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Seasonal variation of peripheral blood leukocyte telomere length in Costa Rica: a population based observational study

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Abstract

Objectives—Peripheral blood leukocyte telomere length is increasingly being used as a biomarker of aging, but its natural variation in human populations is not well understood. Several other biomarkers show seasonal variation, as do several determinants of leukocyte telomere length. We examined whether there was monthly variation in leukocyte telomere length in Costa Rica, a country with strong seasonal differences in precipitation and infection.

Methods—We examined a longitudinal population based cohort of 581 Costa Rican adults age 60 and above, from which blood samples were drawn between October 2006 and July 2008. Leukocyte telomere length was assayed from these samples using the quantitative PCR method. Multivariate regression models were used to examine correlations between month of blood draw and leukocyte telomere length.

Results—Telomere length from peripheral blood leukocytes varied by as much as 200 base pairs depending on month of blood draw, and this difference is not likely to be due to random variation. A moderate proportion of this association is statistically accounted for by month and region specific average rainfall. We found shorter telomere length associated with greater rainfall.

Conclusions—There are two possible explanations of our findings. First, there could be relatively rapid month-to-month changes in leukocyte telomere length. This conclusion would have implications for understanding the natural population dynamics of telomere length. Second, there could be seasonal differences in constituent cell populations. This conclusion would suggest that future studies of leukocyte telomere length use methods to account for the potential impact of constituent cell type.

Keywords

telomere length; seasonality; lymphocytes; infection; rainfall

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Introduction

In this population based observational study we examine seasonal variation of mean levels of peripheral blood leukocyte telomere length (LTL): a biomarker that has been proposed as a cellular-level marker of aging (Bakaysa and others, 2007). The study objective was motivated by four distinct literatures. First, a number of biomarkers associated with health have been demonstrated to exhibit seasonal variation. While this has been best characterized for blood pressure (Brennan and others, 1982; Rose, 1966; Woodhouse and others, 1993a), it has also been shown for other biomarkers such as C-reactive protein (Chiriboga and others, 2009; Sung, 2006) and serum lipids (Woodhouse and others, 1993b), as well as mortality itself (Crombie and others, 1995; Gemmell and others, 2000). Most evidence suggests that temperature and infection are the drivers of these variations, and that the seasonal differences are not universal but are dependent on both social and environmental factors (Lewington and others, 2012; van Asten and others, 2012). Second, behavioral factors, such as physical activity and dietary patterns that have been associated with LTL (Cassidy and others, 2010; Krauss and others, 2011; Puterman and others, 2010) also have been shown to have seasonal variation (de Castro, 1991; Tucker and Gilliland, 2007). Third, prior studies have shown that mean leukocyte telomere length can vary over relatively short periods of time, as short as months (Svenson and others, 2011). Finally, prior work has shown that LTL also differs in different peripheral blood cell types (Lin and others, 2010a), so differences in constituent cells could also lead to short term variation in LTL. Cell subtypes fluctuate with infection (Hillaire and others, 2011), which has a seasonal nature in many locations, including in Costa Rica (Herrero-Uribe and Vargas-Martínez, 1988; Salas-Chaves and Alfaro-Bourrouet, 2005).

Telomeres are specialized DNA-protein structures at the ends of eukaryotic chromosomes that protect chromosome ends and prevent the degradation of coding regions of DNA that would otherwise result from the inability of DNA replication enzymes to copy the end of a DNA strand. With each round of DNA replication there is an associated loss in telomere length. Telomere length can be increased with the activity of a specialized cellular reverse transcriptase, telomerase. While telomerase levels are high during embryonic development, in adults telomerase activity is lower and generally thought to be confined to stem cells and progenitor cells (Hiyama and Hiyama, 2007). Thus the biological basis for telomere length as a biomarker of aging is based upon its function as a metric of cellular age, which is directly dependent on cellular proliferation, differentiation and oxidative stress.

Despite a multi-decade search for a biomarker of aging, defined as "a biological parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capacity at some late age than will chronological age," (Baker and Sprott, 1988) no such factor has been identified (Der and others, 2012). While not meeting this definition (Der and others, 2012), many studies have shown LTL to be associated with health and functional status outcomes independent of chronological age (Bakaysa and others, 2007; Brouilette and others, 2007), although others, particularly when examining LTL in older populations or with limited follow-up time, have not (Mather and others, 2010; Mather and others, 2011). A recent review identified 124 cross-sectional and 5 longitudinal studies of LTL that examined associations with age, finding the relationship

was very consistent, but that variations of the rate of decrease are still unclear, in large part due to lack of longitudinal studies (Muezzinler and others, 2013). While LTL is impacted by genotype (Codd and others, 2010), external factors such as stress and socioeconomic position have also been found correlated with LTL in humans (Adler and others, 2012; Batty and others, 2009; Cherkas and others, 2006; Epel and others, 2009a; Epel and others, 2004; Epel and others, 2010b; Lee and others, 2002).

Assay of telomere length from peripheral blood leukocytes has both practical and biological advantages that have lead to its increasingly common use in population-based studies as a marker of biological age (Mather and others, 2011). Blood is obtained less invasively from individuals in the course of large population based studies as compared to tissue samples. Biologically, mean telomere length of leukocytes offers a window into the physiological aging of the organism that in particular captures immune system aging independently of calendar age. There are, however, some challenges to inference from this metric because peripheral blood contains five different types of leukocytes that contribute to the assayed LTL: neutrophils, lymphocytes, eosinophils, basophils and monocytes. Neutrophils and lymphocytes makeup the majority of cells. Within lymphocytes, differences in telomere length and telomerase activity have been characterized specific to B cells (which release antibodies), CD4+ T cells (which activate and regulate T and B cells) and CD8+ T cells (which target virus infected cells). Most notably, CD8+CD28-have shorter telomere lengths, and B cells have longer telomere length (Lin and others, 2010b). Within individual correlation between LTL in these cell types ranged from 0.36 to 0.67 (Lin and others, 2010a), although earlier work examining telomere length in neutrophils and T lymphocytes suggested that differences in length by cell type were minimal enough to support the use of the total leukocyte cell population to study telomere length dynamics (Robertson and others, 2000). Thus while overall LTL has been shown to be useful, it is a metric that is impacted by both mean LTL within cell subtypes, and also the proportion of cell subtypes within the sample.

Telomerase activity, responsible for the lengthening of telomeres, is highly variable in peripheral blood, dependent in part on whether T and B cells are undergoing differentiation (Hiyama and others, 1995). In non-differentiating resting B and T cells, while telomerase activity is generally low, it is cell type -specific (Lin and others, 2010b), and also undergoes substantial upregulation when T and B cells are stimulated by pathogens during an immune response (Hiyama and others, 1995). This is important for allowing the massive clonal expansion of T and B cells that is required to mount a suitable immune response. However, the extent of upregulation of telomerase activity is not of a sufficient magnitude to prevent some telomere length attrition with clonal expansion, and thus decreases in telomere length are observed among the related cell types (Monteiro and others, 1996), although exceptions have been found for certain types of infections and certain T cell subsets (Maini and others, 1999). Memory T cells have generally been shown to exhibit shorter telomere length than naïve T cells, but this differs by type (Weng, 2008).

Despite increased use of mean telomere length assayed from peripheral blood leukocytes as an indicator of biological aging, there is still a lack of information on the natural variation at the level of national populations. Opportunities for examination of this are rare because

there are very few population based longitudinal studies in which telomere length has been assayed on a large enough number of individuals to assess variation in mean peripheral blood LTL (Aviv and others, 2009; Chen and others, 2011; Ehrlenbach and others, 2009; Epel and others, 2009c; Farzaneh-Far and others, 2010; Nordfjall and others, 2009). In addition, comparison of absolute telomere length across studies is hindered because assays for telomere length are generally only internally consistent – population based comparisons are best performed within a single batch of assays from the same lab. The largest population based study of LTL has shown associations between short telomere length and specific causes of death, but natural variation in telomere length in this population has not been described (Weischer and others, 2012). A notable exception to the lack of study of natural variation of LTL is a study that examined variation in LTL between 11 countries in Europe, finding that variability is substantial (Eisenberg and others, 2011).

The objective of the current study is to examine whether there are relatively short time period (monthly) variations in telomere length occurring in Costa Rica, a context where there are well-documented seasonal differences in environmental conditions associated primarily with rainfall. Our exploration of this was based on the literature of seasonal variation of other biomarkers and behaviors related to LTL, short-term LTL dynamics, and knowledge of short -term fluctuation in constituent lymphocyte populations. There was not sufficient prior evidence to suggest a direction or temporal patterning to the variation, thus our analysis is best characterized as exploratory and descriptive, rather than hypothesis testing.

Methods

Study sample

We examined LTL in a sub-sample of 612 elderly individuals drawn from the nationally representative Costa Rican Study on Longevity and Healthy Aging (CRELES). CRELES is a longitudinal study based on a national sample of 2,827 residents of Costa Rica aged 60 and older in 2005, with oversampling of the oldest old (Rosero Bixby and others, 2010). The CRELES sample was selected from the 2000 census database using a multi -stage sampling design and complemented with a 100% sample of near-centenarians and centenarians of the Nicoya region that rendered 91 additional participants in this longitudinal study. The mean age of the overall CRELES sample was 76 with a range from 60 to 109. The ability of the CRELES sample to provide unbiased descriptions of characteristics of elderly Costa Ricans and of their mortality has been assessed elsewhere (Rosero Bixby and others, 2010; Rosero-Bixby and Dow, 2009). The "Committee on Science and Ethics" of the University of Costa Rica approved the study, and all participants provided written informed consent. We randomly drew a sub-sample of CRELES data for assaying telomere length, selecting approximately 200 individuals in each of three different age strata: 60–75, 76–94, and 95 and over, thus implicitly oversampling older individuals. All analyses control for age to account for the nature of the sampling. We included in the subsample the DNA from blood extracted during two waves of visits to participants in the CRELES panel, which mostly took place in 2005 and 2007. There were 365 individuals who had telomeres measured at two time points and 247 with only one measurement; 86 because of death before the second

wave visit. Reasons for the 161 individuals missing a second observation were: lost of follow up (n=59), refusal to providing the fasting blood sample (n=28), and the rest due to losses in laboratory procedures including 23 samples in which the DNA concentration was not appropriate for measuring LTL. Individuals who were alive and missing a second observation do not differ significantly from those with two observations in terms of age, sex, rural residence or level of education. The mean length of time between samples was 598 days, with a minimum time between samples of 365 days and maximum time between samples of 903 days.

Telomere length assay

To measure mean LTL, quantitative PCR assay was used to determine the relative ratio of telomere repeat copy number to single-copy gene copy number (t/s ratio), with the analyses using the average of two assays per DNA extraction sample. The LTL assay lab was blind to the sample characteristics, and samples were assayed without association between date of survey or geographic origin. The inter-assay coefficient of variability for LTL was 3.7%. The correlation of LTL between samples was 0.57. Some of this strength of correlation in our study may be due to measurement error that was inherent to the quantitative PCR telomere length assay (Steenstrup and others, 2013).

Twenty-three individuals (nine from the first wave and 14 from the second wave) did not have sufficient DNA quantity to perform the assays, leaving an assayed sample of 977. Individuals without sufficient DNA quantity did not differ significantly by any demographic characteristics. The equation for conversion from T/S ratio to base pairs used was base pairs = 3274 + 2413*(T/S) based on prior work (Farzaneh-Far and others, 2010). This conversion ratio is likely to differ between labs, and even between assays within the same lab, and thus exact base pair values we report should be used as an approximation of actual telomere length. While this does not impact the internal validity of the analyses presented here, the absolute level of base pair length should not be used in direct comparison to other studies. The assays were conducted in the Blackburn Laboratory at the University of California, San Francisco.

Covariates

Exact ages were computed from birth dates taken from participants' id-card, double checked against the national database of births (in this way we avoid the problem of exaggeration of self-reported age). Other demographic covariates, including education, were obtained through in-person interview. All blood and urine specimens were collected in participants' homes. Rural was defined by the Costa Rican 2000 census definition, which included administrative, historical and physical criteria (such as the presence of city blocks and streets and electric, water and trash collection services) to establish city limits (INEC, 2004). Educational attainment was categorized into three groups: less than three years of education, from three to six years of education (elementary school comprises six grades), and at least one year of high school. Body mass index (BMI) was calculated from measured weight and height. Sitting systolic and diastolic blood pressure were measured twice during the interview and the average reading was used. Triglycerides and C-reactive protein (CRP) were measured from fasting serum; glycosylated hemoglobin (HbA1c) was measured from

whole blood. HbA1c (range 4.3% to 14.7%) was determined in two laboratories. The first laboratory used High Yield Liquid Chromatography (HPLC). The second used inhibition with latex agglutination. The first laboratory was taken as the standard. Results from the second lab oratory were adjusted with an equation estimated by regression in a validation lot of 40 samples. CRP(range 0.36 to 99.1 mg/L) was determined in two laboratories. The first laboratory used aggregation of particles covered with monoclonal antibodies and the Dade Bohering BN System automated equipment. In the second laboratory a high-sensitivity CRP method was used with the automated equipment KONELAB. The first laboratory was established as the standard laboratory and results of the second laboratory were adjusted with an equation estimated by regression in a validation lot of 40 samples. We linked mean values of temperature (minimal and maximal) and rainfall to each study observation on the basis of location and month of blood draw. We defined 15 regions, one for each of the 15 meteorological observation sites, using historical information of mean rainfall and temperatures per month over a period of approximately 20 years as provided by the Costa Rica Meteorological Institute (IMN, 2013). The use of minimum and maximum average temperature was based on the use of these measures for the examination of seasonal differences in other biomarkers (Brennan and others, 1982). The maps in figure 1 show the mean level of rainfall in the 15 regions in a representative month of the dry season (December) and the rainy season (July). The mean of the regional rainfall by month was 164 mm. The map also shows the location of the sites that collected the climate data and the location of the individuals in the sample contacted during the corresponding month. The seasonal difference in rainfall depended on both region and month (IMN, 2013).

Models

We used ordinary least squares regression models to examine predictors of telomere length. All errors reported are robust standard errors. Our choice of covariates to include in the regression models were based on rules derived from the use of directed acyclic graphs (Glymour, 2006). Briefly, this means that we included covariates that based on prior knowledge we believed had the potential to cause both the exposure and outcome, but are not on the hypothesized causal pathway between exposure and outcome, and are not caused by the outcome. The factors included: BMI, BMI squared, diastolic blood pressure, systolic blood pressure, triglycerides, HbA1c and CRP (Bekaert and others, 2007; Epel and others, 2009b; Fitzpatrick and others, 2007; Valdes and others, 2005; Zannolli and others, 2008). We also included BMI squared to account for the potentially non-linear relationship between BMI and exposure and outcome. Analyses accounted for the clustered nature of the sample since some individuals were measured twice. We fit two types of models, a random effect model to account formultiple observations across individuals, and an individual fixed effect model. The fixed effect model statistically controls for all measured and unmeasured non-time-varying confounding factors because it examines within individual differences. The smoothed plots of difference in telomere length by month of blood draw and mean rainfall per day were fit using generalized additive mixed models with a maximum three degrees of freedom regression spline for the association with month, conditional on rurality, Nicoya region, age and wave of data collection. Generalized additive mixed models fit multiple linear segments to data, using a penalized fit algorithm to prevent overfitting the data (Wood, 2006).

Results

From the first wave of the CRELES subsample, we estimate a LTL average for elderly Costa Ricans of 5,289 base pairs (95% confidence interval: 5,257 – 5,323). Table 1 presents a description of telomere length in base pairs in the unweighted sample. Consistent with prior findings, average telomere length is slightly longer among women and is shorter at older ages. In Costa Rica, those living in rural areas and in Nicoya have longer telomeres. Individuals with seven or more years of education have longer telomere length. There were also shorter telomere lengths when blood was drawn during months and in regions that had historically greater daily average rainfall.

Figure 2 shows the results of a generalized additive mixed model of month of blood draw with telomere length as the dependent (outcome) variable, controlling for rural area of residence, Nicoya region, age and wave of data collection. We find a statistically significant (p<0.01) pattern to mean telomere length by month over the years our data were collected, going from the shortest average telomere length in May to July to the longest average telomere length in November to January. The variability by month (difference between month with longest and shortest telomere length) is approximately 200 base pairs.

Table 2 shows the results of models of the association of month of blood draw with mean LTL. May is the month with the shortest average telomere length so it was used as the comparison group. In model 1, controlling only for Nicoya region and rural region, telomere length in January, June and November were statistically significantly longer. After additional controls for demographic covariates these estimates remained similar but were no longer statistically significant except in November. Controlling for other biomarkers (Model 2 versus Model 3) and maximum and minimum temperature in month of blood draw (Model 4) did not result in any attenuation of coefficients. In model 5, we additionally control for the monthly average of daily rainfall in the month and region of blood draw, and the substantially higher and statistically significant longer telomere length in November is attenuated. One cm higher daily rainfall is associated with 121 base pairs shorter LTL. Overall, the attenuation of coefficients is greatest for December through April.

Table 3 shows the results from a parallel set of models, but now fitting the model with individual fixed effects. These models, as contrasted with the model fit presented in table 2, examine within individual associations. Since all individuals in the dataset were in the same location at both measures, this allows us to now factor out the potential confounding effects of any non-time varying place or individual characteristics, and identify associations based on the time-varying correlations of month and rainfall with LTL. These models show an even stronger association between average cm of daily rainfall in the region and month and LTL. The model-based estimate of the association of rainy season with LTL is 370 base pairs shorter with 1 cm greater of rainfall, which is statistically significant at p<0.01. If model 5 is fit without indicators for month (results not shown), cm of rain remains statistically significantly associated with shorter telomere length, although with a smaller effect estimate of 172 base pairs (p<0.01). The associations with month differ somewhat from those from the random effect model presented in table 2, likely due to the fact that

within the individual there are no geographic differences in rainfall since individuals did not move between observations.

Figure 3 shows the results of a generalized additive mixed model of monthly and regional mm of rain per month with LTL as the dependent (outcome) variable, controlling for rural area of residence, Nicoya region, age and wave of data collection. This smoothed model allows us to account for some of the instability in association by borrowing information from adjacent measurement of rainfall in order to see systematic patterns in the data (Wood, 2006). We find a regular pattern to LTL by rainfall over the years our data were collected, with shorter telomere length associated with greater average rain per day. The difference between no rain and the greatest rain per day is approximately 2 00 base pairs of LTL.

Discussion

We provide initial evidence for the first report of a seasonal pattern of telomere length derived from peripheral blood leukocytes in humans. This pattern is characterized by shortest telomere length in May through July, and longest telomere lengths in November through January. When we statistically control for monthly region specific rainfall at the time of blood collection, the association with month of blood draw is partially attenuated. In within individual (fixed effect) regression models a 1 cm difference in average daily rainfall is associated with 370 base pairs shorter telomere length (p<0.01).

There are three general classes of explanation for why this variation may occur, and why it maybe associated with rainfall: first, it may be an artifact of blood data collection and/or analysis methods, second, it may be due to relatively rapid telomere length changes, third, it may be due to differences in leukocyte cell types by month. We believe that it is unlikely that the differences in telomere length by month and rainy season are due to artifacts of data collection or statistical analyses. We have a relatively large sample size that includes multiple observations in each region and month resulting in stable estimates of mean differences in peripheral blood LTL. Our within-individual analyses, where we see the greatest association with mm of rain, additionally control for any non-time varying individual characteristics. Standard procedures for this large demographic survey were used for the transport and storing of samples that did not vary by region or season and all LTL assays were done blind to sample characteristics and at the same time in the same lab.

The second possible explanation is that there were changes in telomere length by season. Given current knowledge of telomere length dynamics, it is unclear whether upregulation of telomerase in peripheral leukocytes would be rapid enough and substantial enough to account for these differences. While telomerase is active (and appears highly variable) in peripheral blood, it is not known whether it is of a sufficient magnitude to create such changes in LTL over a relatively short period of time. Recent experimental evidence in humans on acute (1 hour) changes in telomerase activity of up to 18% (Epel and others, 2010a) suggests that this explanation remains a reasonable possibility. In addition, results from a study with multiple measures of LTL in humans showed significant changes in telomere length within a six month period (Svenson and others, 2011). Based on these findings, an "oscillation hypothesis" was proposed, where there are short-term fluctuations

in LTL around an overall trend that generally declines with age. While there has been some criticism that increases in LTL could be a statistical artifact, recent methodological work using simulated data is consistent with the fact that even small samples are sufficiently powered to detect true telomere elongation (Simons and others, 2013). In addition to infection, both physical activity and dietary patterns have seasonal variation (de Castro, 1991; Tucker and Gilliland, 2007), and both of these factors have been shown to be associated with LTL (Cassidy and others, 2010; Krauss and others, 2011; Puterman and others, 2010).

The third possible explanation is that there may be differences in the composition of leukocyte cell types by month, and because different populations of cell types have different mean telomere lengths, this could result in the observed differences in mean LTL telomere length by month. Naïve CD4 lymphocyte telomeres have been shown to be up to 2.5 kb longer than memory CD4 lymphocytes, consistent with the number of replications of each type of cell (Rufer and others, 1998). Other work has shown that B cells have the longest telomere length and CD8+ CD28-T cells have the shortest telomere length (Lin and others, 2010a). This particular pattern is consistent with the changes in peripheral blood cell types that accompany some types of infection, thus producing a mix of cells with a mean shorter telomere length during acute immune response to infection. Experimental work in mice infected with *Salmonella* has shown rapid shortening of telomere length with infection (Ilmonen and others, 2008). Prior work in Costa Rica has shown higher levels of infection during times of the highest rainfall (Herrero-Uribe and Vargas-Martínez, 1988; Salas-Chaves and Alfaro-Bourrouet, 2005). We cannot, however, definitively distinguish between these two substantive explanations. Further observational and experimental work will be necessary to determine which of these hypothesized mechanisms is responsible for the seasonal variation that we document in this analysis.

There are several limitations to our current study and analysis. We cannot assay for the composition of lymphocytes in each individual blood sample. Because of this, we are not able to determine whether it is the differential distribution of cell types by season that is driving the differences in telomere length per month and the association with precipitation. We also only have data on regional average precipitation by month, and this measurement error is most likely to lead to some underestimation of effect sizes of rain fall. We do not have data on infection in order to understand the extent that these patterns could be explained by an acute immune response in study participants at the time of blood draw. Our analysis included controlling for participant's CRP, an indicator of acute infection. This did not meaningfully change results, whether used as a continuous measure (as shown), or as a dichotomous variable indicating infection, 10 mg/L and above (data not shown). Finally, our analysis is best described as exploratory, because we did not have strong priors about the direction or nature of the seasonal patterns. This increases the possibility of type 1 error of finding a pattern when it does not actually exist. Therefore, while our findings are suggestive, they should be repeated in other contexts and data sources in order to be confirmed.

The generalizability of our results to other contexts is unclear. If driven primarily by the seasonal variation in precipitation and associated population average levels of acute

infection, it is likely that such patterns could be observed in other countries that experience seasonally differential burdens of infection, although this is likely to depend on the type of infection and the extent of seasonal difference. Similarly, if behavioral factors are behind seasonal differences in LTL, we would expect similar results only in contexts with similar seasonality in these behavioral factors. Comparing seasonal patterns of LTL in different contexts may provide indirect evidence for the source of the variation we observed.

Our findings that show substantial temporal variation in LTL add to prior findings that have found substantial spatial variation (Eisenberg and others, 2011). In our current study, we used an individual fixed effect analysis approach to minimize the extent to which the variation could be explained by non-time varying sociodemographic factors. In the prior study of spatial variation, the impact of sociodemographic factors was controlled through using a restricted age and gender sample, and by controlling for national level measures of population structure. We are not aware of other studies that were able to explore natural variation due to the difficulties in obtaining demographically comparable samples with telomere length assayed with a similar protocol.

Our findings have implications for future studies that utilize peripheral blood leukocytes. Our results also provide evidence of the extent to which there is natural variation (presumably due to external factors) that may (but not necessarily) confound attribution of LTL as a marker of morbidity and mortality. There are several possible approaches to controlling for the potential confounding of cell type. Ideally, future studies should adopt the practice of examining telomere length within specific cell types (Lin and others, 2010b). When this is not possible, for example in large scale field based population studies, data from DNA methylation arrays may be used to adjust coefficients for cell type composition (Houseman and others, 2012). Absent the possibility of either of these more direct approaches, studies should control for one specific type of external factor that impacts celltype composition: type and presence of infection at the time of blood draw. Utilization of one or more of the se approaches in future work will help to clarify reasons for the seasonal variation in LTL we have observed.

Acknowledgments

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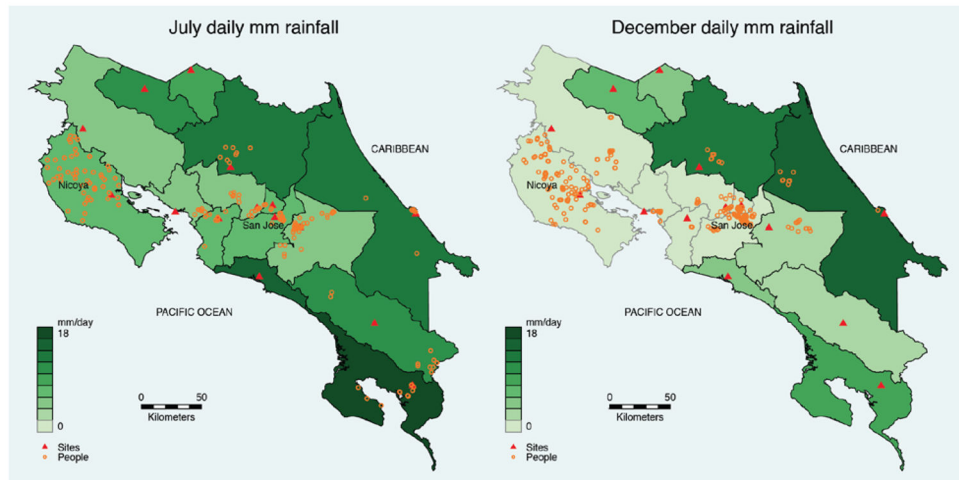
Figure 1.
Maps of average daily rainfall in regions of Costa Rica in July and December. The darkness of green shows the daily average rainfall in mm in July (left panel) and December (right panel), with darker green indicating greater average rainfall. The red triangles show sites of rain data collection. The orange circles show location of samples in that particular month.
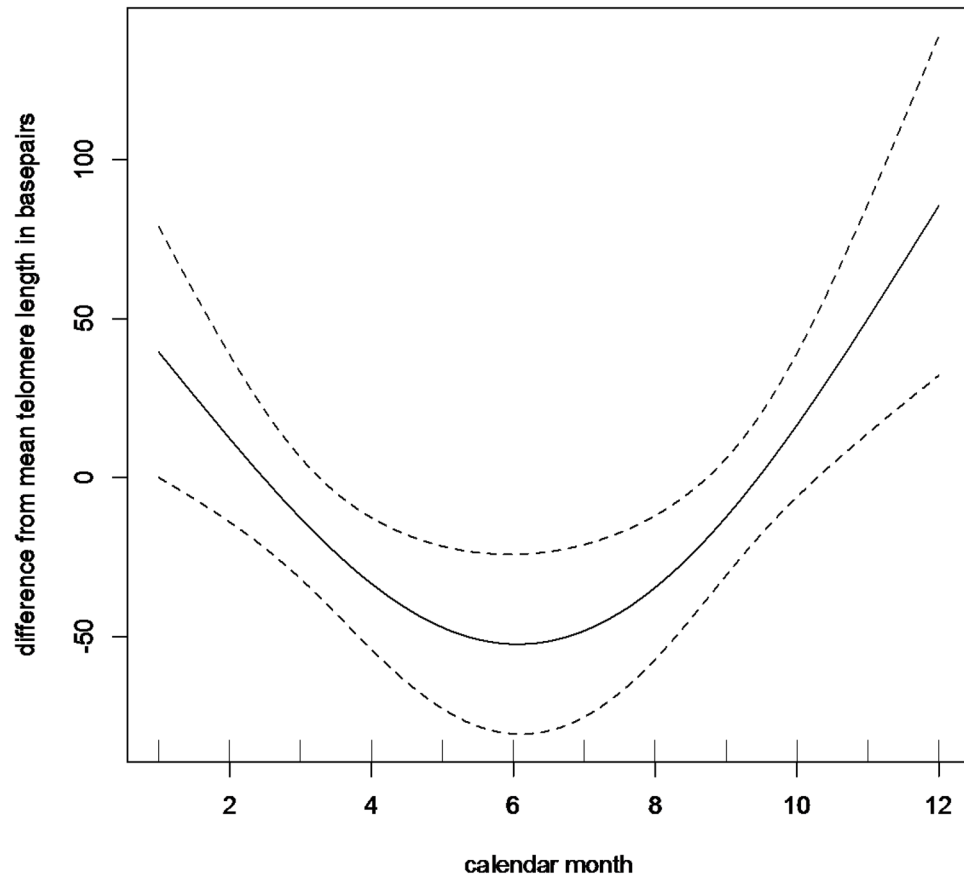
Figure 2.
Smoothed modeled relationship of mean telomere length and month of blood draw. The mean relationship is shown as solid line as estimated from a generalized additive mixed model controlling for rural, Nicoya, age and data collection by month of blood draw, CRELES. Dashed lines show 95% confidence intervals. Horizontal axis shows month (1=January, 2=February, …, 12=December) and the vertical axis shows number of base pairs difference from the mean leukocyte telomere length in the population.
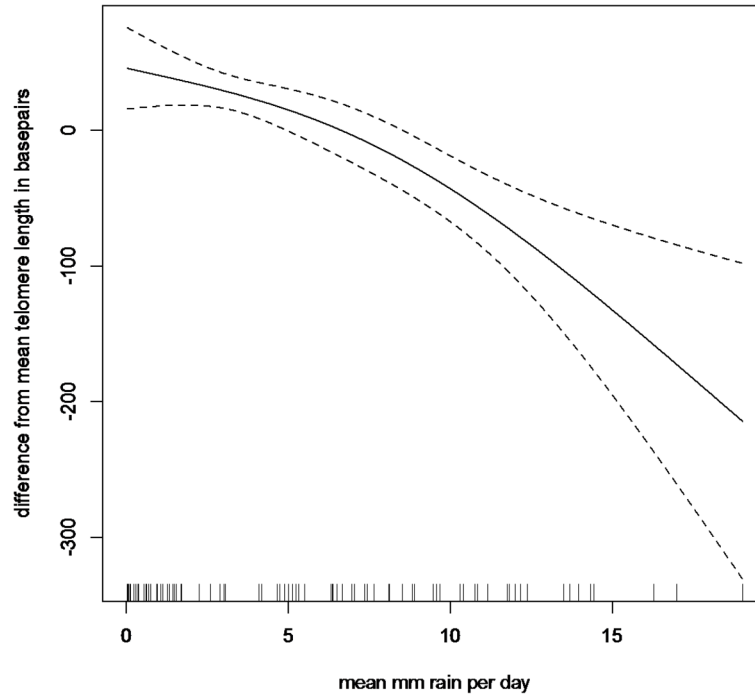
Figure 3.
Smoothed modeled relationship of mean telomere length and average historical days of rain in month and region where blood draw was from. Mean relationship shown as solid line as estimated from a generalized additive model controlling for rural, Nicoya, age and wave of data collection by mean mm of rain per day, CRELES. Dashed lines show 95% confidence intervals. Horizontal axis shows average mm of rain per day and vertical axis shows number of base pairs difference from the mean telomere length in the population. Tick marks on horizontal axis show where there are values for mm of rain per day.

Table 1

Mean telomere length (in basepairs) and standard errors by demographic and health characteristics, CRELES study sample, age 60 and above

	Mean telomere length
Total (n=977)	5154 (13)
Gender	
Men (n=457)	5120 (19)
Women (n=520)	5185 (17)
Age	
60–74 (n=343)	5338 (25)
75–94 (n=319)	5130 (19)
95+ (n=315)	5017 (20)
Urban	
Urban (n=501)	5124 (16)
Rural (n=476)	5186 (20)
Region	
Nicoya (n=358)	5195 (23)
Other (n=619)	5131 (15)
Education	
<3 years (n=462)	5150 (19)
3–6 years (n=440)	5155 (19)
>=7 years (n=75)	5174 (46)
Average rain per day (in mm)	
0.032 – 1.5 (n=332)	5205 (23)
1.6 – 6.5 (n=360)	5149 (21)
6.6 – 19 (n=285)	5100 (22)
Maximum mean temperature in month (degrees Celsius)	
22.7 – 25.9 (n=328)	5110 (20)
26.0 – 32.1 (n=325)	5183 (23)
32.2 – 36.0 (n=324)	5170 (23)
Minimum mean temperature in month (degrees Celsius)	
13.5 – 17.9 (n=330)	5121 (21)
18.0 – 22.8 (n=350)	5177 (22)
22.9 – 25.3 (n=297)	5164 (24)

N in the table indicates the number of observations, not the number of unique individuals. The number of unique individuals is 581. The distribution by age is 195 individuals age 60–74, 180 individuals age 75–94, and 206 individuals age 95 and above.

Table 2

Random effects modeled association of month of blood draw with telomere length as compared to May as comparison month with additional control for region (Model 1), region and demographic covariates (Model 2), region, demographic covariates and biomarkers (Model 3), region, demographic covariates, biomarkers, minimum temperature and maximum temperature (Model 4), region, demographic covariates, biomarkers, minimum temperature, maximum temperature and mm of daily rainfall (Model 5).

	Model 1	Model 2	Model 3	Model 4	Model 5
January	139 (63)*	82 (59)	88 (60)	95 (62)	−16 (73)
February	71 (74)	31 (67)	32 (72)	47 (74)	−59 (84)
March	111 (75)	81 (68)	76 (70)	81 (72)	−20 (83)
April	73 (82)	69 (78)	72 (81)	63 (81)	−20 (86)
May (comparison)	-	-	-	-	-
June	152 (68)*	98 (61)	94 (64)	107 (66)	74 (66)
July	−59 (67)	50 (62)	112 (69)	118 (69)	84 (71)
August	19 (73)	0 (65)	10 (67)	8 (66)	5 (67)
September	56 (69)	3 (65)	6 (69)	18 (71)	20 (70)
October	13 (73)	−17 (70)	−16 (75)	−2 (77)	13 (77)
November	216 (67)**	195 (62)**	200 (64)**	215 (67)**	147 (70)*
December	132 (68)	92 (64)	108 (69)	115 (70)	32 (76)
Maximum Temp (C)				3.0 (9.5)	0.18 (9.5)
Minimum Temp (C)				5.3 (12)	11 (12)
cm of Rainfall					−121 (51)*

Standard errors in parentheses. All errors are robust standard errors and account for clustered sample by individual. Biomarkers controlled for in model 3, 4 and 5 were BMI, BMI squared, diastolic blood pressure, systolic blood pressure, triglycerides, HbA1c and C-reactive protein. Sample size is 977 observations for models 1 and 2 and 861 observations for models 3, 4 and 5 due to missing data on some biomarkers. Restricting models 1 and 2 to complete case analysis of 861 observations results in no meaningful differences in coefficients.

*
indicates p<0.05,

**
indicates p<0.01

Table 3

Within individual Fixed Effect models of month of blood draw with telomere length as compared to May as comparison month with additional control for region (Model 1), region and demographic covariates (Model 2), region, demographic covariates and biomarkers (Model 3), region, demographic covariates, biomarkers, minimum temperature and maximum temperature (Model 4), region, demographic covariates, biomarkers, minimum temperature, maximum temperature and mm of daily rainfall (Model 5).

	Model 1	Model 2	Model 3	Model 4	Model 5
January	137 (85)	140 (83)	136 (85)	18 (114)	−253 (130)
February	82 (105)	74 (101)	72 (112)	−97 (148)	−327 (154)*
March	103 (101)	83 (97)	90 (98)	−29 (120)	−285 (131)*
April	78 (104)	42 (100)	57 (95)	−21 (95)	−177 (109)
May (comparison)	-	-	-	-	-
June	80 (116)	48 (112)	75 (117)	140 (120)	136 (123)
July	8 (84)	−75 (80)	−33 (77)	−25 (85)	−25 (88)
August	116 (125)	79 (119)	153 (126)	51 (130)	64 (137)
September	−118 (118)	−96 (113)	−124 (112)	−153 (113)	−91 (115)
October	−35 (102)	21 (108)	21 (112)	9 (125)	115 (133)
November	114 (99)	122 (96)	97 (96)	66 (112)	−83 (122)
December	103 (84)	144 (85)	118 (89)	74 (111)	−194 (128)
Maximum Temp (C)				31 (26)	21 (26)
Minimum Temp (C)				−135 (72)	−128 (71)
cm of Rainfall					−370 (93)**

Standard errors in parentheses. All errors are robust standard errors from individual fixed effect regression models. Biomarkers controlled for in models 3, 4 and 5 were BMI, BMI squared, diastolic blood pressure, systolic blood pressure, triglycerides, HbA1c and C-reactive protein. Sample size is 977 observations for models 1 and 2 and 861 observations for models 3, 4 and 5 due to missing data on some biomarkers. Restricting models 1 and 2 to complete case analysis of 861 observations results in no meaningful differences in coefficients.

*
indicates p<0.05,

**
indicates p<0.01