RESEARCH ARTICLE

Decline in telomere length by age and effect modification by gender, allostatic load and comorbidities in National Health and Nutrition Examination Survey (1999-2002)

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Abstract

Background

This study aims to assess the decline in telomere length (TL) with age and evaluate effect modification by gender, chronic stress, and comorbidity in a representative sample of the US population.

Methods

Cross-sectional data on 7826 adults with a TL measurement, were included from the National Health and Nutrition Examination Survey, years 1999–2002. The population rate of decline in TL across 10-year age categories was estimated using crude and adjusted regression.

Results

In an adjusted model, the population rate of decline in TL with age was consistent and linear for only three age categories: 20–29 (β = -0.0172, 95% CI: -0.0342, -0.0002), 50–59 (β = -0.0182, 95% CI: -0.0311, -0.0054) and 70–79 (β = -0.0170, 95% CI: -0.0329, -0.0011) years. The population rate of decline in TL with age was significantly greater for males and those with high allostatic load and a history of comorbidities. When the population rate of decline in TL was analyzed by gender in 10-year age bins, a fairly consistent yet statistically non-significant decline for males was observed; however, a trough in the rate was observed for females in the age categories 20–29 years (β = -0.0284, 95% CI: -0.0464, -0.0103) and 50–59 years (β = -0.0211, 95% CI: -0.0391, -0.0032). To further elucidate the gender difference observed in the primary analyses, secondary analyses were conducted with reproductive and hormonal status; a significant inverse association was found between TL and parity, menopause, and age at menopause.

Conclusions

TL was shorter with increasing age and this decline was modified by gender, chronic stress and comorbidities; individuals with chronic morbidity and/or chronic stress and females in their twenties and fifties experienced greater decline. Female reproductive factors, i.e., parity and menopause, were associated with TL.

Introduction

Telomeres, nucleoprotein structures located at the ends of eukaryotic chromosomes, protect the end of the chromosome from degradation and end-to-end fusion [1]. With each somatic cell division, there is a gradual attrition of the telomere, resulting in telomere length shortening with increasing age [1]. Telomere length (TL) has been proposed as a candidate biomarker of aging [2] whereby longer TL is an indicator of healthy aging. Preservation of TL among healthy individuals, in comparison to those with multiple morbidities, is thought to be one of the several pathways by which the development of chronic diseases and mortality can be explained. Although the association between health status and TL has been fairly well established, it is unclear how quickly TL declines with increasing age, or whether there is any effect modification by gender, chronic stress and morbidities, all of which influence telomere dynamics [3,4,5,6].

Although TL shortens with increasing age [7], TL decreases are not directly proportional with age. Gender, chronic stress, and comorbidities may modify the relationship between age and TL. Females have longer telomeres than males [6]. Chronic psychosocial stress, depression, anxiety, and childhood trauma was associated with shorter TL [4,8,9,10,11]. Perceived stress was associated with lower telomerase activity, and shorter telomere length among healthy premenopausal women [4]. An association between childhood trauma and shorter telomere length in adulthood has been reported [9]. Stressful life events within the last five years was associated with associated with shorter telomeres in Netherlands Study of Depression and Anxiety [12]. A systematic review aimed to examine whether chronic social stress is associated with telomere length throughout the life course, concluded that chronic social stress was associated with shorter telomeres in both early and adult exposures [13]. Further, evidence suggests that shorter telomeres is associated with greater cortisol reactivity to stress, central elements of the physiological stress response system [14,15]. "Allostatic load," also called the wear and tear in the body [16], has been proposed as a conceptualization of cumulative stress exacted on the body through attempts to adapt to life's demands [17]. Stress, an inevitable condition of human existence, has been associated with poor health outcomes [16]. Limited research has looked at the relationship between TL and allostatic load [3].

TL has been linked to various morbidities such as diabetes [18], heart disease [19,20,21,22], hypertension [23] cancer [24], and depression [25], as well as overall mortality [26,27]. A meta-analysis of 62 population based studies found a non-significant association between short telomeres and overall risk of cancer but an increased risk for gastrointestinal tumor and head and neck cancer indicating that telomeres may play diverse roles for risk in different cancers [24]. In contrast, a Mendelian Randomization Study reported an increased risk for several cancers with longer telomeres but reduced risk for cardiovascular diseases [28]. Another meta-analysis reported negative association between depression and TL [25]. The Charlson comorbidity index (CCI) is commonly used to provide a cumulative weighted score of 17 comorbid conditions [29]. The measure of decline in TL with age by allostatic load and comorbidities

could be useful particularly in elucidating the biologic pathway by which chronic stress and comorbidities can affect TL and accelerate the rate of aging. Once this pathway is better understood, this information can be used to develop effective prevention measures in at-risk populations. Therefore, we aim to assess the decline in TL with age and evaluate any effect modification by gender, chronic stress and comorbidities in a representative sample of the US population. Guided by the results of the primary analyses and in order to further elucidate the observed gender differences, secondary analyses were conducted to examine the association between TL, parity and menopause.

Methods

Study design

National Health and Nutrition Examination Survey (NHANES) is a cross-sectional nationally representative survey of the US civilian noninstitutionalized population, conducted using complex, multistage, stratified, clustered sampling [30,31]. Details of NHANES methodology have been reported elsewhere [30,31]. We used data from the cycles 1999–2000 and 2001–2002; combining them following the National Center for Health Statistics (NCHS) recommendations [31].

Study participants

NHANES 1999–2000 and 2001–2002 included a total of 9965 and 11,039 participants, respectively. For our analyses, we included those aged $\geq$ 20 years who had a measure of telomere length, for a total of 7826 individuals from both the cycles, NHANES 1999–2002.

Ethical approval

NHANES was approved by the NCHS Research Ethics Review Board (https://www.cdc.gov/nchs/nhanes/irba98.htm). All participants provided written informed consent. The Institutional Review Board at the University of Nevada Las Vegas approved the current study.

Measurements

Telomere length. Telomere length in leukocytes was measured from whole blood using the quantitative polymerase chain reaction method, described in detail elsewhere [32,33]. The Mean T/S ratio, which is the measure of TL relative to standard reference DNA, were provided in the NHANES dataset. During data analysis, the T/S ratio was converted to kilobase pairs (kbp) using the following formula: $(3,274 + 2,413 * (T/S))/1000$.

Allostatic load. Chronic stress, a hypothesized effect modifier, was measured in terms of allostatic load (AL), quantified using nine biomarkers of cardiovascular, inflammatory, and metabolic system functioning. The nine biomarkers with corresponding cutoffs [34,35] were: systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, heart rate $\geq$ 90 beats/minute, total cholesterol level $\geq$ 240 mg/dL, high-density lipoprotein (HDL) cholesterol < 40mg/dL, BMI $\geq$ 30 kg/m2, glycosylated hemoglobin $\geq$ 6.4%, C-reactive protein $\geq$ 0.3 mg/dL, and albumin < 3.8 g/dL. Each measure was coded as a dichotomous variable at the cutoff (1, if the respondent had indicated the condition; 0, if otherwise). The cumulative score of the nine indicators was then converted into a dichotomous variable, with high AL defined as an AL score $\geq$ 3. The same cutoff values and measures have been used to quantify allostatic load with the NHANES dataset in previous studies [34,35].

Comorbidity. We calculated the Charlson Comorbidity Index [29], another hypothesized effect modifier, to account for the impact of any comorbid conditions on telomere length.

Different health conditions included in calculating CCI, their definations and corresponding weights in the calculation, are provided in S1 Table. Because a score of ≥4 points is associated with an estimated 53% 10-year mortality, a weighted combined index score of ≥4 points was used to define a history of chronic comorbid conditions [29].

Other covariates. Covariates for this study, selected based on the literature, were race/ethnicity, educational attainment, socioeconomic status (SES), and physical activity levels [6,33,36].

Age (in years and 10-year categories), gender, race/ethnicity (nominal: Hispanic, NH white, NH Black, and others including multi-racial); educational level (ordinal: <12th grade, high school graduate/some college, and college graduate or above); and marital status (nominal: married/living with partner, divorced/widowed/separated, and never married) were self-reported by the participants. SES was measured on a continuous scale, in terms of poverty income ratio (PIR). PIR, the ratio of family income to the poverty threshold, calculated following the U.S. Department of Health and Human Services' poverty guidelines and described in detail elsewhere [31]. BMI, the ratio of weight/height^2 measured in kg/m^2, was available as a continuous measure and was categorized as normal weight (<25 kg/m^2), overweight (25- <30 kg/m^2) and obese (≥30 kg/m^2). Physical activity was defined as participants' self-reported participation in at least 10 minutes of moderate or vigorous activity or muscle strengthening activities in the previous 30 days [33]. Following the definition for menopause proposed by McKinlay [37], menopause was defined as one or more of the given criteria: over 55 years, had a hysterectomy or both ovaries removed, and menopause as the reason for no periods in the past 12 months. Age at menopause as well as number of pregnancies resulting in live birth were self-reported by women.

Statistical analyses

Sample weights were adjusted according to NHANES guidelines to generate a nationally representative sample [31]. For nominal variables, percentages with 95% CIs are provided; for continuous variables, unadjusted means with standard error (SEM) are provided (Table 1). TL was divided into quartiles based on the weighted population distribution. Covariate characteristics, between the quartiles of TL, were compared using Rao-Scott Chi-Square tests and analysis of variance. Linear regression with TL (kbp) as the outcome was used to assess the association with socio-demographic factors, biomarkers of allostatic load, and reproductive factors among females. Initially, univariate models were evaluated, then a group of covariates (age, gender, race/ethnicity, education, PIR, and physical activity) were added to the model.

The main outcome of this study is rate of decline in TL, which would best use a longitudinal design study. Since this is a cross-sectional study, we aim to examine the cross-sectional rates of TL decline as a proxy for rate of decline in TL by age. For this we are assuming that an individual with a TL at a certain age will, later in life, have the same telomere length as another individual at an older age with similar covariates. Therefore, the rate we are measuring is a population rate of decline in TL, not an individual rate of decline. To measure the population rate of decline in TL in different age categories, we used 10-year bins and calculated the slope of the rate of decline in TL as a function of age in each bin, using a linear regression with TL (kbp) as outcome and age (years) as predictor. All population rates of decline in TL are adjusted for gender, ethnicity, physical activity, CCI, and allostatic load. A two-tailed p-value less than 0.05 was considered statistically significant. Data analyses were performed using the survey procedures that account for the weights and complex survey design of NHANES, in SAS 9.4 (SAS Institute Inc., Cary, NC).

Table 1. Socio-demographic characteristics of the study participants by quartiles of leukocyte telomere length- NHANES 1999–2002.

	Overall (N = 7826)		Quartiles, % (95% CI)				
	N	% (95% CI)	One (N = 2415)	Two (N = 2120)	Three (N = 1633)	Four (N = 1658)	p-Value
			Range: 4.2-<5.4 kbps	Range:5.4-<5.8 kbps	Range: 5.8-<6.2 kbps	Range: ≥6.2 kbps	
Age, years, mean ±SEM	7826	45.2 ±0.4	54.6 ±0.7	46.2 ±0.8	41.5 ±0.7	37.4 ±0.8	<0.001 [a]
LTL, kbp, mean ±SEM [b]	7826	5.8 ± 0.04	5.1 ±0.0	5.6 ±0.0	6.0 ±0.0	6.7 ±0.0	<0.001 [a]
Gender							
Male	3770	48.6 (47.6–49.5)	50.7 (48.3–53.2)	48.0 (45.1–50.9)	47.7 (45.5–49.9)	47.7 (45.2–50.3)	0.287
Female	4056	51.4 (50.5–52.4)	49.3 (46.8–51.7)	52.0 (49.1–54.9)	52.3 (50.1–54.5)	52.3 (49.7–54.8)	
Race/Ethnicity							
Hispanic	2292	13.8 (9.7–17.8)	11.8 (6.0–17.7)	13.4 (9.3–17.4)	14.2 (9.7–18.6)	15.9 (11.1–20.6)	<0.001
NH White	3965	72.8 (69.0–76.5)	77.4 (71.8–82.9)	74.7 (70.5–78.9)	71.1 (66.4–75.8)	67.2 (62.4–72.0)	
NH Black	1333	9.4 (7.2–11.6)	7.2 (5.2–9.2)	8.1 (5.6–10.6)	9.8 (7.6–12.1)	12.8 (9.4–16.3)	
Other	236	4.1 (2.7–5.4)	3.6 (1.8–5.5)	3.9 (2.7–5.0)	4.9 (3.1–6.7)	4.0 (1.8–6.3)	
Educational Status							
<12th Grade	2640	21.3 (19.4–23.2)	26.3 (23.4–29.2)	20.6 (18.3–22.9)	20.8 (17.4–24.2)	17.1 (15.3–18.9)	<0.001
High School/Some College	3733	54.6 (51.7–57.5)	53.0 (49.1–56.9)	55.2 (51.6–58.8)	52.1 (47.1–57.1)	57.9 (53.4–62.5)	
College Graduate	1441	24.1 (20.7–27.5)	20.7 (16.8–24.6)	24.2 (20.7–27.7)	27.1 (21.6–32.7)	24.9 (19.8–30.1)	
Marital Status							
Married/Living with Partner	4759	65.1 (63.0–67.2)	68.8 (66.4–71.1)	67.3 (64.2–70.3)	63.9 (60.5–67.3)	59.5 (55.7–63.3)	<0.001
Divorced/Widowed/Separated	1566	17.5 (16.2–18.9)	23.0 (21.0–24.9)	19.4 (17.3–21.6)	14.4 (11.9–16.8)	12.1 (9.8–14.4)	
Never Married	1123	17.4 (15.6–19.1)	8.3 (6.8–9.8)	13.3 (10.8–15.8)	21.8 (19.1–24.4)	28.4 (25.4–31.4)	
Family PIR, mean ±SEM	7128	3.0 ±0.1	3.0 ±0.1	3.1 ±0.1	3.1 ±0.1	2.8 ±0.1	<0.001 [a]
BMI, kg/m², mean ±SEM	7577	28.0 ±0.1	28.7 ±0.2	28.2 ±0.2	27.8 ±0.2	27.4 ±0.3	<0.001 [a]
Physical Activity							<0.001
Physically active	4552	66.3 (63.5–69.1)	59.4 (55.7–63.2)	66.6 (62.8–70.4)	69.2 (66.6–71.8)	70.7 (66.5–74.8)	
Physically inactive	3271	33.7 (30.9–36.5)	40.6 (36.8–44.3)	33.4 (29.6–37.2)	30.8 (28.2–33.4)	29.3 (25.2–33.5)	
Allostatic load, mean± SEM [b]	7826	2.4 ±0.0	2.7 ±0.0	2.5 ±0.0	2.4 ±0.0	2.2 ±0.1	<0.001 [a]
CCI, mean ±SEM [b]	7826	1.5 ±0.0	2.2 ±0.1	1.5 ±0.1	1.2 ±0.1	1.0 ±0.1	<0.001 [a]

[a]: p-values from one-way ANOVA; all others from a Chi-square test.

[b] SEM values of 0 indicates a value <0.1. Abbreviations: BMI: body mass index, CCI: Charleston's comorbidity index, CI: confidence interval, Kbp: kilo base pairs, LTL: leucocyte telomere length, NH: Non-Hispanic, PIR: poverty income ratio, SEM: standard error of mean. Values expressed are % (95% CI) unless otherwise stated.

Results

Participant's characteristics

The mean (±SE) age and TL of the participants were 45.2±0.4 years and 5.8±0.04 kbp, respectively (Table 1). Participants with shorter TL, i.e., in lower quartiles, were older, NH White, married, and had higher BMI, allostatic load, and CCI. A gradual decreasing trend in mean age, BMI, allostatic load and CCI was noted from lowest to highest quartiles (Table 1).

Telomere length and associated factors

In analyses adjusted for age, gender, race/ethnicity, education, PIR, and physical activity (Table 2), increasing age, and higher heart rate, BMI, and C-reactive protein were associated with lower TL. Likewise, NH Black compared to NH White and unmarried compared to married/living with a partner had higher TL. A further stratified analysis was conducted to assess the role of age and gender in the association between marital status and TL. We found that the marital status and TL association was significant only for younger (20–29 years) females (β:

Table 2. Multivariable regression for factors associated with telomere length- NHANES 1999–2002.

	Model 1 unadjusted			Model 2 adjusted [a]		
	β	**95%CI**	**p-value**	**β**	**95%CI**	**p-value**
Age	**-0.0146**	**-0.0162, -0.0130**	**<0.001**	**-0.0142**	**-0.0157, -0.0127**	**<0.001**
Gender (Reference = Male)						
Female	0.0252	-0.0265, 0.0769	0.328	0.0460	-0.0015, 0.0934	0.0569
Race/Ethnicity (Reference = NH White)						
Hispanic	0.0681	-0.0692, 0.2054	0.319	-0.0053	-0.137, 0.1264	0.9352
NH Black	**0.1687**	**0.0802, 0.2572**	**<0.001**	**0.1186**	**0.028, 0.2092**	**0.0121**
Other	0.0396	-0.1005, 0.1797	0.568	-0.0141	-0.1502, 0.122	0.8334
Educational Status (Reference = High School)						
<12th Grade	**-0.1203**	**-0.1825, -0.0581**	**<0.001**	-0.0679	-0.1365, 0.0006	0.052
College Graduate	-0.0023	-0.0689, 0.0642	0.943	0.0305	-0.0357, 0.0967	0.354
Marital Status (Reference = Married/with Partner)						
Divorced/Widowed/Separated	**-0.1111**	**-0.1655, -0.0566**	**<0.001**	-0.0128	-0.0722, 0.0465	0.6614
Never Married	**0.3162**	**0.2422, 0.3901**	**<0.001**	**0.0979**	**0.0233, 0.1726**	**0.0119**
Family PIR	-0.0127	-0.0354, 0.0100	0.261	-0.0048	-0.0275, 0.018	0.6699
Physical Activity (Reference = Physically Active)						
Physically Inactive	**-0.1158**	**-0.1653, -0.0663**	**<0.001**	-0.0151	-0.0537, 0.0236	0.4316
Biomarkers of allostatic load						
Systolic blood pressure	**-0.0030**	**-0.0044, -0.0015**	**<0.001**	0.0011	-0.0001, 0.0023	0.0631
Diastolic blood pressure	**0.0041**	**0.0022, 0.0060**	**<0.001**	0.0001	-0.0018, 0.0018	0.9755
Heart rate	-0.0008	-0.0026, 0.0009	0.325	**-0.0021**	**-0.0036, -0.0006**	**0.0074**
Total cholesterol	-0.0002	-0.0007, 0.0004	0.516	-0.0001	-0.0007, 0.0004	0.5865
High-density lipoprotein cholesterol	**0.0027**	**0.0009, 0.0044**	**0.004**	**0.0023**	**0.0005, 0.0041**	**0.0134**
BMI (kg/m2)	**-0.0093**	**-0.0133, -0.0052**	**<0.001**	**-0.0068**	**-0.0105, -0.0031**	**0.0007**
Glycosylated hemoglobin	**-0.0370**	**-0.0573, -0.0167**	**<0.001**	-0.0004	-0.0257, 0.0249	0.9751
C-reactive protein	**-0.0538**	**-0.0993, -0.0083**	**0.022**	**-0.0558**	**-0.0903, -0.0214**	**0.0025**
Albumin	0.1664	-0.3897, 0.7225	0.545	0.2317	-0.6846, 1.1481	0.609
Allostatic load (Reference = Low)						
High	**-0.1735**	**-0.2226, -0.1244**	**<0.001**	**-0.0697**	**-0.1166, -0.0228**	**0.005**
Charleston Comorbidity Index	**-0.0723**	**-0.0823, -0.0624**	**<0.001**	-0.0044	-0.0173, 0.0086	0.4971

DV: Telomere length in kbp

[a] Adjusted for age, gender, race/ethnicity, education, PIR, and physical activity. Abbreviations: BMI: body mass index, CI: confidence interval, NH: Non-Hispanic, PIR: poverty income ratio. p-value less than 0.05 are bold.

0.5194, 95%CI: 0.1129–0.9258, p-value: 0.0141) and older (80 years and above) females (β: 0.2770, 95%CI: 0.1217–0.4323, p-value: 0.001). Although female gender demonstrated a positive trend and family PIR demonstrated an inverse trend with TL, the findings did not reach the statistical significance of <0.05 (Table 2).

Of the nine biomarkers of allostatic load, after controlling for covariates, only the regression coefficients of heart rate, HDL cholesterol, BMI, and C-reactive protein had the 95% CIs that did not include the null of no association (Table 2); while a higher heart rate, BMI, and C-reactive protein was associated with shorter TL, a higher value of HDL cholesterol preserved it. In an unadjusted analysis, a one-unit difference in allostatic load and CCI were associated with 17.3% and 7.2% decrease in TL, respectively. However, the estimates for CCI did not remain statistically significant when controlled for covariates. Specifically, age had a strong confounding effect on our estimates because most of the covariates with a significant coefficient in

unadjusted models retained their statistical significance when adjusted for other covariates except age (S2 Table).

Cross-sectional population rates of telomere length decline

The population rates of decline in TL stratified by 10-year age bins showed that decline in TL with age was initiated early in life (20–29 years) and was consistent and linear for only three age categories: 20–29 (β = -0.0172, 95% CI: -0.0342, -0.0002), 50–59 (β = -0.0182, 95% CI: -0.0311, -0.0054) and 70–79 (β = -0.0170, 95% CI: -0.0329, -0.0011) years. The population rate of decline in TL was sharp in the age category 70–79 years; whereas, in the oldest age category examined (80–89 years), the rate of decline (β = -0.0096, 95% CI: -0.0412, 0.0220) was highly variable and did not follow a linear pattern. Likewise, the population rate of decline in TL stratified by allostatic load and comorbid conditions showed a significantly greater rate of decline among those with high allostatic load (β = -0.0122, 95% CI: -0.0143, -0.0101), and history of comorbid conditions (β = -0.0133, 95% CI: -0.0164, -0.0102).

Gender stratified analyses, adjusted for ethnicity, physical activity, CCI, and allostatic load, (Fig 1) showed a significantly greater rate of decline for males (β = -0.0153, 95% CI: -0.0171, -0.0135) compared to females (β = -0.0128, 95% CI: -0.0153, -0.0103). When the population rate of decline in TL was further stratified by gender in 10-year age bin (Table 3), we observed a fairly consistent population rate of decline in TL for male; none of the regression coefficients were statistically significant (Table 3). Interestingly, for females, a peak in the population rate of decline in TL was observed in the age categories of 20–29 years (β = -0.0284, 95% CI: -0.0464, -0.0103) and 50–59 years (β = -0.0211, 95% CI: -0.0391, -0.0032). Although women in the age categories of 30–39 years and 60–69 years displayed a minimal reduction in TL, the estimates were not statistically significant (Table 3).

To explore the heterogeneity in the population rate of decline in TL among females, we conducted additional analyses for women's reproductive history, particularly parity and menopause, since ages 20–29 and 50–59 years are common ages for parity and menopause, respectively. After controlling for ethnicity, education, PIR, and BMI, the number of live children a woman had was negatively associated with TL (β = -0.0318, 95% CI: -0.0510, -0.0125). Further, compared to nulliparous women, decline in TL was noted for women with live births (S1 Fig). Similarly, women's menopausal status (β = -0.2875, 95% CI: -0.3517, -0.2232), age at menopause (β = -0.0077, 95% CI: -0.0130, -0.0023) as well as years passed since menopause (β = -0.0095, 95% CI: -0.0127, -0.0063) were inversely associated with TL. Further, variation in slope of decline in TL with years passed since menopause was noted by women's weight status (S2 Fig). However, these findings were no longer statistically significant once we controlled for age (S3 Table).

Discussion

Overall, TL significantly reduced with age, but this decline was modified by gender, chronic stress and comorbidities; those with chronic morbidities and chronic stress experienced greater decline. Gender stratified analysis was more interesting, showing a fairly consistent and statistically non-significant rate for male but a substantial trough in TL for females in the age categories 20–29 and 50–59 years. Female reproductive factors, i.e., parity and menopause, were associated with TL.

Decline in TL with age is unanimously supported by the literature [33,38]. Heterogeneity in rate of decline in TL is suggested by a previous study from the Erasmus Rucphen Family (ERF) data, which found a significant reduction in variance in TL from young adulthood to old age by using the TL of grandchildren as a proxy for participants' TL at childhood [38]. For females,
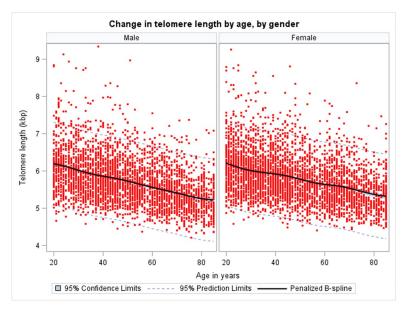
Fig 1. Telomere Length by Age and Gender in NHANES 1999–2002. (a) Male (β = -0.0153; p-value <0.001), **(b) Female** (β = -0.0129; p-value <0.001). Estimates, obtained from linear regression with telomere length (kbps) as outcome and age (years) as predictor, are adjusted for ethnicity, physical activity, allostatic load and comorbidity index.

https://doi.org/10.1371/journal.pone.0221690.g001

a trough in the adjusted population rate of decline in TL was noted in early (20–29) and mid-life (50–59) years. In general, for females the 20–29 and 50–59 age bands are periods of important hormonal changes, as these are the common ages for parity and menopause, respectively. Consistent with previous studies, we found a significant negative association between TL and parity, menopausal status, and age at menopause [39]. The observed inverse relationship between TL and parity is supported by the life history theory, which postulates that the energy used during reproduction reduces the energy available for tissue maintenance given that the amount of energy an organism can mobilize at any given time is finite, and that poor tissue maintenance, in turn, leads to faster cellular degradation and aging [40]. Biologically, estrogen deficiency or over-activity may cause either ovarian tissue aging or tumorigenesis, respectively, through estrogen regulation of telomere remodeling [41]. Therefore, telomere attrition rate should accelerate after menopause in response to a decrease in estrogen [41]. In vitro,

Table 3. Decline in telomere length by gender, across age groups, NHANES 1999–2002.

Age group, years	Male			Female		
	β	95% CI	p-value	β	95% CI	p-value
20–29	-0.0080	-0.0367, 0.0208	0.5763	**-0.0284**	**-0.0468, -0.0099**	**0.0038**
30–39	-0.0078	-0.0290, 0.0135	0.4621	-0.0012	-0.0214, 0.0191	0.9084
40–49	-0.0060	-0.0290, 0.0170	0.5985	-0.0130	-0.0321, 0.0060	0.1724
50–59	-0.0166	-0.0337, 0.0005	0.0571	**-0.0216**	**-0.0396, -0.0036**	**0.0202**
60–69	-0.0124	-0.0333, 0.0085	0.2342	0.0040	-0.0179, 0.0258	0.7134
70–79	-0.0215	-0.0439, 0.0010	0.0599	-0.0146	-0.0326, 0.0034	0.1079
80 and above	-0.0116	-0.0377, 0.0145	0.3700	-0.0075	-0.0519, 0.0369	0.7334

Estimates are slope from a linear regression with telomere length (kbps) as outcome and age (years) as predictor, by age category and gender, adjusted for ethnicity, allostatic load, and comorbidity index. p-value less than 0.05 are bold.

https://doi.org/10.1371/journal.pone.0221690.t003

telomerase activity is upregulated by estrogen [42,43]. Population-based studies also provide evidence that greater estrogen exposure, as measured by the use of hormone therapy [44], and longer duration of reproductive years [45], are related to significantly higher TL in postmeno-pausal women. Oxidative stress and proinflammatory cytokines have also been associated with telomere shortening [46,47]; thus, antioxidants aid in the attenuation of telomere shortening [48,49]. Estrogen is known to have antioxidant properties [50,51]; thus, the conferred protec-tive role may be due to its ability to lower oxidative stress and reduce inflammation [52,53].

This study found that one-unit increases in allostatic load were associated with 7% decreases in TL after controlling for covariates. Further analyses stratified by allostatic load lev-els showed a greater decline in TL among those with higher allostatic load. The allostatic load model posits that repeated or inadequate physiological adaption to social and environmental stress over time results in dysregulation of cortisol (via dysfunction of the hypothalamic-pitui-tary-adrenal axis) and catecholamines (via the sympathetic nervous system), which may in turn result in dysfunction of the body's cardiovascular, immune, and metabolic systems [16]. Therefore, it is likely that TL may serve as an important cellular-based indicator of systemic allostatic load.

The associations between TL and various morbidities such as diabetes [18], heart disease [19,20,21,22], hypertension [23] cancer [24], and depression [25], as well as overall mortality [26,27] have been established. In this study, one-unit increases in comorbidities, as measured by the Charlson Comorbidity Index, was associated with 7.2% decreases in TL, although find-ings lost significance after adjusting for age. Regardless, there is biological plausibility linking comorbidities with TL. Additionally, since TL is strongly correlated with chronological age, the latter had a powerful confounding effect on most of our estimates which lost statistical sig-nificance when adjusted for age. Further, analyses stratified by comorbidity levels showed a greater decline in TL among those with four or more comorbidities. It has been suggested that telomere shortening might contribute to various morbidities through pathways involving cel-lular senescence, chronic inflammation and endothelial dysfunction [54,55].

Many of the covariates we selected were associated with TL. TL in our study was positively correlated with HDL cholesterol and inversely correlated with BMI and heart rate [36]. The existing literature on race/ethnicity and TL is inconsistent [33,56]. In our study, NH Black had higher allostatic load compared to NH White (OR = 1.32, 95% CI = 1.13–1.53), after adjust-ment for age, gender, and PIR. Despite the higher allostatic load, in overall and stratified analy-sis by allostatic load status, NH Black had higher TL than NH White. Although unexpected given that African-Americans experience greater stress in various life domains, sociocultural factors such as social support and religion/spirituality may enhance resilience when dealing with psychological distress for this group [57]. These factors may nurture coping efficacy which in turn fosters an ability to manage adversity [57]. More research is needed to under-stand relationships between high effort coping styles and TL for racial and ethnic populations in the United States. Another surprising finding in our study was never having been married was associated with longer TL than being married. This finding conflicts with other studies that showed being married is associated with longer TL [58,59]. In general, being unmarried is associated with poor health outcomes, presence of systemic inflammation [60,61] increased mortality risk, and a shorter lifespan [60,61,62,63]. Although the protective role of marriage is not completely understood, it has been hypothesized that the social, emotional, and financial support provided by a spouse/partner acts as a buffer to life stressors [64]. Even among labora-tory animals, social isolation was related to increased oxidative stress [65] which in turn is related to telomere attrition. In our stratified analyses, we found that age and gender played a role in the association between marital status and TL. Thus, different mediators and

moderators may explain the inconsistency observed in the relationship between marital status and TL attrition, which should be explored in future research.

Strengths, limitations and implications

The strengths of the study include a large sample size, a nationally representative sample of non-institutionalized American adults, rigorous methodology and the comprehensive quality control procedures of NHANES. The statistical analyses have been adjusted for study weights and complex survey design to reduce errors in estimation. The cross-sectional design of the study is the primary limitation, and no causation should be inferred from this study. Limitations were also observed for the quantification of allostatic load and CCI. Currently no gold standard measure exists to quantify allostatic load, and while our approach was similar to others [34,35], the extent to which the measures of allostatic load actually reflect the complex concept of "wear and tear" is uncertain, which may impact the accuracy of the measurement, and consequently, the quality of the evidence generated. Of the 17 comorbidities used in the original CCI, we were unable to include the measures of hemiplegia and metastatic cancer due to unavailability of data on these conditions in NHANES 1999–2002. Some of the disease statuses used in calculating CCI were self-reported. Lastly, the possibility of residual confounding due to unmeasured covariates cannot be ruled out.

If the findings from this current study could be replicated with longitudinal data, there may be several important implications. First, our findings imply that interventions aimed at preserving TL should be targeted at younger ages, not just at old age. The heterogeneity in decline in TL and absence of a linear pattern in the oldest age category (≥80 years) may also partially explain the lack of statistical association between TL and survival, as seen in some studies conducted among the elderly. For women, a trough in decline in TL with age was noted in early and midlife years. Although 20–29 and 50–59 years are common ages for first childbirth and menopause, respectively, the underlying cause of the decline in these age categories is still not clear and should be addressed by future research.

Conclusions

We found shorter TL with increasing age; this decline was modified by gender, chronic stress and comorbidities. Females in their twenties and fifties and those with chronic morbidities and/or chronic stress experienced greater TL decline. Female reproductive factors, i.e., parity and menopause, were associated with TL. Given the cross-sectional design of our study, future research should attempt to replicate our findings, specifically those related to parity and menopause, in a longitudinal design. Females in their twenties and fifties are potential subgroups of interest for any interventions or programs aimed at preserving TL.

Supporting information

S1 Fig. Association between telomere length and parity in NHANES 1999–2002. Y-axis represents the slope of change in telomere length with the number of live births. Regression coefficients adjusted for ethnicity, education, poverty income ratio, and body mass index.
(TIF)

S2 Fig. Association between telomere length and years passed since menopause, by BMI status of women in NHANES 1999–2002. The slope for each weight category is: Normal weight (β = -0.0101; p = 0.001), Overweight (β = -0.0111; p = 0.005) and Obese (β = -0.0076; p = 0.007). Estimates adjusted for ethnicity, education, poverty income ratio, and body mass

index.
(TIF)

S1 Table. Comorbid conditions included in Charlson's comorbidity index.
(DOCX)

S2 Table. Multivariable regression for factors associated with telomere length- NHANES 1999–2002.
(DOCX)

S3 Table. Multivariable regression for women's reproductive history associated with telomere length- NHANES 1999–2002.
(DOCX)

Acknowledgments

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