Sociodemographic correlates and family aggregation of leukocyte telomere length in adults and children from Mesoamerica

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Abstract

Objective: Telomere length is a biomarker of cumulative stress and inflammation related to chronic disease risk. We examined the associations of leukocyte telomere length (LTL) with sociodemographic and anthropometric variables and estimated LTL family aggregation in Central America, a region with a high burden of chronic disease where LTL has not been studied.

Methods: We conducted a cross-sectional study of 174 school age children and their parents in the capital cities of Belize, Honduras, Nicaragua, Costa Rica, Panama, and the city of Tuxtla-Gutierrez in Mexico. We measured LTL by quantitative PCR in DNA extracted from whole blood. We compared the distribution of LTL by categories of sociodemographic and anthropometric characteristics using linear regression. Family aggregation was estimated with correlation coefficients and intraclass correlations.

Results: In mothers, LTL was positively associated with age (P, trend<0.0001) and height (P=0.0002). Among fathers, LTL was inversely associated with food insecurity (P, trend=0.0004). In children, boys had 0.10 log units shorter LTL than girls (95% CI: -0.17, -0.03; P=0.004). LTL was inversely associated with parental education (P, trend=0.01) and positively associated with paternal age at birth (P, trend<0.0001), maternal LTL (P, trend=0.007), and paternal LTL (P, trend=0.02). LTL varied significantly by country of origin among all family members. Aggregation was greatest between children and their mothers, and mostly occurred at the country, rather than family, level.

Conclusion: LTL is associated with age and height in women; food insecurity in men; and sex, parental education and LTL, and paternal age at birth among children.

Keywords: leukocyte telomere length, children, parents, sociodemographic characteristics, Central America

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Introduction

Telomeres are repeated DNA sequences at the ends of chromosomes. They protect chromosomes against loss of genomic DNA during replication and cell division, and prevent chromosome ends from fusing together (Blackburn, 2005). Every time a somatic cell divides, its telomeres undergo incomplete replication at the ends of DNA strands and, as a result, telomeres grow shorter over the lifetime of the cell (Blackburn, 2005; Starkweather et al., 2014). Leukocyte telomere length (LTL) in humans decreases with chronological age, inflammation, and damage to DNA from oxidative stress (Aviv, 2009). Hence, LTL could be a useful marker of biological aging and of risk for chronic diseases resulting from environment and lifestyle conditions that induce inflammation and oxidative stress (Aviv, 2009). Short LTL is associated with risk factors including chronic psychological stress (Epel et al., 2004; Næss and Kirkengen, 2015), smoking (Valdes et al., 2005), and dietary exposures (Paul, 2011). The nature of the associations between LTL and chronic diseases is less clear. Some studies found associations between short LTL and risk of myocardial infarction, stroke (Fitzpatrick et al., 2007), type II diabetes (Salpea et al., 2010; Zhao et al., 2013), and cancer (Wentzensen et al., 2011). Other studies have found no association (Fernández-Alvira et al., 2016; Schürks et al., 2013) or have only found associations in population subgroups (Needham et al., 2015; O'Donnell et al., 2008). A large meta-analysis (Haycock et al., 2014) found an inverse association between LTL and coronary heart disease, but the evidence for an association with cerebrovascular disease was less clear. The meaning of LTL as a biomarker of chronic disease risk is still uncertain.

Many studies have demonstrated links between telomere length and demographic, anthropometric, and socioeconomic characteristics. These studies have generally found that men (Gardner et al., 2014), obese persons (Müezzinler et al., 2014), and people of low socioeconomic

status (Robertson et al., 2013) have comparatively short telomeres. However, with the exception

of two studies from Costa Rica (Rehkopf et al., 2013, 2014), few investigations have been

conducted in Latin American countries, and much of the existing literature may not be applicable

to these populations. Because chronic illnesses, particularly cardiovascular diseases, are among

the leading causes of death in this region (Rivera-Andrade and Luna, 2014), examining the

distribution of a novel marker for these conditions according to sociodemographic characteristics

could inform the identification of high risk groups that may benefit from preventive measures.

Furthermore, studying correlates of LTL in a new population with high rates of chronic disease

may help to elucidate the relations between these diseases and LTL.

There is evidence that LTL is partly heritable (Broer et al., 2013), although the extent to

which genetics and environment each influence LTL is unclear. Additionally, parental

characteristics may influence LTL in children as early as in utero, infancy, and throughout

childhood (Shalev et al., 2013). For these reasons, we studied LTL and sociodemographic

characteristics in children and their parents in a setting where this biomarker has not been

thoroughly investigated.

The aim of this study was to examine family aggregation of LTL and the associations

between LTL and demographic, anthropometric, and socioeconomic traits of school-age children

and their parents from six Central American countries. We hypothesized that there would be

LTL aggregation between children and each of their parents as a result of shared genetics and

environment. There would be less aggregation between spouses as a result of shared environment

in adulthood only. We hypothesized that age, male sex, higher BMI, and smoking would be

associated with shorter LTL. Finally, we studied several indicators of socioeconomic status (SES)

and predicted that lower SES would be associated with shorter LTL.

Methods

Study design and population

The Nine Mesoamerican Countries Metabolic Syndrome Study (NiMeCoMeS) has been described in detail elsewhere (Villamor et al., 2016). Briefly, we conducted a cross-sectional study of nutrition and cardiovascular health among school-age children and their parents in nine countries of the Mesoamerican region. We aimed to recruit 30 families from the capital cities of Belize, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, and the Dominican Republic, and from the city of Tuxtla Gutiérrez in Chiapas, Mexico. Between July 2011 and November 2013 we identified 4 to 10 public primary schools in the periurban areas of each city, roughly representing the four cardinal directions. Study teams requested the list of enrolled students from each school and randomly selected approximately 80 children ages 7 to 12 years per country as potentially eligible for recruitment. They visited the schools and interviewed the children to confirm additional eligibility criteria, including living with both biological parents, not being pregnant or having a pregnant mother, and not having a sibling already invited to participate. Parents of potentially eligible families received a written invitation to attend a meeting, held at the school, in which the study aims and procedures were explained. At the end of this meeting, each family received a written informed consent form and was afforded one week to consider participation in the study. Signed consent forms were received at the schools and assent to participate was confirmed from the children by a research team member. The final number of enrolled families was 267 (Mexico, 31; Belize, 31; Guatemala, 31; El Salvador, 30; Honduras, 30; Nicaragua, 31; Costa Rica, 27; Panama, 26; and the Dominican Republic, 30).

The study protocol and procedures were approved by the Institutional Review Boards (IRB) of collaborating institutions in each of the nine countries and by the University of Michigan Health and Behavioral Sciences IRB.

Data collection

Families who consented to participate received an appointment for a home visit by the research team, or for a visit to a health center if they preferred. Each family member was asked to fast for at least 6 hours before the visit. During these visits, each participant completed a questionnaire containing information on sociodemographic characteristics including age, education level, and socioeconomic status indicators; as well as health and dietary habits. The Latin American and Caribbean Food Security Scale (Pérez-Escamilla et al., 2007) was administered to the mothers. The survey consists of 16 yes-no questions regarding food insecurity experiences in the household during the previous 3 months and has been validated for use in this region.

At this same visit, investigators obtained anthropometric measurements including height, weight, and waist circumference. Height was measured without shoes to the nearest millimeter with the use of portable Seca stadiometers (Seca, Hamburg, Germany). Weight was measured in light clothing to the nearest 100 g with use of Tanita scales (Tanita, Tokyo, Japan). Waist circumference was measured at the end of an unforced exhalation with the use of a metric tape, accurate to the nearest millimeter. In adults, it was measured at the midpoint between the lowest border of the ribcage and the iliac crest, and in children it was measured above the uppermost lateral border of the right ilium. All measures were obtained in triplicate. At the end of the visit, researchers obtained biological samples from each participating family member. Approximately 7.5 mL of whole blood were collected in EDTA tubes and cryostored at -20˚C on the same day.

The time until samples were frozen was the same for all countries. The samples were transported

frozen from their respective countries to the Institute of Nutrition of Central America and

Panama (INCAP) in Guatemala City, Guatemala, and from there were transported frozen to the

University of Michigan for DNA extraction and analysis. Due to logistical constraints, samples

for DNA extraction were only collected in participants from Belize, Honduras, Nicaragua, Costa

Rica, Panama, and Mexico.

DNA extraction and telomere length measurement by qPCR

We extracted DNA from whole blood using a Qiagen Qiasymphony and QIAsymphony

DSP DNA Midi Kits (Qiagen, Valencia, CA, USA). Because data collection was carried out

through a 14 month span, storage time between collection and DNA extraction was longest for

samples from participants in Honduras and Nicaragua, and shortest for samples from participants

in Panama and Costa Rica. After DNA extraction, all LTL measurements were made within 2

months. We measured LTL using a monochrome multiplex qPCR procedure which has been

described in detail elsewhere (Cawthon, 2009). Briefly, all qPCR reactions were carried out

using 10 ng of input DNA, iQ^{TM} SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules,

CA, USA), and two sets of primers (Invitrogen, Carlsbad, CA, USA, sequences described in

(Cawthon, 2009)): telg/telc (to amplify telomeres) and albu/albd (to amplify albumin, a single-

copy gene). The same batch of standard DNA (extracted from MCF-7 cells) was serially diluted

to make two standard curves on each plate, one for telomeres and one for albumin, with seven

points per curve (at concentrations of 291.6, 97.2, 32.4, 10.8, 3.6, 1.2, and 0.4 ng/uL). These

curves were used to quantify the telomere (T) and albumin (S) signals for each reaction, and LTL

was calculated as the T/S ratio. This ratio gives a measurement of telomere length relative to

that of the standard DNA, and is comparable for all samples measured against the same standard

DNA. All samples were run in triplicate, and all plates included a positive control sample to

assess inter-assay variation, as well as a no template control to detect spurious amplification.

Intra-assay coefficients of variation (CV; calculated as the mean of all individual CVs from a

given plate, where individual CV is calculated as the standard deviation among a set of T/S

triplicates divided by the mean of those triplicates) for all plates were less than 16%. Inter-assay

CV (calculated as the standard deviation of all plate-specific T/S means for the control sample

divided by the mean of those means) was 37%.

To further assess LTL measurement accuracy, we sent a random selection of 47 samples

for testing at Dr. Elizabeth Blackburn's laboratory at the University of California, San Francisco.

This laboratory has a well-established protocol for performing LTL measurement (Lin et al.,

2010) and is widely considered to produce high-quality data. Their LTL measurements of

samples from the National Health and Nutrition Examination Survey have been reviewed for

quality by the Centers for Disease Control and Prevention and approved for public availability

(Needham et al., 2015). The samples that we sent covered the entire range of T/S ratios as

measured at our laboratory. The agreement between our measurements and those from the

Blackburn laboratory was excellent (Spearman correlation: 0.88, intraclass correlation

coefficient: 0.74).

Definition of outcome and exposures

The primary outcome was LTL. We calculated the median of the three LTL

measurements for each participant (Villamor and Bosch, 2015) and log-transformed the variable

to achieve normality. The primary exposures were sociodemographic and anthropometric

variables: age, sex (for children), height, body mass index (BMI), country of origin, education

level, parity, smoking status, monthly household income, and household food insecurity. We

used the median of 3 replicate measures for each anthropometric indicator in the analyses

(Villamor and Bosch, 2015). Height was categorized into sex-specific quartiles for the adults.

BMI was calculated as weight in kg divided by the square of height in meters. In adults, it was

categorized as <25, 25 - <30, or 30+ according to the World Health Organization (1995). A BMI

<18.5 kg/m^2 was not considered since there were very few adults under this cut point. In

children, we estimated height- and BMI-for-sex-and-age Z-scores according to the World Health

Organization growth references (de Onis, 2007). Parental education level was categorized

according to the number of completed school years (incomplete elementary, 1-5 years; complete

elementary, 6 years; incomplete secondary, 7-11 years; complete secondary, 12 years; and post-

secondary, ≥13 years). Smoking status was classified as never/ever in adults. For children,

passive smoking was defined as any known smoke exposure from a parent during the child's

lifetime vs. none. We divided household income into country-specific tertiles of low, medium,

and high. A household food insecurity score was created as the sum of all affirmative answers to

the survey and categorized as none (0), mild (1-5), moderate (6-10), or severe (11-16). For the

analysis of LTL among children, we also considered maternal and paternal LTL as exposure

variables, categorized into sex-specific quartiles.

Statistical analysis

We conducted separate analyses for mothers, fathers, and children because telomere

length dynamics vary by age and sex (Starkweather et al., 2014). We first compared the

distribution of log LTL across categories of the sociodemographic and anthropometric correlates

by estimating the mean log LTL. For ordinal variables, we estimated tests for linear trend with

the use of linear regression models in which a variable representing ordinal categories of the

characteristic was introduced as a continuous covariate. For nominal variables, we conducted χ^2 score tests. We tested for interaction by sex by including cross-product terms between sex and each sociodemographic predictor. Because we chose sociodemographic covariates based on existing literature and with a priori hypotheses, we did not adjust for multiple comparisons (Perneger, 1998). Next, we estimated adjusted differences and 95% confidence intervals in mean log LTL by categories of predictors from multivariable linear regression models. These models included as covariates those correlates that were significantly associated with log LTL at $p < 0.05$, or that were considered relevant from a mechanistic viewpoint. One multivariable model was fitted separately for each family member. For men, the model included age, food security, and country of origin. The model for women included age, height, and country of origin. For children, the model included age, sex, household educational attainment, maternal and paternal LTL, father's age at child's birth, and country of origin. Although age was not statistically significantly associated with LTL in men or children, we kept it in the models because age is a well-known predictor of LTL. All models were fitted using generalized estimating equations with empirical standard errors, which are robust to heteroskedasticity and non-normality (White, 1980). We also conducted supplemental analyses using untransformed LTL as the outcome.

To examine family aggregation, we calculated Pearson correlations of log LTL between children and each parent, and between mothers and fathers. Using a mixed effects model of log LTL with random intercepts for country and family, and fixed effects for age and sex, we calculated adjusted intraclass correlation coefficients (ICC). The ICC indicates the proportion of the total variance in LTL that is explained by variance between clusters at each level (country of origin and family nested within country). A high ICC would imply that persons randomly selected from the same country or the same family are likely to have similar LTL.

We conducted all analyses using Statistical Analysis Software version 9.4 (SAS Institute,

Cary, NC, USA).

Results

The analytic sample comprised all participants from the six countries from whom whole blood was available. Samples were available from 174 mothers, 172 fathers, and 164 children. Among adults, 50.3% were women. The mean (±SD) age was 37.0 ± 6.4 years for women and 40.4 ± 8.4 years for men. Among children, the mean (±SD) age was 9.9 ± 1.7 years and 52.4% were female. Mean (±SD) LTL in mothers, fathers, and children was 2.27 ± 0.86 (log mean 0.76 ± 0.36), 2.14 ± 0.74 (log mean 0.70 ± 0.34), and 3.35 ± 1.07 (log mean 1.16 ± 0.33), respectively.

Women's LTL. In bivariate analysis, mothers' LTL was inversely associated with age and parity and positively associated with height (**Table 1**). The lowest values were in women from Belize and Honduras, and the highest in Mexican, Panamanian, and Costa Rican women.

[Table 1 here]

In multivariable analysis (**Table 2**), mothers aged 45+ years had a 0.33 log units shorter mean LTL than did mothers younger than 30 years of age (P=0.0008), and women from Mexico had a mean LTL 0.41 log units longer than did women from Belize (P<0.0001). Mean LTL in the lowest height quartile was 0.18 log units significantly shorter than that in quartiles 2, 3, and 4 combined (95% CI: -0.28, -0.09; P=0.0002). BMI, smoking status, education level, household income, household food insecurity, and parity were not significantly associated with LTL after adjustment. The within-country associations of age and LTL were all inverse; the strongest associations were in Panama and Costa Rica, and the weakest in Nicaragua (**Supplemental Figure 1**).

Men's LTL. Among fathers, food insecurity was inversely associated with LTL in bivariate analyses (**Table 1**). Men from Costa Rica, Belize, and Honduras had the longest LTL while men from Nicaragua and Mexico had the shortest. In multivariable analysis (**Table 2**),

men from households with severe food insecurity had a mean log LTL 0.29 units shorter than did men from food secure households (P=0.0002). Fathers from Belize had a mean log LTL 0.35 units longer than did fathers from Nicaragua (P=0.0004). Height, BMI, smoking status, education level, and household income were not associated with LTL. Age was also not significantly associated with LTL, but was retained in the adjusted model because of its known influence on this biomarker. When stratified by country, the age-LTL associations were mainly parallel and slightly inverse; there was a slight positive association in men from Panama and a stronger inverse association in men from Honduras (**Supplemental Figure 1**).

[Table 2 here]

Children's LTL. In bivariate analysis, children's LTL was inversely associated with male sex and the parents' highest education level and positively associated with maternal telomere length (**Table 3**). The longest mean log LTL was among children from Panama and Mexico and the shortest in children from Honduras (P<0.0001). In multivariable analysis, boys had a 0.10 log units shorter LTL than girls (P=0.004). Children in households with at least one parent with a post-secondary education had a mean LTL 0.13 log units shorter than children whose parents only had incomplete elementary schooling (P=0.04). Further adjustment for BMI-for-age Z score did not change the association with parental education level. Telomere lengths of both parents were positively associated with the children's LTL; the association was stronger with the mothers' LTL. Paternal age at child's birth was also positively associated with LTL after adjustment. Mean LTL among children born to fathers ≥40 years of age was 0.27 log units longer than mean LTL in children whose fathers were less than 25 years old (P=0.0001). Children from Panama had a mean LTL 0.61 log units longer than children from Honduras (P<0.0001). Children's LTL was not significantly associated with mother's parity, parental

smoking, or parental anthropometry (data available on request). It was also not associated with children's age, height-for-age, BMI-for-age, household income, household food insecurity, or the mother's age at the child's birth.

[Table 3 here]

Aggregation. Children's log LTL was significantly positively correlated with their mothers' (Pearson's R=0.27, P=0.0004) and was not correlated with their fathers' (Pearson's R=0.004, P=0.96) (**Table 4**). Mothers' and fathers' log LTL were weakly, inversely correlated (Pearson's R=-0.19, P=0.01). Similarly, the age- and sex-adjusted ICCs were highest for mothers and children only (country-level ICC=28.8%, family-level ICC=32.1%). ICCs were nearly identical for the whole family (country- and family-level ICC=10.5%) and for fathers and children only (country- and family-level ICC=10.7%). In all cases, the family-level ICC was either identical to or only minimally larger than the country-level ICC.

[Table 4 here]

All results from supplemental analyses using untransformed LTL were similar to results using log LTL (**Supplemental Tables 1-4**).

Discussion

In this cross-sectional study of families from Mesoamerica, longer LTL was associated with younger age and height in women; and with food security in men. In children, longer LTL was associated with female sex, lower parental education level, parental LTL, and paternal age at child's birth. LTL differed significantly by country of origin in each group.

Shorter LTL can serve as a biomarker of biological aging and chronic disease risk (Starkweather et al., 2014). We found an inverse, monotonic relation between age and LTL in women only. This is unexpected, since most studies have found that age is inversely associated with LTL in both sexes (Müezzinler et al., 2013). Because measurement procedures were identical for all study participants, differential measurement error should not explain this finding. Differences in the direction and strength of the age-LTL association between men and women might be related to the age distribution of the study populations, since LTL attrition rates vary differently by sex across the lifespan (Dalgård et al., 2015; Iwama et al., 1998). The lack of an association between age and LTL in children could be explained by the relatively narrow age range of children in our study.

We found that food insecurity in fathers was related to shorter LTL. This association had not been reported in other populations. It could be the result of life stress associated with an inability to consistently secure adequate food (Epel et al., 2004; Liu et al., 2014), or from specific dietary deficiencies that could affect LTL (Paul, 2011). For example, intake patterns typical of households with food insecurity, including low intake of vegetables, legumes, and nuts (Tarasuk, 2001; Weigel and Armijos, 2015), have been associated with shorter telomere length in previous studies (Lee et al., 2015). It is uncertain why the association between food insecurity and LTL was restricted to fathers. Household food insecurity may have different consequences

for fathers, mothers, and children. For example, fathers might experience more psychological stress associated with food insecurity than do mothers or children. Food insecurity could be acting as a proxy measure for aspects of SES such as occupational status that affect fathers differently than they do mothers or children. Alternatively, fathers' diets may be affected differently than other family members' by food insecurity. It is also possible that the association seen in fathers is spurious.

Children's LTL was inversely associated with parental education. Our findings are in agreement with one previous study of adults from six cities in the United States that found an inverse association of LTL with fathers' educational attainment among Hispanic study participants (Carroll et al., 2013). One possible explanation of the inverse association observed in our study could be related to the nutrition transition that these countries are undergoing. Through this transition, the burden of nutrition-related chronic diseases and their risk factors progressively shifts from people of higher SES to those living in poorer conditions, as countries undergo economic development. This transition tends to affect women before men. Countries in Central America are at a relatively early stage of the transition considering that the prevalence of chronic disease risk factors is higher in men of lower SES and women and children of higher SES (Villamor et al 2016). Some obesogenic dietary factors that are typically consumed by children of higher SES (Nettleton et al., 2008) are predictors of short telomere length. One could hypothesize that an effect of parental education on LTL could be mediated through obesity. Adjusting for children's BMI did not change the association of parental education with LTL. However, this is a crude means of assessing mediation. In addition, BMI may not adequately capture all of the relevant consequences of the nutrition transition. We cannot rule out that LTL in children may be an indicator of adherence to unhealthy dietary or other lifestyles among the

better-off. In contrast with the findings among children, education level was not associated with

LTL in adults. It is possible that the education level of adults does not represent the same SES

construct to them as parental education level does to children.

In our study, women in the shortest quartile of height had significantly shorter LTL than

did taller women. Two previous studies examined the relation between adult height and

telomere length, and both found no association (Cui et al., 2013; Pearce et al., 2012). One

potential explanation for this discrepancy is that an association between height and LTL may

exist only when comparing people of very short stature with taller ones. In the previous

investigations women were not as short as those in our study. Very short final height in

Mesoamerica is the consequence of stunting during childhood, which is attributable mainly to

dietary deficiencies, a high burden of infection, and low SES (Silventoinen, 2003; Steckel, 2009;

Subramanian et al., 2011). Experiments in mice have shown that chronic infection leads to

shortened LTL (Ilmonen et al., 2008). It is possible that some of the determinants of short stature

could also impair LTL.

We found that country was a strong predictor of LTL in adults and children. This finding

is novel for Mesoamerica. One other study of inter-country variation examined average telomere

length among men of similar age from 11 European countries and found a three-fold difference

in mean LTL between the countries with highest and lowest means (Eisenberg et al., 2011).

Although it is likely that our findings arose to some extent from true differences in LTL by

country, it is also of note that timing of blood collection varied significantly across months of the

year, and that month of collection was highly collinear with country. This is important because a

study of LTL in Costa Rica found significant variation by the month in which LTL was

measured (Rehkopf et al., 2014). There are other reasons to explain the association between

country and LTL. It may reflect differences in lifestyle characteristics or ecological exposures that were not captured by the individual level variables we measured in the study. For example, LTL could be affected by stress associated with political and social unrest and conflict that took place in Nicaragua from the late 1970's through 1990's or in Chiapas, Mexico in the 1990's, by national health and social welfare policies, or by differences in standards of living between countries of different income levels.

In this population, the association between maternal and child LTL was stronger than the association between paternal and child LTL. This was consistent when comparing average LTL of children across quartiles of their parents' LTL, and when estimating the correlations and ICCs of LTL between children and each parent. The stronger association of child LTL with maternal, rather than paternal, LTL is in agreement with results from a large meta-analysis of six European and Australian cohorts (Broer et al., 2013) but contrary to studies in Swedish and Amish populations (Njajou et al., 2007; Nordfjäll et al., 2009). Variation across studies could be the result of differences in parenting techniques and behaviors between countries.

LTL was weakly, inversely correlated between mothers and fathers. This finding differs from the six cohorts meta-analysis which showed a positive correlation between spouses' LTL (Broer et al., 2013). The inverse correlation may be driven by the opposite associations of country and LTL in men and women. Alternatively, men in Mesoamerica may be buffering their partners from the influence of some environmental predictors of LTL.

There were little differences between the ICCs at country and family levels for any configuration of family members. This indicates that family membership does not explain additional variance in LTL, conditional on age and sex, beyond what is explained by country of origin. In other words, members of the same family are no more likely to have similar LTL than

persons from the same country. Since influences on LTL that operate at the country level are most likely non-genetic, this implies that environmental and lifestyle factors are important determinants of LTL in these populations.

Our study has many strengths. First, it provides new information on telomere length in countries where this biomarker has not been studied before. Second, because we collected data on parents and children within the same households, we were able to study associations between children's LTL and their parents' characteristics. Third, we had an opportunity to examine associations controlling for important confounders. Also, the measure of LTL had high external validity considering the high correlations with measures obtained at a reference laboratory. Finally, LTL was associated with many factors in the direction expected according to previous studies.

There are also some limitations. The cross-sectional nature of the design prevents making causal inferences. Also, the sample sizes within each country were small and we lacked samples from some of the countries originally included in the study. This prevented us from conducting country-specific analyses. The inter-assay CV for our LTL measurements was high. This could indicate a high degree of random measurement error of LTL in our study. Random error in a continuous outcome would not have biased the mean differences in LTL by categories of the exposures, but could have lowered the precision of the estimates and reduced the statistical power to detect associations.

In conclusion, we found that LTL is associated with age and height in women; food insecurity in men; and sex, parental education and LTL, and paternal age at birth among children. There are important between-country differences in LTL, and little additional between-family variation. Children's LTL is more similar to their mothers' than their fathers'. Further studies are

needed to assess the specific nature of these relations, particularly whether diet and other

lifestyle characteristics explain a potential effect of household food insecurity and parental

educational attainment on telomere length.

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Author contributions

KSF performed the data analysis and KSF and EV drafted the manuscript. EV, AVR, and MRZ designed the Nine Countries Metabolic Syndrome Study, AVR and MRZ oversaw data collection. KSF, ECJ, and KMR optimized the telomere measurement protocol and quantified LTL, and LSR provided necessary laboratory expertise and resources. All authors read and approved the final version of the manuscript.

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Table 1. Mean (±SD) log LTL according to categories of sociodemographic characteristics in adults from Mesoamerica

Characteristics	Mothers			Fathers		
	N	Unadjusted mean log LTL ± SD		N	Unadjusted mean log LTL ± SD	
Overall	174	0.76	± 0.36	172	0.70	± 0.34
Age (years)						
<30	27	0.86	± 0.39	12	0.75	± 0.34
30 - <35	33	0.89	± 0.34	35	0.70	± 0.35
35 - <40	63	0.74	± 0.35	51	0.69	± 0.38
40 - <45	34	0.69	± 0.34	35	0.69	± 0.35
45+	17	0.52	± 0.33	39	0.70	± 0.30
P, trend[a]		0.0002			0.74	
P, interaction[b]			0.16			
Height quartile (sex-specific medians, cm)						
Q1 (mothers=149.1, fathers=159.3)	43	0.58	± 0.37	42	0.71	± 0.36
Q2 (mothers=153.8, fathers=165.3)	43	0.84	± 0.34	44	0.70	± 0.28
Q3 (mothers=157.2, fathers=169.8)	45	0.82	± 0.31	43	0.69	± 0.35
Q4 (mothers=163.2, fathers=176.9)	43	0.77	± 0.38	43	0.71	± 0.39
P, trend		0.03			0.90	
P, interaction			0.07			
BMI (kg/m^2)						
<25	30	0.82	± 0.43	33	0.80	± 0.32
25-<30	65	0.71	± 0.38	87	0.69	± 0.32
30+	79	0.76	± 0.32	52	0.66	± 0.40
P, trend		0.71			0.10	
P, interaction			0.48			
Education level						
Incomplete elementary	25	0.58	± 0.34	14	0.74	± 0.45
Complete elementary	24	0.82	± 0.32	27	0.64	± 0.39
Incomplete secondary	50	0.83	± 0.32	51	0.69	± 0.37
Complete secondary	31	0.73	± 0.43	26	0.69	± 0.28
Post secondary	40	0.75	± 0.36	51	0.75	± 0.28
P, trend		0.32			0.44	
P, interaction			0.37			

Parity						
1	16	0.88	± 0.39			
2	56	0.79	± 0.35			
3	60	0.75	± 0.37			
4+	41	0.67	± 0.35			
P, trend		0.03				
Smoking status						
Never smoked	138	0.75	± 0.36	70	0.74	± 0.33
Ever smoked	36	0.76	± 0.36	101	0.68	± 0.35
P[c]		0.96			0.22	
P, interaction			0.59			
Household income level, adjusted for country						
Low	31	0.86	± 0.37	31	0.75	± 0.38
Medium	71	0.68	± 0.37	69	0.68	± 0.33
High	69	0.78	± 0.35	69	0.70	± 0.35
P, trend		0.65			0.70	
P, interaction			0.56			
Food security						
No insecurity	74	0.75	± 0.37	74	0.79	± 0.27
Mild insecurity	44	0.81	± 0.35	44	0.73	± 0.37
Moderate insecurity	37	0.64	± 0.39	35	0.64	± 0.39
Severe insecurity	19	0.88	± 0.27	19	0.40	± 0.28
P, trend		0.84			<0.0001	
P, interaction			0.009			
Country of origin						
Belize	29	0.50	± 0.28	28	0.81	± 0.40
Honduras	30	0.55	± 0.42	29	0.78	± 0.24
Nicaragua	31	0.83	± 0.27	31	0.43	± 0.35
Costa Rica	27	0.87	± 0.30	27	0.82	± 0.30
Panama	26	0.89	± 0.37	26	0.72	± 0.37
Mexico	31	0.91	± 0.29	31	0.68	± 0.26
P		<0.0001			0.001	
P, interaction			<0.0001			

[a]From linear regression models with log LTL as the outcome and a variable representing ordinal categories of the characteristic introduced as a continuous predictor.

[b]From a χ^2 score test for the cross-product term of interaction between sex and the sociodemographic predictor.

[c]From a χ^2 score test.

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Table 2. Adjusted differences in log LTL according to categories of sociodemographic characteristics in adults from Mesoamerica

Characteristics	Mothers Adjusted difference (95% CI)[a]		Fathers Adjusted difference (95% CI)[b]	
Age (years)				
<30		Reference		Reference
30 - <35	-0.03	(-0.20, 0.14)	0.06	(-0.17, 0.29)
35 - <40	-0.17	(-0.32, -0.02)	-0.10	(-0.32, 0.13)
40 - <45	-0.23	(-0.39, -0.07)	-0.15	(-0.39, 0.09)
45+	-0.33	(-0.52, -0.14)	-0.08	(-0.31, 0.16)
P, trend[c]		<0.0001		0.08
P, interaction[d]			0.43	
Mother's height (cm)				
140.6-151.9		Reference		
152.0-155.7	0.22	(0.10, 0.33)		
155.8-159.2	0.20	(0.07, 0.32)		
159.3-181.9	0.12	(0.00, 0.24)		
P, trend		0.11		
P, interaction			0.07	
Food security				
No insecurity				Reference
Mild insecurity			-0.05	(-0.18, 0.08)
Moderate insecurity			-0.14	(-0.28, -0.01)
Severe insecurity			-0.29	(-0.44, -0.14)
P, trend				0.0004
P, interaction			0.05	
Country of origin				
Belize		Reference		Reference
Honduras	0.04	(-0.12, 0.21)	-0.04	(-0.22, 0.13)
Nicaragua	0.24	(0.11, 0.38)	-0.35	(-0.54, -0.16)
Costa Rica	0.33	(0.19, 0.48)	-0.03	(-0.21, 0.15)
Panama	0.34	(0.18, 0.50)	-0.10	(-0.30, 0.10)
Mexico	0.41	(0.27, 0.54)	-0.20	(-0.36, -0.03)
P[e]		<0.0001		0.004
P, interaction			<0.0001	

Footnotes to Table 2

[a]From a linear regression model adjusted for age, height, and country of origin.

[b]From a linear regression model adjusted for age, food insecurity, and country of origin.

[c]From linear regression models with log LTL as the outcome and a variable representing ordinal categories of the characteristic introduced as a continuous predictor.

[d]From a χ^2 score test for the cross-product term of interaction between sex and the sociodemographic predictor.

[e]From a χ^2 score test.

Table 3. Mean (±SD) and differences in log LTL according to categories of sociodemographic characteristics in children from Mesoamerica

Characteristics	N	Unadjusted mean log LTL ± SD	Adjusted difference (95% CI)[a]
Overall	164	1.16 ± 0.33	
Age (years)			
<9	58	1.14 ± 0.36	Reference
9 - <11	53	1.14 ± 0.30	0.02 (-0.06, 0.10)
11+	53	1.19 ± 0.32	0.03 (-0.05, 0.12)
P, trend[b]		0.45	0.46
Sex			
Female	86	1.20 ± 0.34	Reference
Male	78	1.11 ± 0.31	-0.10 (-0.17, -0.03)
P[c]		0.08	0.005
Height-for-age Z score			
<-1	36	1.20 ± 0.30	
-1 - <0	58	1.11 ± 0.34	
0 - <1	45	1.19 ± 0.34	
1+	25	1.15 ± 0.34	
P, trend		0.98	
BMI-for-age Z score			
<-1	23	1.08 ± 0.32	
-1 - <0	36	1.15 ± 0.37	
0 - <1	46	1.20 ± 0.32	
1+	59	1.15 ± 0.31	
P, trend		0.39	
Household highest education level			
Incomplete elementary	13	1.24 ± 0.23	Reference
Complete elementary	16	1.26 ± 0.32	-0.01 (-0.16, 0.13)
Incomplete secondary	45	1.24 ± 0.24	-0.06 (-0.20, 0.08)
Complete secondary	28	1.03 ± 0.37	-0.15 (-0.30, 0.00)
Post secondary	62	1.11 ± 0.36	-0.13 (-0.26, -0.01)
P, trend		0.006	0.01

Household income level,
adjusted for country

Low	29	1.29	±	0.35		
Medium	68	1.09	±	0.32		
High	63	1.18	±	0.30		
P, trend			0.42			

Food insecurity

No insecurity	72	1.16	±	0.32		
Mild insecurity	41	1.11	±	0.35		
Moderate insecurity	33	1.18	±	0.34		
Severe insecurity	18	1.19	±	0.33		
P, trend			0.75			

Maternal age at child's birth

<20	18	1.15	±	0.31		
20 - <25	34	1.15	±	0.32		
25 - <30	63	1.15	±	0.31		
30 - <35	31	1.13	±	0.38		
35+	18	1.24	±	0.36		
P, trend			0.57			

Maternal telomere length

Q1	40	1.00	±	0.30	Reference	
Q2	41	1.17	±	0.33	0.04	(-0.06, 0.14)
Q3	41	1.19	±	0.30	0.06	(-0.04, 0.15)
Q4	40	1.27	±	0.33	0.15	(0.04, 0.25)
P, trend			0.0002		0.007	

Paternal age at child's birth

<25	42	1.13	±	0.29	Reference	
25 - <30	46	1.16	±	0.36	0.10	(-0.02, 0.22)
30 - <35	35	1.11	±	0.30	0.13	(0.03, 0.24)
35 - <40	20	1.23	±	0.29	0.15	(0.02, 0.27)
40+	21	1.21	±	0.42	0.27	(0.13, 0.41)
P, trend			0.29		<0.0001	

Paternal telomere length

Q1	40	1.22 ± 0.27		Reference
Q2	40	1.05 ± 0.37	-0.02	(-0.13, 0.08)
Q3	41	1.18 ± 0.36	0.10	(-0.01, 0.21)
Q4	40	1.19 ± 0.27	0.09	(-0.02, 0.20)
P, trend		0.82		0.02

Country of origin

Belize	27	1.17 ± 0.21	0.42	(0.29, 0.54)
Honduras	28	0.69 ± 0.21		Reference
Nicaragua	31	1.24 ± 0.32	0.53	(0.38, 0.69)
Costa Rica	25	1.22 ± 0.24	0.42	(0.30, 0.55)
Panama	25	1.33 ± 0.28	0.61	(0.48, 0.73)
Mexico	28	1.31 ± 0.22	0.54	(0.44, 0.65)
P		<0.0001		<0.0001

[a]From a linear regression model adjusted for age, sex, parents' highest education level, paternal age at child's birth, maternal and paternal telomere length, and country of origin.
[b]From linear regression models with log LTL as the outcome and a variable representing ordinal categories of the characteristic introduced as a continuous predictor.
[c]From a χ^2 score test.

Table 4. Family correlations and intraclass correlation coefficients of log LTL

	All	Mothers and children	Fathers and children	Mothers and fathers
Correlation[a]	-	0.27	0.004	-0.19
ICC[b] (%)				
Country-level	10.5	28.8	10.7	6.1
Family-level	10.5	32.1	10.7	6.1

[a]Pearson product-moment correlation.

[b]From a linear mixed model with random intercepts for country and family, and fixed effects for sex and age. ICCs calculated as either the variance of country-specific intercepts (for country-level ICC) or the sum of variances of country- and family-specific intercepts (for family-level ICC) divided by the sum of all variance components.