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Leukocyte telomere length is inversely associated with a metabolic risk score in Mesoamerican children

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ABSTRACT

Objective: Leukocyte telomere length (LTL) may be involved in the etiology of the metabolic syndrome (MetS). We examined the associations of LTL with MetS and its components among Mesoamerican children and their adult parents, in a region where MetS prevalence is high.

Methods: We conducted a cross-sectional study of 151 children aged 7-12 y and 346 parents from the capitals of Belize, Honduras, Nicaragua, Costa Rica, Panama, and Chiapas State, Mexico. We quantified LTL by qPCR on DNA extracted from whole blood. In children, we created an age- and sex-standardized metabolic risk score using waist circumference (WC), the homeostasis model of insulin resistance (HOMA-IR), blood pressure, serum high-density lipoprotein (HDL) cholesterol, and serum triglycerides. In adults, MetS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III definition. We estimated mean differences in metabolic risk score and prevalence ratios of MetS across quartiles of LTL using multivariable-adjusted linear and Poisson regression models, respectively.

Results: In children, every 1 LTL z-score was related to an adjusted 0.05 units lower (95% CI: -0.09, -0.02, *P*=0.005) MetS risk score, through WC, HOMA-IR, and HDL. Among adults, LTL was not associated with MetS prevalence; however, every 1 LTL z-score was associated with an adjusted 34% lower prevalence of high fasting glucose (95% CI: 3%, 55%, *P*=0.03).

Conclusions: Among Mesoamerican children, LTL is associated with an improved metabolic profile; among adults, LTL is inversely associated with the prevalence of high fasting glucose.

Keywords: Metabolic syndrome, leukocyte telomere length, Mesoamerica, children

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INTRODUCTION

The metabolic syndrome (MetS) consists of a set of related cardiometabolic risk factors that strongly predict the incidence of cardiovascular disease, type-2 diabetes (T2D), and mortality (Grundy, 2008). In Latin America, the prevalence of MetS among adults is higher than in many high-income countries (Márquez-Sandoval et al., 2011). MetS components can develop in childhood (Zimmet et al., 2007), and evidence from Latin American and other low- and middle-income countries suggests that MetS has become highly prevalent among children (Caceres, Teran, Rodriguez, & Medina, 2008; Kelishadi, 2007), partly due to the childhood obesity epidemic in this region (Rivera et al., 2014). For example, in Mexican children aged 6-12 years, 44% of obese children had MetS, and more than one third of normal-weight children had ≥ 1 MetS component (Guzmán-Guzmán, Salgado-Bernabé, Muñoz Valle, Vences-Velázquez, & Parra-Rojas, 2015). Children with MetS exhibit signs of early atherosclerosis (Rumińska et al., 2016), and childhood MetS is a strong predictor of both cardiovascular disease (Morrison, Friedman, & Gray-McGuire, 2007) and T2D (Morrison, Friedman, Wang, & Glueck, 2008) later in life. Hence, identifying modifiable predictors of metabolic dysfunction is critical to address the growing epidemic of chronic disease.

Telomeres are repeated DNA sequences at the ends of chromosomes that protect against loss of genomic DNA during replication and cell division. Leukocyte telomere length (LTL) in humans decreases with chronological age, inflammation, and damage to DNA from oxidative stress (Aviv, 2009). LTL is inversely associated with the risk of cardiovascular disease (Haycock et al., 2014) and diabetes (Zhao, Miao, Wang, Ding, & Wang, 2013), and likely plays a role in the pathogenesis of cancer (Shay, 2016). In addition, shorter LTL has been linked with higher mortality risk in many cohort studies (Arbeev et al., 2020; Wang, Zhan, Pedersen, Fang, & Hägg,

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2018). Thus, LTL may be a useful biomarker of biological age and risk of chronic disease, particularly cardiometabolic conditions. In adults, LTL has been positively associated with metabolic health (Chen et al., 2009; Demissie et al., 2006; Harte et al., 2012; Huzen et al., 2014; Nordfjäll et al., 2008; Révész, Milaneschi, Terpstra, & Penninx, 2016; Révész, Milaneschi, Verhoeven, Lin, & Penninx, 2015; Révész, Milaneschi, Verhoeven, & Penninx, 2014), although associations with obesity are less consistent (Tzanetakou, Katsilambros, Benetos, Mikhailidis, & Perrea, 2012). In children, LTL may be related to individual MetS components (Al-Attas et al., 2010; Chen et al., 2009), but studies of LTL and MetS are lacking.

The link between LTL and metabolic health is complex; available evidence indicates that their causal relation is likely bidirectional (Kirchner et al., 2017). In light of the increasing burden of MetS, and because most prior studies of LTL and MetS have focused on MetS as the exposure, we aimed to test the causal scenario in which LTL may affect MetS. We conducted a cross-sectional study among families from nine countries in Mesoamerica to examine associations between LTL and MetS among school-aged children and their adult parents in an under-studied population where the prevalence of MetS is high (Villamor, Finan, Ramirez-Zea, & Roman, 2017).

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METHODS

Study Design and Population

This investigation was conducted in the context of the Nine Mesoamerican Countries Metabolic Syndrome (NiMeCoMeS) Study, a cross-sectional study of school-aged children and their parents from the capital cities of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Panama, Costa Rica, and Belize, and the city of Tuxtla Gutiérrez in Chiapas, Mexico. Participants were recruited between 2011 and 2013 in family groups (n = 267) that consisted of one child between ages 7 and 12 years and both biological parents. Additional criteria for enrollment involved not being pregnant or having a pregnant mother, and not having a sibling already invited to participate.

The Institutional Review Boards (IRB) of collaborating institutions in each of the nine countries and the University of Michigan Health and Behavioral Sciences IRB approved all study procedures. All adults provided written informed consent to participate for themselves and their children. Children confirmed their agreement to participate before enrollment.

Data Collection

Recruitment and study procedures have been described in detail elsewhere (Villamor et al., 2017). Briefly, children were recruited at primary schools and invited to attend a research visit with their parents at a study clinic. At this visit, participants completed questionnaires that inquired on sociodemographic characteristics, including their age, education level, smoking status, and indicators of socioeconomic status (SES) such as the number of household assets and household food security status per the Latin American and Caribbean Food Security Scale (ELCSA) (Pérez-Escamilla, Melgar-Quiñonez, Nord, Álvarez Uribe, & Segall-Corrêa, 2007). Trained study personnel obtained measurements of height, weight, and waist circumference

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using standardized procedures and calibrated instruments. Waist circumference was measured to the nearest millimeter at the end of an unforced exhalation using an inelastic measuring tape. In adults, it was measured at the midpoint of the lower end of the ribcage and the iliac crest; in children, it was measured above the uppermost lateral border of the right ilium. All anthropometric measures were obtained in triplicate. Blood pressure was measured in triplicate while seated, with at least 1 minute between each reading, and the final blood pressure value was calculated as the average of the second and third readings. Finally, a fasting blood sample was collected by antecubital venipuncture. On the day of collection, samples were transported on ice to laboratories in the participants' country for processing and cryostorage at -20° C. Blood samples were then transported frozen to the Institute of Nutrition of Central America and Panama in Guatemala City, Guatemala, where they were stored at -70° C prior to biochemical analysis. An aliquot of whole blood was transported frozen in one batch to the University of Michigan where it was stored at -70° C until analysis. The samples underwent only one thawing cycle for DNA extraction, under homogeneous conditions. Due to logistical constraints, samples for DNA extraction were only collected in participants from Belize, Honduras, Nicaragua, Costa Rica, Panama, and Mexico.

Laboratory Methods

Serum insulin was measured using chemiluminescent immunoassay with an Immulite 1000 system (Siemens Healthcare Diagnostics Products, Tarrytown, NY, USA); plasma glucose concentrations were quantified using an automated chemistry analyser (Cobas c111 system; Roche Diagnostics, Mannheim, Germany); and serum lipid profiles were determined using enzymatic colorimetric assays on the same system.

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We extracted DNA from whole blood using Qiagen Qiasymphony and QIAsymphony DSP DNA Midi Kits (Qiagen, Valencia, CA, USA). LTL was determined at the University of Michigan using a monochrome multiplex qPCR procedure that has been described in detail elsewhere (Cawthon, 2009; Flannagan et al., 2017); an in-depth description of these methods per the Telomere Research Network recommendations ("Minimum Reporting Recommendations for PCR-based Telomere Length Measurement," 2019) is also included as **Supporting Information Appendix S1**. Briefly, all qPCR reactions were carried out using 10 ng of input DNA, iQ™ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and two sets of primers (Invitrogen, Carlsbad, CA, USA, sequences described by Cawthon (Cawthon, 2009)): telg/telc (to amplify telomeres) and albu/albd (to amplify albumin, a single-copy gene). The same batch of standard DNA was serially diluted to make two standard curves on each plate, one for telomeres and one for albumin, with seven points per curve. 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. All samples were run in triplicate , and all plates included a positive control sample to assess inter-assay variation, as well as a no templ ate control to detect spurious amplification. We calculated the intraclass correlation coefficient of the 3 replicates (ICC=0.92) using a one-way, random-effects, absolute agreement model (McGraw & Wong, 1996). Intra- and inter-assay coefficients of variation were 16% and 37%, respectively. To further document the quality of our LTL measurements, we tested a random set of 47 samples at Dr. Elizabeth Blackburn's reference laboratory at the University of California San Francisco. The correlation between T/S values obtained at both labs was very high (r=0.88, **Supporting Information Figure S1**). LTL data also had high internal validity; for example, child values' correlation with their mother's (r=0.25) was within the expected range (Broer et al., 2013; Eisenberg, 2014). Moreover, associations with

sex (**Supporting Information Table S1**) were in the expected direction per existing literature (Ly et al., 2019; Müezzinler, Zaineddin, & Brenner, 2013).

Definition of exposure

We estimated the median of the 3 LTL replicate measurements per participant (Villamor & Bosch, 2015) to use as the exposure variable in the analyses. We then transformed LTL values to z-scores, as recommended (Verhulst, 2020), separately for children and adults using each subgroup as the corresponding standard. LTL z-scores were categorized into quartiles and also considered as a continuous variable.

Definition of outcomes

Children. Because the metabolic syndrome is undefined in children <10 years old, we estimated a continuous metabolic risk score using values on the same criteria used in adults, as previously recommended (Eisenmann, 2008). We used the homeostasis model assessment of insulin resistance (HOMA-IR) as the measure of insulin resistance and mean arterial pressure (MAP) as the blood pressure criterion. We first obtained sex- and age-standardized scores for WC, HOMA-IR, MAP, high-density lipoprotein (HDL) cholesterol and serum triglycerides (TAG) by performing the regression of the natural logarithmic transformation of each of these variables on sex and log-transformed age with the use of linear regression models. The standardized regression residuals for WC, HOMA-IR, MAP and TAG, plus the HDL cholesterol residual multiplied by −1, were then averaged to create a metabolic risk score, where higher values reflect a worse metabolic profile.

Adults. The primary outcome of interest was the presence of MetS according to the National Cholesterol Education Program's ATP III criteria. Abdominal obesity was defined as WC>102 cm in men or WC >88 cm in women; high fasting glucose as ≥100 mg/dl; high blood

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pressure as systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg or treatment with an antihypertensive drug; low HDL cholesterol as serum concentration <40 mg/dl in men or <50 mg/dl in women, or drug treatment for reduced HDL cholesterol; and high TAG as serum concentration ≥150 mg/dl or drug treatment for elevated TAG. MetS was defined as the presence of three or more of these criteria.

Covariates

In children, we estimated sex-specific height-for-age z-scores according to the World Health Organization reference (de Onis et al., 2007). Parental smoking was defined as ever smoking by one or both parents. In adults, smoking status was classified as never, past, or current. The number of household assets was determined as the sum of affirmative responses to owning a car, bicycle, refrigerator/freezer, gas stove, electric stove, blender, microwave, washing machine, color television, sound set, computer, or Internet. The presence of severe food insecurity in the household was defined as a sum of affirmative answers ≥11 to the 16 questions on food security experiences over the prior 3 months in the ELCSA survey.

Data analyses

There were 151 children and 346 adults with non-missing data on both LTL and metabolic syndrome outcomes and these constituted the analytic sample. All analyses were conducted separately for children and adults.

Children. We compared the distribution of the metabolic risk score overall and for each component across quartiles of LTL z-score using means and standard deviations. Next, we estimated mean metabolic risk score differences with 95% CI between LTL z-score quartiles using bivariate linear regression. We then fit two adjusted models, one with country of origin which was the largest source of variability in LTL in this population (Flannagan et al., 2017) and

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a multivariable-adjusted model involving additional independent predictors of the metabolic syndrome (Villamor et al., 2017) or correlates of LTL that are not affected by it (to avoid over-adjustment), such as parental MetS and parental LTL. In addition to country, these included the presence of metabolic syndrome in the parents, child's height-for-age z-score, mother's height and LTL z-score, father's height and LTL z-score, and parental smoking. We also adjusted for SES by including the number of household assets as a continuous variable, as well as an indicator variable for severe food insecurity. The metabolic risk score was standardized for sex and age using the study population as the reference; therefore, estimates of association are intrinsically adjusted for these variables. Because the multivariable-adjusted associations between LTL and metabolic risk score seemed to follow a dose-response relation, we also estimated the difference in metabolic risk score per 1 z-score difference in LTL.

Adults. In bivariate analyses, we compared the prevalence of MetS and its components across quartiles of LTL z-score. Next, we estimated prevalence ratios with 95% CI. These were first adjusted for country alone and subsequently for independent predictors of MetS or its components plus correlates of LTL which included sex, age and height, and smoking status (Flannagan et al., 2017), as well as SES, as indicated by household assets and food insecurity. Robust estimates of variance were specified in all models to account for within-family correlations among adults. All analyses were performed using SAS version 9.4.

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RESULTS

Children. Mean ± SD age of children was 9.9 ± 1.6 y; 53% were girls (**Supporting Information Table S2**). Mean ± SD LTL and metabolic risk score were 3.32 ± 1.05 and -0.01 ± 0.23, respectively. In bivariate analysis, LTL was not associated with the metabolic risk score (**Table 1**); however, after adjustment for country, LTL was inversely related to this score. In multivariable analysis, every 1 LTL z-score was associated with an adjusted mean 0.05 units lower risk score (*P*=0.005) (**Table 1**). LTL was inversely associated with the WC score (-0.03 adjusted mean units per 1 LTL z-score, *P*=0.01) and the HOMA-IR score (-0.12 adjusted mean units per 1 LTL z-score, *P*=0.03), and positively associated with the serum HDL score (0.06 adjusted mean units per 1 LTL z-score, *P*=0.002). LTL was not significantly related to the MAP or the serum TAG scores. Regression coefficients for adjustment covariates are included in **Supporting Information Table S3**.

Adults. Mean ± SD age of adults was 38.7 ± 7.6 y; 50.3% were women (**Supporting Information Table S4**). Mean ± SD LTL was 2.20 ± 0.80; the prevalence of MetS was 35.6%. LTL was not significantly associated with the MetS (**Table 2**); however, there was a linear, inverse relation between LTL and high fasting glucose in bivariate and multivariable analyses. Every 1 LTL z-score was associated with an adjusted 34% lower prevalence of high fasting glucose (*P*=0.03). LTL was not significantly related to other metabolic syndrome components. Parameter estimates for adjustment covariates are presented in **Supporting Information Table S5**.

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DISCUSSION

In this cross-sectional study of Mesoamerican families, we found that LTL was inversely related to a MetS risk score in children, mainly through inverse associations with WC and HOMA-IR, and a positive association with serum HDL cholesterol. Among adults, LTL was inversely related to the prevalence of high fasting glucose in a dose-response manner.

Few studies have explored the association between LTL and metabolic health among children, although some have reported inverse associations between childhood obesity, a component of MetS, and LTL (Buxton et al., 2011; Clemente et al., 2019; Lamprokostopoulou et al., 2019). In a lifestyle intervention among overweight and obese adolescents, those with higher LTL at baseline had a larger decrease in body weight (García-Calzón et al., 2014). Meanwhile, in a randomized trial of a different lifestyle intervention in children with abdominal obesity, higher baseline LTL predicted larger decreases in blood glucose during the intervention (Morell-Azanza, Ojeda-Rodríguez, Azcona-SanJulián, Zalba, & Marti, 2020), in line with our findings for HOMA-IR. In the Bogalusa heart study, higher childhood HDL was associated with higher adult LTL (Chen et al., 2009), which is consistent with the positive association between LTL and HDL in our study, although we did not follow children into adulthood. In a cross-sectional study of children in middle childhood, WC was the strongest predictor of LTL in girls, whereas systolic blood pressure was the most important predictor in boys (Al-Attas et al., 2010). These results are consistent with the inverse relation between LTL and WC in Mesoamerican children; however, we did not find an association with MAP. Differences in study populations, as well as adjustment approaches, might explain these discrepancies.

In adults, most studies have reported a positive relation between LTL and metabolic health. Cross-sectional studies have found associations between shorter LTL and elevated WC

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(Nordfjäll et al., 2008), TAG (Harte et al., 2012), insulin resistance (Demissie et al., 2006), and hypertension (Demissie et al., 2006). In the Netherlands Study of Depression and Anxiety, shorter baseline LTL was associated with an adverse metabolic profile across all MetS components at baseline, 2 years, and 6 years (Révész et al., 2014). In the same cohort, an adverse baseline profile of MetS components (high WC, elevated glucose, low HDL, and total number of metabolic disregulations) was associated with shorter LTL at baseline and follow-up; also, greater 6-year increase in WC was associated with a greater decrease in 6-year LTL (Révész et al., 2015). In the PREVEND study, higher baseline plasma glucose and waist-to-hip ratio, as well as lower HDL, were associated with greater LTL attrition over follow up (Huzen et al., 2014). More recently, results from the CARDIA study found that HDL was positively associated with LTL during follow-up, and 10-year increase in WC was related to greater telomere attrition (Révész et al., 2018). Finally, in a prospective twin study, baseline LTL was inversely associated with changes in insulin resistance over an average of 12 years of follow-up (Verhulst et al., 2016), in line with our finding of an inverse association between LTL and prevalence of high fasting glucose in adults. Unlike many prior studies, we did not find associations between LTL and other MetS components; however, differences in study design and participant characteristics could be responsible for these divergent results.

Mechanisms linking LTL and metabolic health remain incompletely understood. It has been hypothesized that inflammation and oxidative stress are responsible for associations between LTL and insulin resistance (Gardner et al., 2005). These factors may also explain the connection between LTL and HDL cholesterol, as HDL reduces oxidation and inflammation (Chen et al., 2009). However, other authors have noted that, because relative LTL is largely determined prior to adulthood, metabolic outcomes in adults are likely preceded by differences in

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LTL, as opposed to causing those differences (Verhulst et al., 2016). Evidence from animal models supports a potential impact of LTL on metabolic health. In mice, short telomeres impair beta cell signaling, contributing to hyperglycemia (Guo et al., 2011), and promote mitochondrial dysfunction, which leads to a number of downstream adverse metabolic effects (Sahin et al., 2011). In these models, disruption of a protein that is part of a protective complex at telomeres results in metabolic dysregulation with effects that include glucose intolerance, insulin resistance, and accumulation of abdominal fat (Martínez et al., 2013; Yeung et al., 2013). Ongoing studies will be needed to investigate the extent to which LTL may be a cause or consequence of metabolic health.

Our study has several strengths. The relation between metabolic syndrome and LTL has not been studied in children; thus, our results address an important gap, which is crucial in developing early interventions to improve metabolic health. In addition, our population consisted of people from a region where childhood metabolic syndrome and obesity represent a substantial public health burden. Because we studied families of adult parents and their children, we were also able to compare the LTL-metabolic syndrome associations between children and adults, and we had the opportunity to adjust the results in children for parental LTL.

Some limitations should also be noted. Because this study was cross-sectional, we cannot determine the direction of the association between LTL and MetS. As discussed previously, it is possible that telomere shortening disrupts metabolic function, that metabolic health affects LTL, or that the association is bidirectional. We also cannot discard the possibility of residual confounding, which results in non-causal estimates of association due to the effects of common causes of exposure (LTL) and outcome (MetS) (Bateson & Nettle, 2018; Lash, VanderWeele, Haneuse, & Rothman, 2021). One example of an unmeasured confounder for which we could not

control is MetS during pregnancy; this has been related to shorter telomeres later in childhood (McAninch et al., 2020) and could be a cause of MetS in children through shared genetic and environmental factors. Although we were able to adjust for parental MetS during childhood, it is possible that MetS in pregnancy could introduce additional confounding that we were not able to capture. Measurement error in the ascertainment of exposure, outcome, and covariates is another potential limitation of the study. Furthermore, associations between LTL and metabolic syndrome cannot be quantitatively compared between children and adults because the outcomes are in different scales. Last, generalizability may be limited to populations with comparable distributions of LTL and metabolic outcomes.

In conclusion, higher LTL is associated with a more favorable metabolic profile among Mesoamerican children, specifically through lower WC and HOMA-IR, and higher HDL. In adults, higher LTL is associated with a lower prevalence of high fasting glucose. Prospective investigations of these questions are warranted, especially in children, to enhance strategies for improving metabolic health throughout life.

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AUTHOR CONTRIBUTIONS

JG drafted the manuscript. KSF optimized the telomere measurement protocol and quantified LTL. KSF and EV conceptualized the study. DR, EV, and JG performed the data analysis. MRZ oversaw data collection. MRZ and EV designed the Nine Mesoamerican Countries Metabolic Syndrome Study and obtained funding. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (EV), upon reasonable request.

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Table 1. Metabolic syndrome risk score according to leukocyte telomere length (LTL) z-score in school-age children from Mesoamerica

Metabolic syndrome score	LTL z-score quartile (Q)				Mean difference (95% CI) per 1 LTL z-score[†]	*P*
	Q1 n=37	Q2 n=38	Q3 n=38	Q4 n=38		
Median LTL	2.08	2.93	3.62	4.53		
Overall metabolic risk score						
Mean ± SD	-0.06 ± 0.26	0.06 ± 0.24	-0.01 ± 0.19	-0.03 ± 0.20		
Unadjusted differences (95% CI)	Reference	0.12 (0.01, 0.23)	0.05 (-0.05, 0.16)	0.03 (-0.07, 0.14)	0.00 (-0.04, 0.03)	0.90
Country-adjusted differences	Reference	0.05 (-0.09, 0.19)	-0.01 (-0.15, 0.12)	-0.05 (-0.19, 0.09)	-0.04 (-0.08, 0.00)	0.03
Multivariable-adjusted differences[‡]	Reference	0.02 (-0.12, 0.16)	-0.04 (-0.17, 0.09)	-0.09 (-0.22, 0.05)	-0.05 (-0.09, -0.02)	0.005
Waist circumference score						
Mean ± SD	-0.03 ± 0.14	0.04 ± 0.16	0.01 ± 0.14	-0.02 ± 0.15		
Unadjusted differences (95% CI)	Reference	0.06 (0.00, 0.13)	0.03 (-0.03, 0.09)	0.01 (-0.06, 0.07)	0.00 (-0.03, 0.02)	0.83
Country-adjusted differences	Reference	0.03 (-0.04, 0.10)	0.00 (-0.07, 0.06)	-0.04 (-0.11, 0.03)	-0.02 (-0.05, 0.00)	0.08
Multivariable-adjusted differences[‡]	Reference	0.01 (-0.05, 0.07)	-0.02 (-0.08, 0.04)	-0.04 (-0.10, 0.01)	-0.03 (-0.05, -0.01)	0.01
HOMA-IR score						
Mean ± SD	-0.13 ± 0.71	0.17 ± 0.63	-0.02 ± 0.52	-0.02 ± 0.55		
Unadjusted differences (95% CI)	Reference	0.30 (0.00, 0.60)	0.11 (-0.17, 0.39)	0.11 (-0.18, 0.39)	0.01 (-0.09, 0.10)	0.89
Country-adjusted differences	Reference	0.13 (-0.34, 0.60)	-0.06 (-0.49, 0.37)	-0.10 (-0.54, 0.35)	-0.09 (-0.20, 0.02)	0.12
Multivariable-adjusted differences[‡]	Reference	0.03 (-0.41, 0.48)	-0.13 (-0.54, 0.27)	-0.20 (-0.63, 0.22)	-0.12 (-0.22, -0.01)	0.03
MAP score						
Mean ± SD	0.00 ± 0.14	0.04 ± 0.18	0.02 ± 0.12	0.03 ± 0.16		
Unadjusted differences (95% CI)	Reference	0.04 (-0.03, 0.11)	0.02 (-0.04, 0.08)	0.03 (-0.04, 0.10)	0.00 (-0.02, 0.03)	0.93
Country-adjusted differences	Reference	0.03 (-0.07, 0.12)	-0.01 (-0.10, 0.08)	-0.01 (-0.11, 0.09)	-0.02 (-0.05, 0.01)	0.22
Multivariable-adjusted differences[‡]	Reference	0.03 (-0.06, 0.11)	0.00 (-0.08, 0.07)	0.00 (-0.08, 0.08)	-0.01 (-0.04, 0.01)	0.34

Metabolic syndrome score	LTL z-score quartile (Q)				Mean difference	*P*
Serum HDL score						
Mean ± SD	0.09 ± 0.22	-0.01 ± 0.23	0.04 ± 0.24	0.10 ± 0.27		
Unadjusted differences (95% CI)	Reference	-0.10 (-0.21, 0.00)	-0.05 (-0.16, 0.05)	0.01 (-0.10, 0.12)	0.02 (-0.02, 0.05)	0.40
Country-adjusted differences	Reference	0.01 (-0.08, 0.11)	0.05 (-0.04, 0.14)	0.11 (-0.01, 0.22)	0.05 (0.01, 0.10)	0.01
Multivariable-adjusted differences[‡]	Reference	0.01 (-0.08, 0.10)	0.07 (-0.01, 0.16)	0.13 (0.02, 0.23)	0.06 (0.02, 0.10)	0.002
Serum triglycerides score						
Mean ± SD	-0.06 ± 0.40	0.04 ± 0.41	0.00 ± 0.36	-0.03 ± 0.37		
Unadjusted differences (95% CI)	Reference	0.10 (-0.08, 0.28)	0.06 (-0.11, 0.24)	0.04 (-0.14, 0.21)	0.00 (-0.06, 0.05)	0.97
Country-adjusted differences	Reference	0.09 (-0.12, 0.30)	0.05 (-0.16, 0.26)	0.00 (-0.22, 0.23)	-0.03 (-0.10, 0.03)	0.34
Multivariable-adjusted differences[‡]	Reference	0.04 (-0.18, 0.25)	0.02 (-0.18, 0.22)	-0.06 (-0.28, 0.17)	-0.04 (-0.11, 0.03)	0.24

LTL = leukocyte telomere length. HOMA-IR = homeostasis model assessment of insulin resistance. MAP = mean arterial pressure. HDL = high density lipoprotein. CI = confidence interval.

† From a linear regression model with LTL z-score as a continuous predictor.

‡ From a linear regression model with each score as a continuous outcome, adjusted for country, presence of metabolic syndrome in the parents (2 indicators), child's height-for-age z-score, mother's height and LTL z-score, father's height and LTL z-score (continuous), parental smoking (2 indicators), and socioeconomic status (household assets and an indicator for severe food insecurity). Robust variances were specified in all models.

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Table 2. Metabolic syndrome according to leukocyte telomere length (LTL) z-score in adult parents from Mesoamerica

Metabolic syndrome component	LTL z-score quartile (Q)				Prevalence ratio (95% CI) per 1 LTL z-score[†]	*P*
	Q1 n=86	Q2 n=87	Q3 n=86	Q4 n=87		
Median LTL	1.32	1.86	2.33	3.13		
Metabolic syndrome						
Prevalence, %	31.4	40.2	39.5	31.0		
Unadjusted prevalence ratio (95% CI)	1.00	1.28 (0.86, 1.90)	1.26 (0.84, 1.89)	0.99 (0.64, 1.53)	0.97 (0.84, 1.11)	0.65
Country-adjusted prevalence ratio	1.00	1.30 (0.88, 1.93)	1.23 (0.82, 1.87)	1.01 (0.65, 1.57)	0.98 (0.84, 1.13)	0.76
Multivariable-adjusted prevalence ratio[‡]	1.00	0.83 (0.55, 1.26)	1.06 (0.73, 1.54)	0.79 (0.52, 1.20)	0.99 (0.85, 1.15)	0.88
Abdominal obesity						
Prevalence, %	50.0	49.4	47.7	44.8		
Unadjusted prevalence ratio (95% CI)	1.00	0.99 (0.73, 1.33)	0.95 (0.70, 1.30)	0.90 (0.64, 1.25)	0.96 (0.86, 1.08)	0.50
Country-adjusted prevalence ratio	1.00	1.03 (0.77, 1.37)	0.98 (0.71, 1.34)	0.91 (0.66, 1.27)	0.97 (0.86, 1.09)	0.63
Multivariable-adjusted prevalence ratio[‡]	1.00	0.86 (0.65, 1.14)	1.04 (0.81, 1.33)	1.07 (0.80, 1.43)	0.92 (0.82, 1.04)	0.17
High fasting glucose						
Prevalence, %	11.6	10.3	9.3	3.5		
Unadjusted prevalence ratio (95% CI)	1.00	0.89 (0.38, 2.08)	0.80 (0.34, 1.90)	0.30 (0.08, 1.05)	0.65 (0.45, 0.95)	0.02
Country-adjusted prevalence ratio	1.00	0.87 (0.37, 2.05)	0.78 (0.34, 1.82)	0.30 (0.08, 1.07)	0.65 (0.44, 0.96)	0.03
Multivariable-adjusted prevalence ratio[‡]	1.00	0.36 (0.10, 1.33)	1.07 (0.42, 2.74)	1.18 (0.50, 2.83)	0.66 (0.45, 0.97)	0.03
High blood pressure						
Prevalence, %	20.9	28.7	19.8	20.9		
Unadjusted prevalence ratio (95% CI)	1.00	1.37 (0.82, 2.29)	0.94 (0.51, 1.74)	1.00 (0.57, 1.77)	0.95 (0.79, 1.14)	0.58
Country-adjusted prevalence ratio	1.00	1.37 (0.82, 2.28)	0.90 (0.50, 1.64)	0.94 (0.53, 1.66)	0.92 (0.76, 1.11)	0.38
Multivariable-adjusted prevalence ratio[‡]	1.00	1.17 (0.65, 2.13)	1.56 (0.88, 2.76)	1.00 (0.54, 1.83)	0.99 (0.82, 1.21)	0.94

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Metabolic syndrome component	LTL z-score quartile (Q)				Prevalence ratio	*P*
Low HDL cholesterol						
Prevalence, %	75.6	80.5	88.4	79.3		
Unadjusted prevalence ratio (95% CI)	1.00	1.06 (0.92, 1.23)	1.17 (1.02, 1.34)	1.05 (0.90, 1.23)	0.99 (0.93, 1.06)	0.81
Country-adjusted prevalence ratio	1.00	1.07 (0.93, 1.24)	1.18 (1.03, 1.35)	1.09 (0.93, 1.28)	1.01 (0.94, 1.08)	0.85
Multivariable-adjusted prevalence ratio‡	1.00	0.92 (0.81, 1.05)	0.92 (0.82, 1.04)	0.86 (0.75, 1.00)	1.00 (0.93, 1.07)	0.94
High triglycerides						
Prevalence, %	44.2	52.9	58.1	48.3		
Unadjusted prevalence ratio (95% CI)	1.00	1.20 (0.86, 1.67)	1.32 (0.97, 1.78)	1.09 (0.78, 1.53)	0.99 (0.89, 1.11)	0.90
Country-adjusted prevalence ratio	1.00	1.18 (0.85, 1.65)	1.29 (0.95, 1.75)	1.12 (0.79, 1.57)	1.00 (0.89, 1.12)	0.99
Multivariable-adjusted prevalence ratio‡	1.00	0.94 (0.71, 1.24)	0.90 (0.69, 1.17)	0.77 (0.57, 1.03)	1.04 (0.93, 1.17)	0.46

LTL = leukocyte telomere length. HDL = high density lipoprotein. CI = confidence interval.

† From a Poisson regression model with LTL z-score as a continuous predictor.

‡ From a Poisson regression model with metabolic syndrome or each component as a dichotomous outcome, adjusted for country, sex, age and height (continuous), smoking status (indicators for past or current), and socioeconomic status (household assets and an indicator for severe food insecurity). Robust variances were specified in all models.

Table S1. Associations of age and sex with mean ± SD leukocyte telomere length (LTL) z-score in school-age children and their adult parents from Mesoamerica

Age, years	Females		Males	
	n	Mean ± SD LTL z-score	n	Mean ± SD LTL z-score
Children				
5 to <9	24	0.06 ± 1.27	26	-0.29 ± 0.88
9 to <11	29	-0.07 ± 0.81	24	-0.08 ± 0.94
11 to <14	27	0.26 ± 1.11	21	-0.04 ± 0.84
Adults				
<30	27	0.45 ± 1.42	11	0.15 ± 0.95
30 to <35	33	0.45 ± 1.07	35	-0.08 ± 0.97
35 to <40	63	0.02 ± 0.95	51	-0.07 ± 1.01
40 to <45	34	-0.13 ± 0.89	35	-0.12 ± 0.84
45 to <50	10	-0.54 ± 0.92	19	-0.08 ± 0.70
≥50	7	-0.52 ± 0.79	20	-0.17 ± 0.94

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Table S2. Characteristics of school-age children from Mesoamerica, by sex

Variable	Total N = 151	Females n = 80 (53.0%)	Males n = 71 (47.0%)
Age (years), mean ± SD	9.9 ± 1.6	10.1 ± 1.6	9.7 ± 1.7
LTL, mean ± SD			
T/S ratio	3.32 ± 1.05	3.43 ± 1.13	3.20 ± 0.94
Z-score	-0.03 ± 0.98	0.08 ± 1.06	-0.14 ± 0.88
Country, n (%)			
Honduras	28 (18.5)	15 (18.8)	13 (18.3)
Nicaragua	31 (20.5)	18 (22.5)	13 (18.3)
Panama	18 (11.9)	7 (8.8)	11 (15.5)
Costa Rica	20 (13.2)	14 (17.5)	6 (8.4)
Mexico	27 (17.9)	14 (17.5)	13 (18.3)
Belize	27 (17.9)	12 (15.0)	15 (21.1)
Parental MetS, n (%)			
Neither parent	59 (40.1)	35 (44.3)	24 (35.3)
One parent	69 (46.9)	35 (44.3)	34 (50.0)
Both parents	19 (12.9)	9 (11.4)	10 (14.7)
Child's HAZ, mean ± SD	-0.10 ± 1.06	-0.07 ± 1.00	-0.14 ± 1.12
Mother's height (cm), mean ± SD	155.9 ± 6.1	155.2 ± 5.4	156.7 ± 6.7
Father's height (cm), mean ± SD	167.4 ± 7.8	167.9 ± 7.7	166.9 ± 8.0
Parental smoking, n (%)			
Neither parent	51 (34.5)	25 (31.3)	26 (38.2)
One parent	74 (50.0)	41 (51.3)	33 (48.5)
Both parents	23 (15.5)	14 (17.5)	9 (13.2)
Maternal LTL z-score, mean ± SD	0.00 ± 0.97	0.11 ± 1.00	-0.13 ± 0.93
Paternal LTL z-score, mean ± SD	-0.06 ± 0.94	-0.19 ± 0.82	0.08 ± 1.06

LTL = leukocyte telomere length. SD = standard deviation. MetS = metabolic syndrome. HAZ = height-for-age z-score.

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Table S3. Multivariable-adjusted model for the associations between leukocyte telomere length (LTL) and metabolic outcomes among children[†]

Covariate	Mean metabolic score difference (95% confidence interval)					
	MetS score	WC score	HOMA-IR score	MAP score	HDL score	Triglycerides score
LTL z-score, per 1 z	-0.05 (-0.08, -0.02)	-0.03 (-0.05, -0.01)	-0.11 (-0.21, -0.01)	-0.02 (-0.05, -0.01)	0.06 (0.02, 0.10)	-0.03 (-0.10, 0.03)
Country						
Honduras	Ref	Ref	Ref	Ref	Ref	Ref
Nicaragua	0.18 (0.08, 0.28)	0.07 (0.01, 0.13)	0.45 (0.16, 0.75)	0.11 (0.02, 0.20)	-0.10 (-0.20, 0.00)	0.15 (-0.08, 0.37)
Panama	0.16 (0.03, 0.28)	0.12 (0.05, 0.20)	0.41 (0.02, 0.79)	0.11 (0.03, 0.19)	-0.05 (-0.20, 0.09)	0.10 (-0.15, 0.34)
Costa Rica	0.18 (0.04, 0.32)	0.09 (0.02, 0.16)	0.40 (0.01, 0.78)	0.08 (-0.01, 0.18)	-0.16 (-0.31, -0.02)	0.16 (-0.08, 0.39)
Mexico	0.19 (0.06, 0.32)	0.08 (0.00, 0.15)	0.43 (0.08, 0.79)	0.04 (-0.05, 0.13)	-0.22 (-0.35, -0.09)	0.16 (-0.06, 0.38)
Belize	0.11 (0.01, 0.22)	0.13 (0.08, 0.19)	0.35 (0.01, 0.68)	-0.01 (-0.08, 0.06)	-0.16 (-0.28, -0.05)	-0.06 (-0.26, 0.14)
Parental MetS						
Neither parent	Ref	Ref	Ref	Ref	Ref	Ref
One parent	0.04 (-0.02, 0.10)	0.02 (-0.01, 0.06)	0.00 (-0.17, 0.18)	0.00 (-0.05, 0.05)	-0.13 (-0.20, -0.05)	0.06 (-0.06, 0.19)
Both parents	0.13 (0.02, 0.25)	0.03 (-0.03, 0.08)	0.10 (-0.23, 0.43)	0.03 (-0.06, 0.12)	-0.27 (-0.37, -0.16)	0.24 (0.03, 0.44)
Child's HAZ, per 1 z	0.11 (0.09, 0.14)	0.11 (0.09, 0.13)	0.33 (0.24, 0.42)	0.05 (0.02, 0.07)	-0.02 (-0.06, 0.02)	0.07 (0.01, 0.13)
Mother's height, per 1 cm	-0.01 (-0.02, 0.00)	0.00 (-0.01, 0.00)	-0.03 (-0.04, -0.01)	0.00 (-0.01, 0.00)	0.01 (0.00, 0.01)	-0.01 (-0.02, 0.00)
Father's height, per 1 cm	-0.01 (-0.01, 0.00)	0.00 (-0.01, 0.00)	-0.02 (-0.03, -0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)	-0.01 (-0.02, 0.00)
Parental smoking						
Neither parent	Ref	Ref	Ref	Ref	Ref	Ref
One parent	-0.04 (-0.10, 0.03)	-0.02 (-0.06, 0.02)	-0.02 (-0.21, 0.16)	-0.06 (-0.11, 0.00)	0.01 (-0.07, 0.09)	-0.08 (-0.21, 0.05)
Both parents	-0.09 (-0.19, 0.00)	0.00 (-0.07, 0.07)	-0.15 (-0.39, 0.10)	-0.08 (-0.15, 0.00)	-0.04 (-0.14, 0.06)	-0.28 (-0.48, -0.07)
Maternal LTL z-score, per 1 z	0.03 (-0.01, 0.07)	0.01 (-0.01, 0.03)	0.08 (-0.01, 0.17)	0.00 (-0.03, 0.02)	-0.04 (-0.08, 0.01)	0.02 (-0.05, 0.09)
Paternal LTL z-score, per 1 z	0.00 (-0.04, 0.03)	0.00 (-0.02, 0.02)	-0.01 (-0.11, 0.09)	-0.01 (-0.04, 0.01)	0.00 (-0.04, 0.04)	0.00 (-0.08, 0.08)

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Footnotes to Table S3

LTL = leukocyte telomere length. MetS = metabolic syndrome risk score. WC = waist circumference. HOMA-IR = homeostasis model assessment of insulin resistance. MAP = mean arterial pressure. HDL = high density lipoprotein. HAZ = height-for-age z-score.

† From a linear regression model with LTL z-score as a continuous predictor.

Table S4. Characteristics of adult parents from Mesoamerica, by sex

Variable	Total N = 346	Females n = 174 (50.3%)	Males n = 172 (49.7%)
Age (years), mean ± SD	38.7 ± 7.6	37.0 ± 6.4	40.4 ± 8.4
LTL, mean ± SD			
T/S ratio	2.20 ± 0.80	2.27 ± 0.86	2.14 ± 0.74
Z-score	0.00 ± 1.00	0.08 ± 1.07	-0.09 ± 0.92
Country, n (%)			
Honduras	59 (17.1)	30 (17.2)	29 (16.9)
Nicaragua	62 (17.9)	31 (17.8)	31 (18.0)
Panama	52 (15.0)	26 (14.9)	26 (15.1)
Costa Rica	54 (15.6)	27 (15.5)	27 (15.7)
Mexico	62 (17.9)	31 (17.8)	31 (18.0)
Belize	57 (16.5)	29 (16.7)	28 (16.3)
Height (cm), mean ± SD	161.8 ± 9.1	156.0 ± 6.1	167.8 ± 7.6
Smoking status, n (%)			
Current	208 (60.3)	138 (79.3)	70 (40.9)
Past	105 (30.4)	33 (19.0)	72 (42.1)
Never	32 (9.3)	3 (1.7)	29 (17.0)

LTL = leukocyte telomere length. SD = standard deviation.

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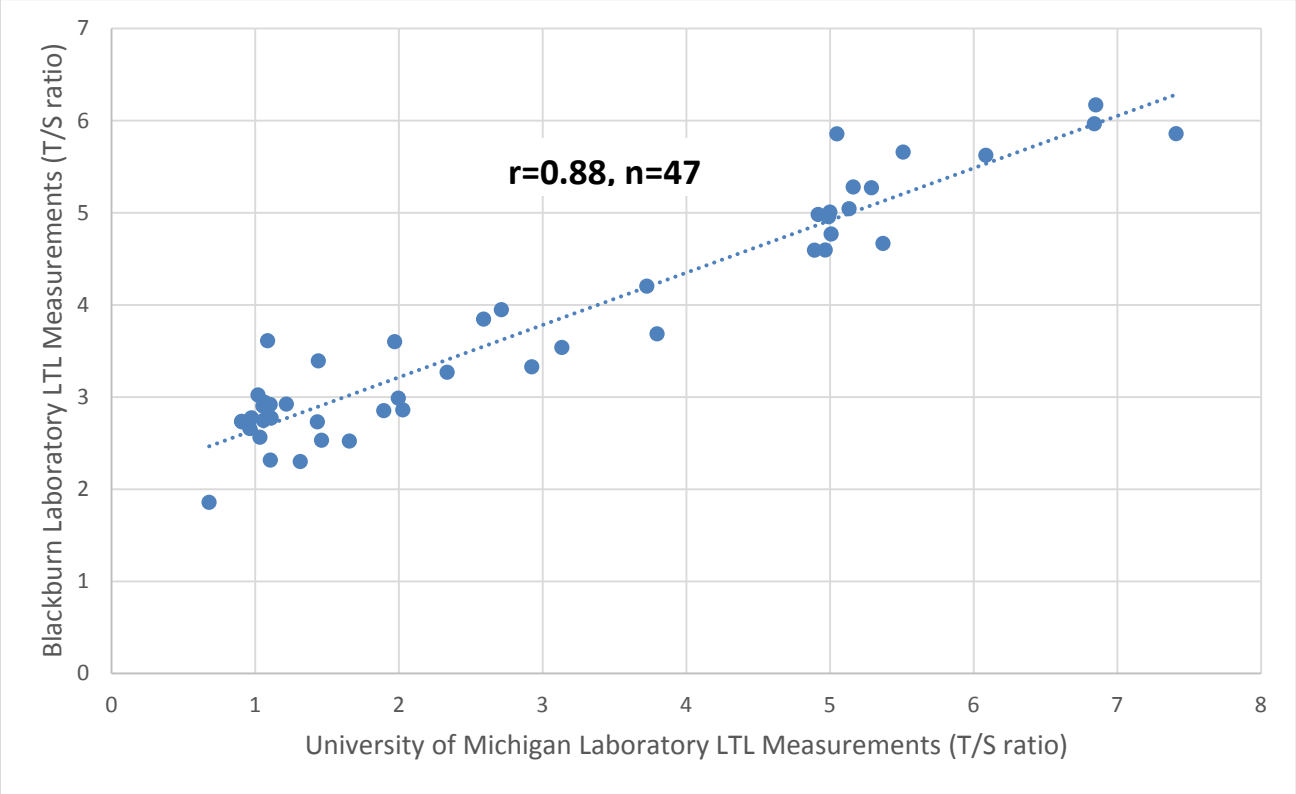
Table S5. Multivariable-adjusted model for the associations between leukocyte telomere length (LTL) and metabolic outcomes among adults

Covariate	Metabolic syndrome component prevalence ratio (95% confidence interval)					
	Metabolic syndrome	Abdominal obesity	High fasting glucose	High blood pressure	Low HDL	High triglycerides
LTL z-score, per 1 z	0.99 (0.85, 1.15)	0.92 (0.82, 1.04)	0.66 (0.44, 0.98)	0.99 (0.82, 1.20)	1.00 (0.93, 1.07)	1.04 (0.93, 1.17)
Sex (male)	0.68 (0.45, 1.01)	0.26 (0.18, 0.37)	0.32 (0.13, 0.82)	1.31 (0.72, 2.38)	1.09 (0.92, 1.29)	1.89 (1.39, 2.58)
Age, per 1 y	1.02 (1.00, 1.04)	1.01 (1.00, 1.02)	1.04 (1.01, 1.08)	1.03 (1.01, 1.05)	0.99 (0.98, 1.00)	1.01 (1.00, 1.02)
Country						
Honduras	Ref	Ref	Ref	Ref	Ref	Ref
Nicaragua	1.64 (1.01, 2.66)	1.54 (1.09, 2.18)	3.59 (0.84, 15.27)	1.48 (0.76, 2.89)	1.14 (0.95, 1.37)	0.93 (0.67, 1.30)
Panama	0.81 (0.42, 1.55)	1.35 (0.90, 2.03)	1.81 (0.37, 8.85)	1.34 (0.62, 2.90)	0.79 (0.61, 1.02)	0.62 (0.44, 0.89)
Costa Rica	1.37 (0.80, 2.35)	0.97 (0.61, 1.55)	2.58 (0.47, 14.26)	2.09 (1.11, 4.00)	1.05 (0.85, 1.30)	0.94 (0.71, 1.23)
Mexico	1.36 (0.80, 2.30)	1.40 (0.96, 2.05)	4.48 (1.03, 19.59)	0.61 (0.26, 1.40)	1.15 (0.97, 1.37)	1.02 (0.77, 1.35)
Belize	1.26 (0.75, 2.11)	1.42 (1.01, 2.00)	4.56 (1.17, 17.75)	0.83 (0.37, 1.85)	1.15 (0.98, 1.36)	0.80 (0.56, 1.14)
Height, per 1 cm	1.03 (1.00, 1.05)	1.02 (1.01, 1.04)	1.05 (0.99, 1.11)	1.01 (0.99, 1.04)	1.00 (0.99, 1.01)	0.99 (0.98, 1.01)
Smoking status						
Never	Ref	Ref	Ref	Ref	Ref	Ref
Past	1.03 (0.75, 1.41)	1.02 (0.81, 1.29)	0.98 (0.40, 2.36)	1.03 (0.65, 1.62)	1.00 (0.88, 1.13)	1.10 (0.90, 1.34)
Current	1.16 (0.68, 1.96)	0.78 (0.42, 1.44)	0.80 (0.13, 5.01)	1.59 (0.87, 2.91)	0.94 (0.78, 1.12)	1.10 (0.78, 1.54)

LTL = leukocyte telomere length. HDL = high density lipoprotein.

† From a Poisson model with LTL z-score as a continuous predictor

Figure S1. Scatterplot from a validation subsample comparing NiMeCoMeS leukocyte telomere length (LTL) measurements performed at the University of Michigan laboratory with measurements performed at the Blackburn laboratory.



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Appendix S1. Telomere Research Network reporting recommendations for PCR-based telomere length measurement.

Sample type, storage, extraction, and integrity:

- Sample type
  - **Whole blood**
- Sample storage conditions, including temperature, duration, and buffer
  - **Samples were collected in EDTA tubes and cryostored at -20 °C on the day of collection. All samples were transported frozen prior to analysis. Because data collection occurred throughout a 14-month period, storage time between collection and DNA extraction varied.**
- DNA extraction method
  - **We extracted DNA from whole blood using a Qiagen Qiasymphony and QIAsymphony DSP DNA Midi Kits (Qiagen, Valencia, CA, USA).**
- DNA storage conditions, including freeze-thaw cycles
  - **DNA was cryostored at -70° C until the time of analysis. All LTL measurements were made within 2 months after DNA extraction. DNA samples were frozen after extraction and thawed before LTL measurement; thus each sample went through approximately 1-2 freeze-thaw cycles between extraction and measurement.**
- Method of documenting DNA quality and integrity
  - **None**
- Percentage of samples specifically tested for DNA quality and integrity
  - **N/A**
- For studies with repeated measures design, report the above for all time points
  - **N/A**

qPCR assay:

- State whether qPCR, MMqPCR, aTL (absolute TL/PCR based) or other PCR based method
  - **MMqPCR**
- PCR machine type
  - **Bio-Rad MyiQ Single Color Real-Time PCR Detection System**
- Source (manufacturer/home-made) of master mix and reagents, and final reaction volume
  - **10 ng of input DNA, iQTM SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and two sets of primers (Invitrogen, Carlsbad, CA, USA). The final reaction volume was 25 µl per well plate.**
- Telomere primer sequences and concentration
  - **telg, ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT and telc, TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA, both with concentration 900 nM**
- Single copy gene name, primer sequences, and concentration
  - **albu, CGGCGGCGGGCGGCGCGGGCTGGGCGGaaatgctgcacagaatccttg and**
    albd, GCCCGGCCCGCCGCGCCCGTCCCGCCGgaaaagcatggtcgcctgtt, both with concentration 900 nM

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- Full PCR program description including temperature, times, and cycle numbers
    - **Stage 1: 15 min at 95°C; Stage 2: 2 cycles of 15 s at 94°C, 15 s at 49°C; and Stage 3: 32 cycles of 15 s at 94°C, 10 s at 62°C, 15 s at 74°C with signal acquisition, 10 s at 84°C, 15 s at 88°C with signal acquisition**
- PCR efficiency of single copy gene and telomere primers
    - **> 90% for both**
- Source and concentration of control samples and standard curve
    - **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/µL). A separate batch of MCF-7 DNA was used as a positive control on all plates at 10 ng/uL.**
- For aTL PCR measurement only: sequence and concentration of oligo standards
    - **N/A**

Data analysis:

- Mean and standard deviation or median and range of telomere lengths
    - **3.32 ± 1.05 in children and 2.20 ± 0.80 in adults**
- Number of sample replicates
    - **All samples were run in triplicate.**
- Level of independence of the replicates (plate vs day vs extraction)
    - **The three replicates were run on the same plate/day, but different wells.**
- Analytic method, considering replicate measurements, to determine final telomere length
    - **Standard curves were used to quantify the telomere (T) and albumin (S) signals for each reaction, and LTL was calculated as the T/S ratio. The median of 3 replicate measures was used as the final LTL value.**
- Method of accounting for variation between sample replicates
    - **We used the median of 3 replicates, which is the optimal method to reduce random variation (Villamor & Bosch, Ann Hum Biol 2015), and calculated average intra-assay CV for all plates (<16%).**
- Method for accounting for well position effects within plates
    - **The replicates were run in different wells within the same plate. We did not correct for well position in these samples.**
- Method of accounting for between-plate effects
    - **We calculated the inter-assay CV (37%) as the standard deviation of all plate-specific T/S means for the control sample divided by the mean of those means.**
- % of samples repeated and % samples failing final QC and excluded from further analyses
    - **Samples repeated: 64/510 (12.5%); samples excluded: 13/510 (2.5%)**
    - **Samples were rerun if the efficiency of the primers was outside 90-110% or if they showed amplification in the negative controls, indicating contamination. Of the 64 repeated samples, 13 were excluded because their amplification curve appeared abnormal or was inconsistent with the rest of the samples on a run, or because they fell outside the range of the standards on the standard curve.**

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- Acceptable range of PCR efficiency for the single copy gene and telomere primers
  - **90-110%**
- ICCs of sample/study groups to address variability (not CV)
  - **We calculated ICC statistics for the triplicate measurements obtained in our laboratory (0.92).**
  - **As a test of validity, we also sent a random selection of 47 samples, covering the entire range of T/S ratios as measured at our laboratory, for testing at Dr. Elizabeth Blackburn's laboratory at the University of California, San Francisco. The agreement between the two measurements was excellent (r= 0.88, ICC=0.74).**
- T/S ratio transformed to a z score prior before comparison across methods/studies
  - **We transformed all the T/S ratios into z-scores prior to analysis.**
- For studies with family samples or repeated measure design: analytic method to account for this
  - **We specified robust estimates of variance in all generalized estimating equation models to account for within-family correlations.**

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