 (−0.04, 0.09)	0.46
Obese age 1y (n=71)		0.06 (−0.15, 0.27)	0.58
No (n=60)	85%		
Yes (n=11)	15%		
BMI z-score age 2y (n=70)	0.78 (0.99)	−0.001 (−0.07, 0.06)	0.97
Obese age 2y (n=70)		−0.04 (−0.26, 0.18)	0.72
No (n=62)	89%		
Yes (n=8)	11%		
BMI z-score age 3y (n=73)	0.79 (1.04)	0.02 (−0.04, 0.07)	0.58
Obese age 3y (n=73)		−0.005 (−0.18, 0.17)	0.95
No (n=59)	81%		
Yes (n=14)	19%		
BMI z-score age 5y (n=70)	0.71 (0.99)	0.005 (−0.06, 0.07)	0.88
Obese age 5y (n=70)		0.07 (−0.12, 0.25)	0.46
No (n=58)	83%		
Yes (n=12)	17%		
Exclusive breastfeeding 4–6 weeks (n=72)		0.05 (−0.07, 0.16)	0.43
No (n=50)	69%		
Yes (n=22)	31%		
Any breastfeeding 6mo (n=70)		**0.14 (0.02, 0.27)**	**0.02**
No (n=20)	29%		
Yes (n=50)	71%		
Any breastfeeding 12mo (n=70)		0.08 (−0.04, 0.20)	0.18
No (n=26)	37%		
Yes (n=44)	63%		
Rapid infant weight gain first 6mo (n=73)		0.004 (−0.12, 0.13)	0.95
No (n=49)	67%		
Yes (n=24)	33%		
High soda intake age 3y (≥4 times per week) (n=69)		−0.07 (−0.21, 0.08)	0.35
No (n=56)	81%		
Yes (n=13)	19%		
High fruit juice intake age 3y (≥4 times per week) (n=69)		−0.02 (−0.15, 0.10)	0.71
No (n=47)	68%		
Yes (n=22)	32%		

		Child Leukocute Telomere Length 3–5yo	
	% or Mean (SD)	β (95% CI)	p-value
High flavored milk intake age 3y (≥3 times per week) (n=69)		−0.07 (−0.19, 0.05)	0.25
No (n=59)	86%		
Yes (n=10)	14%		
High SSB intake age 3y (≥9 times per week) (n=69)		−0.07 (−0.20, 0.06)	0.27
No (n=54)	78%		
Yes (n=15)	22%		
High sweets intake age 3y (≥9 times per week) (n=69)		−0.08 (−0.19, 0.03)	0.15
No (n=54)	78%		
Yes (n=15)	22%		
High combined sugar intake age 3y (>16 times per week) (n=69)		−0.08 (−0.22, 0.05)	0.23
No (n=58)	84%		
Yes (n=11)	16%		
High fast food consumption age 3y (>1x per week) (n=69)		−0.06 (−0.20, 0.08)	0.43
No (n=48)	70%		
Yes (n=21)	30%		

Table 3:

Multivariable Models of Independent Predictors of Child Leukocyte Telomere Length at 3–5 Years, With and Without Paternal Leukocyte Telomere Length

	Child Leukocyte Telomere Length 3–5yo (n=145 total observations, n=66 children)		**Child Leukocyte Telomere Length 3–5yo, including Paternal Leukocyte Telomere Length (n=104 total observations, n=45 children)**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Breastfeeding at 6mo	0.11 (−0.01, 0.23)	0.077	0.13 (−0.004, 0.26)	0.06
Obese at 6mo	**−0.18 (−0.27, −0.08)**	**<0.001**	**−0.15 (−0.27, −0.02)**	**0.02**
Child Sex	**−0.12 (−0.24, −0.01)**	**0.03**	−0.007 (−0.13, 0.11)	0.9
Maternal education	0.05 (−0.08, 0.18)	0.48	0.06 (−0.11, 0.22)	0.49
Maternal Leukocyte Telomere Length (T/S Ratio)	0.26 (−0.03, 0.56)	0.08	0.23 (−0.008, 0.47)	0.058
Paternal Leukocyte Telomere Length (T/S Ratio)	-	-	0.22 (−0.07, 0.52)	0.13
Child age at time of Leukocyte Telomere Length collection (years)	−0.04 (−0.11, 0.03)	0.31	−0.07 (−0.14, 0.007)	0.07