Psychoneuroendocrinology 107 (2019) 70–81

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Cellular response to chronic psychosocial stress: Ten-year longitudinal changes in telomere length in the Multi-Ethnic Study of Atherosclerosis

Helen C.S. Meier[a,*], Mustafa Hussein[a], Belinda Needham[b], Sharrelle Barber[c], Jue Lin[d], Teresa Seeman[e], Ana Diez Roux[c]

[a] Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, 1240 N. 10th St., Milwaukee, WI, 53205, USA
[b] Department of Epidemiology, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI, 48109, USA
[c] Department of Epidemiology and Biostatistics, and the Urban Health Collaborative, Drexel University Dornsife School of Public Health, 3215 Market St., Philadelphia, PA, 19104, USA
[d] Department of Biochemistry and Biophysics, University of California, San Francisco, 600 16th Street, Room S312F Genentech Hall, San Francisco, CA, 94158, USA
[e] Department of Medicine, Division of Geriatrics, University of California, Los Angeles, 10945 Le Conte Avenue, Suite 2339, Los Angeles, CA, 90095, USA

ARTICLE INFO

Keywords:
Chronic psychosocial stress
Telomere length
Cellular aging

ABSTRACT

Previous studies have demonstrated an inverse association between chronic psychosocial stress and leukocyte telomere length (LTL), a potential marker of cellular aging. However, due to paucity of longitudinal data, responses of LTL and the LTL aging trajectory to changes in chronic stress exposure remain less well understood.

Using data from the Stress I and II ancillary studies of the Multi-Ethnic Study of Atherosclerosis, we estimated the 10-year longitudinal (n = 1,158) associations of within-person changes in chronic stress with changes in LTL, as well as the pooled, cross-sectional associations of chronic stress and LTL (total n = 2,231).

We measured chronic stress from both individual and neighborhood-environment sources. At the individual level, we calculated a summary score of each participant's rating of their ongoing (> 6 months) material/social problems as moderately/very stressful on the Chronic Burden Scale. Neighborhood-level stress was measured using a summary score of reverse-coded MESA Neighborhood safety, aesthetic quality, and social cohesion scales. Quantiles of these scores were empirically categorized as high, moderate, or low stress. We then summed these individual- and neighborhood-level categorical variables for a total stress measure. Longitudinal within-person associations were estimated with fixed-effects models, which control for all time-invariant confounding, with additional control for time-varying demographics, lagged behaviors and chronic conditions, and specimen storage duration, as well as correction for regression to the mean.

Change from low to high total chronic stress was associated with telomere shortening by 0.054 units [95% confidence interval: −0.095, −0.013] over 10 years. This was consistent with, though stronger in magnitude than, cross-sectional estimates. Change in individual-level stress was the primary driver of this effect. We also found suggestive evidence that 1) individuals with persistently high stress experienced the *least* shortening of telomeres, and 2) changes in individual-level stress were associated with stronger telomere shortening among women, whereas changes in neighborhood stress were associated with stronger shortening among men. Our findings provide longitudinal support to existing evidence, and point to interesting dynamics in telomere attrition across stress levels and genders.

1. Introduction

Research suggests many negative health consequences of chronic stress. Chronic stress is associated with poor physical and mental health as well as greater susceptibility to infection and diseases associated with inadequate and overactive immune function (McEwen, 2004; Quinlan et al., 2014). Chronic stress has long been considered a key link between low socioeconomic position (SEP) and poor health (Baum et al., 1999; Geronimus et al., 2015; Matthews et al., 2010; Seeman et al., 2010; Surtees et al., 2012). Psychosocial stress may impact aging at the cellular level and telomere attrition is one possible mechanism by which stress influences downstream biological dysregulation producing

* Corresponding author.
E-mail addresses: meierh2@uwm.edu (H.C.S. Meier), husseimh@uwm.edu (M. Hussein), needhamb@umich.edu (B. Needham), smb483@drexel.edu (S. Barber), jue.lin@ucsf.edu (J. Lin), tseeman@mednet.ucla.edu (T. Seeman), avd37@drexel.edu (A. Diez Roux).

https://doi.org/10.1016/j.psyneuen.2019.04.018
Received 28 August 2018; Received in revised form 14 February 2019; Accepted 26 April 2019

negative health consequences.

Telomeres are protein-bound DNA complexes that cap chromosomal tails, preventing chromosomes from fusion and degradation during mitosis, thus preserving genomic integrity (Armanios and Blackburn, 2012; Blackburn et al., 2006). Telomeres shorten with age, ultimately leading to cellular senescence: classically, a state of cellular arrest with no capacity for further division (Allsopp et al., 1992; Aubert and Lansdorp, 2008; Harley et al., 1990). Telomere length is also associated with multiple chronic disease processes (Kong et al., 2013), including obesity, diabetes, cardiovascular disease and mortality (D'Mello et al., 2015; Fitzpatrick et al., 2007; Rode et al., 2015; Sampson et al., 2006; Valdes et al., 2005).

Telomeres may represent a pathway by which stress and other adverse exposures get "under the skin" (Epel and Prather, 2018). A large body of literature supports an inverse association between leukocyte telomere length (LTL) and psychosocial stress, indicating that individuals with higher stress have shorter telomeres on average (Mathur et al., 2016; Oliveira et al., 2016; Quinlan et al., 2014; Theall et al., 2013). Many previous studies, however, are cross-sectional in nature and focus on severe forms of psychosocial stress, such as death of a loved one, job loss, caring for chronically ill children or relatives, intimate partner violence and childhood trauma (Jodczyk et al., 2014; Schaakxs et al., 2015; Starkweather et al., 2014). Less well understood is the relationship between moderate, chronic psychosocial stress and cellular aging (Schutte and Malouff, 2014) and whether moderate, chronic psychosocial stress alters aging-related telomere attrition over time (Puterman et al., 2016). As telomere length has been linked to many human diseases (Kong et al., 2013), sustained, moderate stress acting on telomeres may play an important role in linking socioeconomic disadvantage and health outcomes (Geronimus et al., 2015; Surtees et al., 2012).

The present study examines the relationship between chronic stress exposure, measured at both the individual and neighborhood levels, and LTL in the Multi-Ethnic Study of Atherosclerosis, a longitudinal, ethnically diverse population-based sample. We assessed the longitudinal associations of within-person changes in chronic stress levels with changes in LTL, as well as the cross-sectional associations of chronic stress levels and LTL. Further, we explicitly mapped out how the direction of change in chronic stress since baseline may have driven the direction of change in telomere length. We hypothesized that having higher levels of chronic stress would be associated with shorter telomeres and greater telomere attrition over 10 years.

2. Materials and methods

2.1. Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal study of cardiovascular disease among adults aged 45–84 years at baseline at six field sites (Forsyth County, NC; New York City, NY; Baltimore, MD; St Paul, MN; Chicago, IL; and Los Angeles, CA) in the United States. Persons with a history of clinically overt cardiovascular disease were excluded. The study recruited 6814 participants at baseline. Baseline assessment was conducted from 2000 to 2002, with four follow-up waves occurring at approximately 1.5–2 year intervals (Bild et al., 2002). Telomere length was measured on a subsample of 1295 white, black and Hispanic participants from Stress I and II ancillary studies examining the effects of stress on cardiovascular outcomes at baseline and/or exam 5 (approximately 10 years apart; 2590 total LTL observations). Our cross-sectional analyses pooled data from 1242 participants who had LTL and complete covariate data either at baseline or at exam 5, resulting in 2231 total observations. For longitudinal analysis, we included 1029 participants who had LTL and complete covariate data at *both* baseline and exam 5. The study was approved by the Institutional Review Boards at each site and all participants gave written informed consent.

2.2. Leukocyte telomere length (LTL) (exams 1 & 5)

Telomere length was determined in peripheral blood leukocytes collected in EDTA tubes taken from participants at Exam 1 and Exam 5, with DNA extracted using Gentra Puregene Blood kit (Qiagen) and quantified by NanoDrop (NanoDrop Technologies, Wilmington, DE). Samples were quality verified by assessing purity using the NanaDrop Spectrophotometer followed by Picogreen analysis (Molecular Probes, Eugene, OR). All DNA was of high quality (mean purity A260/280 = 1.77) and of high molecular weight as determined by gel electrophoresis. DNA was stored at −80 °C until further processing. The samples were shipped on dry ice to UCSF, where assay methods were employed to determine relative telomere length adapted from Cawthon (Cawthon, 2002) using the absolute quantification method to obtain the concentrations of the telomere (T) signal and the single copy gene (S) signal. Briefly, using a standardized real time quantitative polymerase chain reaction (qPCR) methodology, 384-well plates were prepared, placing matching subject samples from each time point together on the same plate, and run with the following protocol. 8 standard curves were generated on each plate using 3-fold serial dilution of 8 control DNA samples from various cancer cell lines and used for normalizing batch to batch variations. 1 uL of participant DNA samples, at a concentration of 20–30 ng/uL, were denatured at 96 °C for 10 min using a buffer consisted of 20 mM Tris-HCl, pH 8.4; 50 mM KCl, 6 ng/ul of E. coli DNA stock (Sigma-Aldrich). Each sample well included DNA sample in dilution buffer, a master mix made from combining SYBR Green, Platinum Taq polymerase (Invitrogen), PCR buffer, 50 mM $MgCl_2$ (from Platinum Taq kit), 200 u M dNTP (Roche Applied Science) and DMSO (Sigma-Aldrich), and primers. The primers for the telomere plate were as follows *tel1b* [5′-CGGTTT(GTTTGG)$_5$GTT-3′], used at a final concentration of 100 nM, and *tel2b* [5′-GGCTTG(CCTTAC)$_5$CCT-3′], used at a final concentration of 900 nM. Primers for the single-copy gene (human beta-globin) PCR plate (S) were as follows *hbg1* [5′-GCTTCTG ACACAACTGTGTTCACTAGC-3′], used at a final concentration of 300 nM, and *hbg2* [5′-CACCAACTTCATCCACGTTCACC-3′], used at a final concentration of 700 nM. All primers were purchased from IDT (www.idtdna.com) in standard desalted form. Using the Roche LightCycler 480 for qPCR the plates were run as follows, TEL (T plate): 1 min at 96 °C; 1 s at 96 °C, 60 s at 54 °C, repeated 30 cycles; HGB (S Plate): 1 min at 96 °C; 15 s at 95 °C, 1 s at 58 °C, 20 s at 72 °C, 8 cycles; followed by 1 s at 96 °C, 58 °C for 1 s, 72 °C for 20 s, hold at 83 °C for 5, repeat 35 cycles. Crossing point (CP) data was collected for each well (Cp on Roche LightCycler 480 realtime machine, equivalent to Cq) and used to generate the estimated concentration of the T and S values using the standard curve method with one of the control DNA (same for every plate). Samples were run in triplicate with 0.2% outliners removed. The following quality control criteria were applied. Each assay run (T and S runs) have 96 control wells; any assay runs with 8 or more invalid control wells are considered a failed run and excluded from further analysis. No run failed for the entire sample set. For each of the 8 control DNAs; slope, intercept, midpoint and r-value are calculated using the linear regression algorithm from the scipy Python library. R-values less than 0.95 were considered non-linear, and that particular control DNA for that run is excluded from further analysis. The control DNA values were used to normalize for run-to-run variations. Average of midpoints of valid control DNA dilutions were used as an offset for the run. One of the control DNA sample, a pooled genomic DNA sample from 100 female donors (Aldevron, cat# 5085-25, now discontinued) was used as the reference standard to calculate the T and S concentrations of well. Each fully successfully assayed sample has six T/S values associated with it, assayed three times, on three different days. The inter-assay CV was 2.9% ± 2.1%. The 8 standard curves were used to confirm PCR performance using slope, intercept, midpoint and r-values, calculated using the linear regression algorithm from the SciPy Python library. R-values less than 0.95 were considered non-linear, and that particular control DNA for that run is excluded from further

analysis. Lab personnel were blind to patient information. The correlation coefficient between baseline (exam 1) and follow-up (exam 5) T/S ratios was 0.46 overall, and slightly above and below this number across subgroups by gender (men: 0.38; women: 0.52) and baseline stress levels (low: 0.42; moderate: 0.51; high: 0.61).

2.3. Chronic stress (exams 1 & 3)

We measured chronic stress from both individual-level and neighborhood-level sources. Because individual-level chronic stress data are available in MESA only up to exam 3, we assessed both individual and neighborhood stress (as well as confounders) at exams 1 & *3*, instead of at exams 1 & *5* like LTL. Although having different follow-up periods for chronic stress and LTL could be considered a limitation, having stress exposure temporally preceding LTL measurement in the follow-up period might help mitigate reverse causality concerns.

Individual-level chronic stress was measured via questionnaire at baseline and exam 3 using the Chronic Burden Scale (Bromberger and Matthews, 1996; Pilkonis et al., 1985). Respondents were asked to indicate whether they had experienced any ongoing problems in five domains [health (self), health (loved one), job, relationship, and financial problems and if any ongoing problems lasted 6 or more months]. Respondents rated how stressful each problem was. Respondents were classified as having chronic burden for each of the five domains if they had experienced the circumstance for at least six months and it was moderately or very stressful. We summed the number of domains in which chronic burden was experienced (0, 1, 2 or more) to estimate overall chronic burden (Mujahid et al., 2011).

Exposure to adverse social conditions within the neighborhood environment may be a source of chronic stress (Barber et al., 2016; Kershaw et al., 2015; Mujahid et al., 2011; Steptoe and Feldman, 2001). Neighborhood-related stress was assessed as a summary score of three scales developed in the MESA Neighborhood Ancillary Study: neighborhood aesthetic quality (3-item: trash/litter on streets, noise, and attractiveness), safety (2-item: safety walking day/night, and violence), and social cohesion (4-item: neighbors willing to help, getting along, trustworthy, and sharing same values). Construction of the scales has been described previously (Kershaw et al., 2015; Mujahid et al., 2011, 2007; Needham et al., 2014b). Each original scale was reverse-coded (so that higher values mean greater stress), standardized across baseline and exam 3, and then summed to create the summary score of neighborhood stress (Cronbach's alpha = 0.86).

In the absence of biologically-based demarcations of stress levels, we converted our continuous scales of chronic stress into meaningful, data-driven categorical measures that potentially correspond to breaks in stress association with LTL. This was motivated by the fact that most of the literature on stress and LTL has focused on severe forms of stress (Jodczyk et al., 2014; Schaakxs et al., 2015; Starkweather et al., 2014), suggesting that continuous measures of milder forms of stress, as in this study, may not be informative, i.e., they would capture LTL associations with rather small changes in stress, while masking more important associations with larger, more realistic changes in chronic stress. With categorical measures, we are capable of documenting LTL responses to <u>both</u> relatively small and large changes in stress, as well as potential non-linearity in the effect of stress on LTL. Categorical measures also facilitate combining the different scales of individual-level and neighborhood-level stress into a total stress measure in a straightforward manner. To construct our categorical measures, we performed a two-step process for each of the individual and neighborhood stress scales: 1) modeling LTL association with quantiles of stress in a longitudinal model with a basic set of covariates (time between exams, specimen storage duration, and demographics), then 2) collapsing neighboring stress quantiles with similar magnitudes of association with LTL. This procedure resulted in the following categorical measures: low (0–1), moderate (2–3) and high (4–5) levels of the 6-month individual-level chronic burden scale; and low (−8.25 to −1.90), moderate (−1.91 to 2.14) and high (2.15–7.31) for the neighborhood-level stress scale. These low/moderate/high levels were coded as 0/1/2. Adding across individual and neighborhood stress levels, we created a measure of total stress (range: 0–4), which was then collapsed into a three-level categorical variable of low (0–1), medium (2) and high (3–4) total chronic stress based on the magnitude of associations of these stress levels with LTL. Nonetheless, we assessed LTL associations with the continuous stress measures (as z scores) in secondary analyses for comparability with the literature.

Finally, to help unpack how the direction of change in chronic stress since baseline may have driven the direction of change in LTL in our longitudinal models, we created stress change variables that explicitly classify participants' stress levels as remaining persistently low, moderate, or high; or decreasing, or increasing in magnitude over the 10-year study period.

2.4. Covariates (exams 1 & 3)

Socio-demographic, behavioral and biomedical information was collected via questionnaire at exam 1 and exam 3. Demographic covariates included age, sex, race/ethnicity, education level, time-varying income-wealth index and marital status. Sex was measured as male (referent) or female. Self-reported race/ethnicity was categorized as non-Hispanic black, Hispanic and non-Hispanic white (referent). Education level was categorized as high school or less, some college (including Associate's degree or technical school), or bachelor's or graduate degree. We also constructed an income-wealth index (range: 0–8) from participants' income and wealth data, following Hajat et al. (2010). Briefly, first a 5-point wealth index was created where one point was given for ownership of vehicles, homes, land, or investments (Hajat et al., 2010). Second, an income index was generated using the midpoint of income categories ranging from $2500 to $112,500, dividing by $10,000 and categorized into quintiles (0 poorest, 5 richest) (Hajat et al., 2010). The income-wealth index was generated by summing the 5-point wealth index and 5 category income index (Hajat et al., 2010). The continuous income-wealth index was then categorized as low (0–2), middle (3–5), and high (6–8). Marital status was categorized as married or living with partner vs. else.

Behavioral and biomedical factors previously found to be associated with telomere length were included as potential confounders or mediators (Chen et al., 2009; Jeanclos et al., 2000; Ludlow and Roth, 2011; Nettleton et al., 2008; Strandberg et al., 2011; Valdes et al., 2005). These variables included current alcohol consumption status (yes/no), moderate/vigorous physical activity (tertiles of MET/min/week) and standardized continuous variables for pack-years of cigarette smoking, daily servings of processed meat, BMI, Spielberger anxiety scale, CES-D depression scale, systolic and diastolic blood pressure. Additionally, time-varying indicators for diabetes and cardiovascular disease incidence were included.

2.4.1. Specimen storage duration

DNA samples from baseline and exam 5 were stored for different periods of time before q-PCR for telomere length was performed. Baseline samples were stored for a median of 13 years (range 11.85–13.95) while exam 5 samples were stored for a median of 3 years (range 2.12–3.88). In preliminary analyses, we found storage duration to be associated with increases in measured T/S ratios in a non-linear fashion (by 0.13 units/year in 12–14 years of storage; and by 0.06 units/year in 2–4 years of storage). While this observation seems consistent with the sparse literature (Reichert et al., 2017; Tolios et al., 2015) on specimen storage effects, the mechanism(s) behind this lengthening remain unclear. We therefore assess the sensitivity of our findings to adjusting for storage duration using linear and quadratic terms.

2.5. Statistical analyses

We first examined cross-sectional associations between stress and LTL, pooling together baseline and follow-up data, then performed longitudinal analyses to examine associations of within-person changes in stress with change in LTL using fixed-effects models. In both sets of analyses, associations of LTL with each measure of chronic stress exposure (individual, neighborhood, and total) were analyzed separately in series of pre-specified linear regressions, as detailed below, that document sensitivity of our main estimates to control/correction for sources of confounding. In both sets of analyses, the fully-adjusted "Model 4″ specification provides our main estimates of chronic stress associations with LTL. Using this same specification, we also explored how these associations potentially vary by gender. Since our models involve multiple interaction terms and fixed effects, we recover and report the average associations of stress and LTL integrated over all included terms. All analyses were performed in Stata 14.2 (StataCorp, College Station, TX), and robust standard errors and 95% confidence intervals (95% CIs) were estimated for all model estimates.

2.5.1. Cross-sectional analyses

In the pooled sample of baseline and follow-up data, we estimated the cross-sectional associations of chronic stress and LTL, for comparison with the literature. As is well-known, these association are derived from between-person variation in chronic stress levels, i.e. using individuals as controls for one another. In the most basic linear regression, *Model 1*, we estimated the association of the stress measure with LTL, adjusting for the MESA exam indicator and its interaction with the stress measure, allowing the associations to vary in magnitude across exams. *Model 2* additionally adjusted for main confounders, including age, gender, race/ethnicity, education, income-wealth, and marital status. *Model 3* further adjusted potential confounders, including behaviors (consumption of alcohol, tobacco, and processed meat; BMI, and physical activity), depressive symptoms, anxiety, and incidence of cancer, diabetes, hypertension, or CVD up until exam 3. Finally, our main specification *Model 4* further adjusts for linear and quadratic terms of specimen storage duration.

2.5.2. Longitudinal analyses

Prior research shows that analyses of change in LTL may be confounded by regression to the mean (RTM), especially in the presence of large measurement error, which could induce artificially stronger dependence of LTL change on baseline values (Benetos et al., 2013; Glymour et al., 2005; Verhulst et al., 2013). We corrected our follow-up LTL measurements for RTM, following the formulas in Barnett et al. (2005); Linden (2013); and Verhulst et al. (2013). As also observed in these studies, RTM correction diminishes the dependence of change in LTL on baseline values in our sample (Fig. 1). Our modeling strategy shows longitudinal associations of stress and LTL are sensitive to applying this correction.

We examined the longitudinal associations of chronic stress with LTL using fixed-effects (FE) models. FE models have been mainstay for analysis of panel data in the social sciences, offering the unique advantage of controlling for *all observed and unobserved time-invariant confounding* by deriving associations only from within-person changes, i.e. using each person at t_1 as their own control at t_2 (Angrist and Pischke, 2009b; Halaby, 2004; Schempf and Kaufman, 2012). FE models achieve this control in three equivalent formulations: 1) Dummy Variables: conditioning on person $n - 1$ identifiers and estimating person-specific intercepts; 2) Demeaning/"Absorbing"/"Within" Estimator: modeling changes in the outcome and covariates as deviations from their respective person-specific overall means; or 3) First-Differencing: manually regressing change in the outcome on change in covariates. With two periods, as in our data, these formulations are identical. Gunasekara et al. (2013) provide a very useful tutorial on FE models.

Because of the sweeping control for confounding, the FE model is superior for analyzing longitudinal effects than the random-effects model, generalized estimating equations, and the traditional "change-score" model which typically regresses outcome change on exposure and covariates (e.g. (Bateson et al., 2018)), all of which mix between- and within-person effects in various ways and remain vulnerable to unobserved confounding. Further, the FE model likely mitigates confounding by RTM. That is because FE models are similar to ANCOVA models in effectively mean-centering exposure and covariates (Angrist and Pischke, 2009b; Rabe-Hesketh and Skrondal, 2012), and ANCOVA models have been shown to reduce RTM (Barnett et al., 2005; Linden, 2013). Notwithstanding these advantages, one main downside to FE models is that their power to detect effects inherently hinges on the extent of changes in the exposure; individuals who do not experience change overtime do not contribute to the estimated associations. This is likely an issue for analyses of changes in neighborhood stress which tends to be stable over time.

In a similar fashion to the cross-sectional models above, we analyzed the longitudinal associations of within-person changes in chronic stress with within-person changes in LTL in a series of FE linear regression models. In a hierarchical dataset with two observations for each person, our base model (*Model 0*) simply regressed *observed* time-varying T/S ratios on time-varying stress exposure, adjusting for persons' FEs. *Model 1* was identical to Model 0 but used instead *RTM-corrected* T/S ratios, documenting how the association responds to this correction. Building on Model 1, *Model 2* adjusted time-varying main demographic confounders, including income-wealth and marital status. Time-invariant confounders, e.g. gender and race, are already adjusted via the FEs and cannot be individually added to the model since they are perfectly collinear with the FEs. *Model 3* further added potential time-varying confounders, including behavioral and psychosocial factors and incident conditions. *Model 4*, our main specification, further controls for linear and quadratic terms of specimen storage duration. We could not adjust for potential confounding related to baseline LTL in our FE models; to be properly performed to avoid substantial downward bias, such adjustment would require at least three observations per person (Angrist et al., 2009a), which our dataset unfortunately lacks. However, the extent of confounding control and RTM correction in our FE models address major sources of confounding and possibly obviate the need for baseline LTL adjustment.

Finally, since our main FE models average over all changes in stress, we replaced the time-varying stress measure in a secondary *Model 4* specification with a time-interacted stress measure that explicitly modeled the direction of change in stress (persistently low, moderate, high; decreasing; increasing) to help unpack how the direction of change in chronic stress since baseline may have driven the direction of change in LTL.

3. Results

3.1. Sample characteristics

The cross-sectional and longitudinal sample characteristics are shown in Table 1. The average age at baseline was 61 years and 53% were female. The sample was racially and socioeconomically diverse with a non-white majority (29% non-Hispanic Black, 43% Hispanic) and a range of educational levels (41% less than college, 30% some college, 30% college or higher). In terms of behavioral and biomedical characteristics at baseline, 57% consumed alcohol, the mean pack-years of cigarettes smoked was 8.51 years and the sample BMI mean was 29 kg/m^2. The mean Spielberger anxiety score was 15.8 and the mean CES-D score was 7.9. Hypertension was common (44%), but only 13% had diabetes and 6% reported a cancer diagnosis.

Most participants had low individual-level (68%) and total (59%) chronic stress score, however, 51% had moderate and 25% had high neighborhood stress. In the longitudinal sample, while 32%

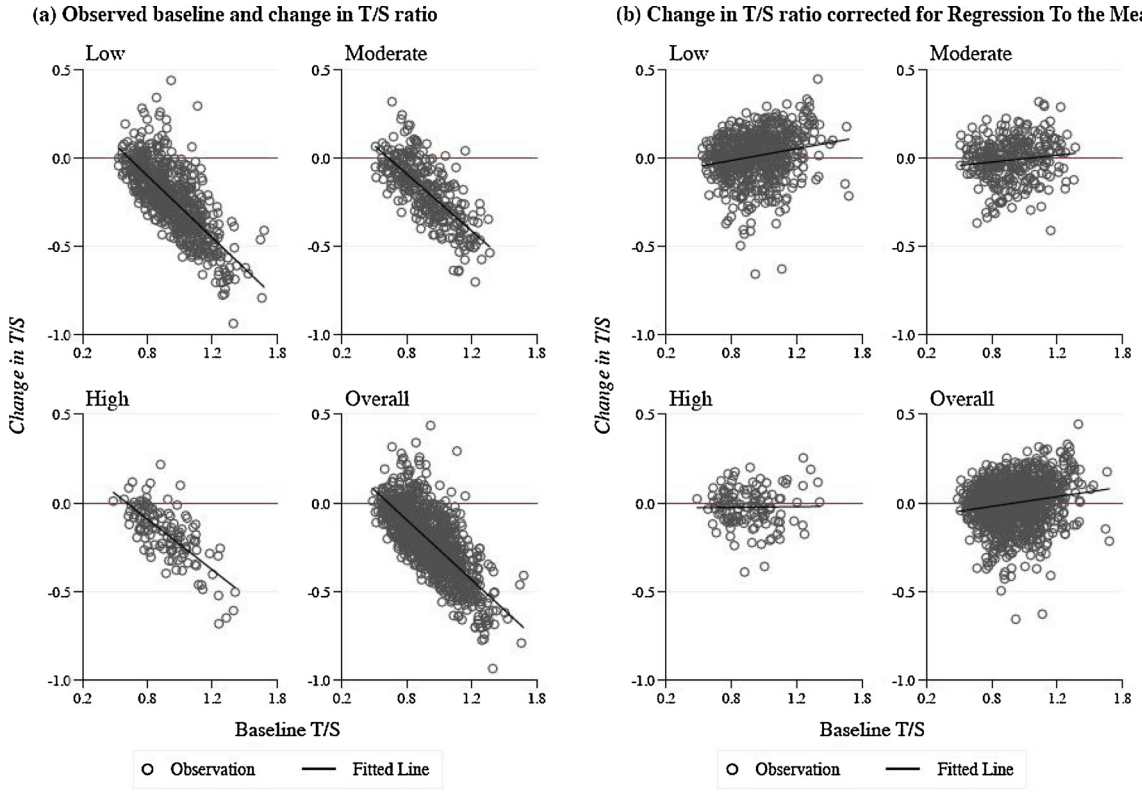

Fig. 1. Correlation between baseline and change in telomere length (T/S ratio) by total baseline stress, with and without correcting for regression to the mean (RTM). Correction for RTM diminishes the strong negative correlation between baseline and change in the T/S ratio: overall (-0.73 to 0.17), low-stress (-0.74 to 0.20), moderate-stress (-0.73 to 0.12), and high-stress (-0.72 vs. 0.01).

experienced some change in their individual-level stress, only 3% experienced change in neighborhood-level stress. Change in total stress was experienced in 26% of participants. Observed mean LTL decreased over the 10-year follow-up period by about 0.2 units. LTL attrition rate was slightly larger in magnitude among women than men, and smaller among blacks than whites (data not shown). Approximately 66% of the sample experienced telomere attrition, defined as LTL decrease greater than 15% from baseline (van Ockenburg et al., 2015), while only 2.5% of the sample's telomeres lengthened (LTL increase greater than 15%).

3.2. Cross-sectional association of stress with telomere length

Pooled cross-sectional associations, adjusted for interdependence of the samples, of chronic stress with LTL are shown in Table 2. In comparison with participants under mild or no overall chronic stress, those with moderate total stress had shorter telomeres, by about 0.022 units [95%CI: −0.038, −0.007] (about 2.7% shorter), whereas those with high total stress had shorter telomeres by about 0.036 units [−0.059, −0.012] (about 4.4% shorter) (Table 2; Model 4). Participants with high individual-level chronic stress had shorter telomeres by about 0.028 units [−0.060, 0.006] relative to their low-stress counterparts. Neighborhood stress was also associated with LTL to a similar degree. These associations were generally robust to covariate adjustment (Table 2; Models 2–4), although control for specimen storage slightly changed estimates in apparently different directions by stress level. These patterns were also evident in models employing z scores of continuous stress measures (Supplemental Table 1), albeit with much smaller magnitudes than even low-to-moderate stress comparisons.

3.3. Longitudinal association of within-person change in stress with change in telomere length

Adjusted longitudinal associations from the FE models are shown in Table 3. It is first important to note that these are strictly within-person associations, inherently driven by those who experienced changes in stress levels between baseline and follow-up exams. For a given person, change from low total stress to high total stress was associated with LTL shortening by 0.054 units [−0.095, −0.013] (about 5.9% shorter), whereas change to moderate stress was associated with shortening by only 0.010 units [−0.035, 0.016] (about 1.1% shorter) (Table 3; Model 4). These changes were likely driven by changes in individual-level stress, which on their own were associated with LTL attrition to comparable degrees. Change from low to high neighborhood stress was associated with LTL shortening by 0.021 units [−0.115, 0.073], whereas change to moderate stress was associated with lengthening by 0.015 units [−0.073, 0.103] (Table 3; Model 4). These associations with changes in neighborhood stress were very imprecise, likely due to the limited change (only ˜3%) in the data. RTM correction appears to have had mixed effects on the associations, driving some away (individual stress) while others towards (neighborhood stress) the null (Model 0 vs. Model 1). Covariate adjustment (Models 1–3) had generally small effects on the associations, except for neighborhood associations which were not stable. Control for storage duration (Model 4) had a similar effect as in the cross-sectional models. These patterns were once again similar to those observed in the FE models with z scores of stress measures, but those estimates were rather small and imprecise (Supplemental Table 2).

In the secondary Model 4 specification using the measures with directional change in stress (Fig. 2), we found the greatest shortening to have occurred among those who experienced increased stress (black bar), followed by those who had persistently low stress (dark gray bar). On the other hand, those with *persistently high stress* since baseline appear to have experienced the least shortening of all (lightest gray bar). Estimates of these changes, however, are very imprecise.

Table 1
Characteristics of the Multi-Ethnic Study of Atherosclerosis (MESA) telomere length study samples in pooled cross-sectional and longitudinal analyses.

	Pooled Cross-Sectional Analysis			Longitudinal Analysis	
	Baseline[a]	*Follow-Up*[a]	*Overall*	*Baseline*[a]	*Follow-Up*[a]
N Observations	1,096	1,135	2231	1029	1029
N Persons	1242			1029	
Observed Telomere Length					
T/S ratio, mean	0.92	0.71	0.81	0.92	0.71
T/S ratio change from baseline,[b] %					
Stable	31.56	31.59	31.58	n/a	31.49
Attrition, > 15%	66.01	65.98	66	n/a	65.99
Lengthening, > 15%	2.42	2.42	2.42	n/a	2.53
Sample storage duration (years), mean	12.94	3.18	7.98	12.96	3.17
Chronic Psychosocial Stress[c]					
Total chronic stress score, mean	1.37	1.34	1.36	1.38	1.34
Individual-level stress: 6-month chronic burden score, mean	1.15	1.1	1.13	1.17	1.09
Neighborhood stress, mean	0.01	−0.04	−0.02	0.05	−0.01
Total chronic stress, %					
Low	59.31	58.5	58.9	58.99	58.21
Moderate	29.2	31.98	30.61	29.15	32.65
High	11.5	9.52	10.49	11.86	9.14
Individual-level stress: 6-month chronic burden score, %					
Low	68.07	69.25	68.67	67.54	70.36
Moderate	27.55	26.26	26.89	28.09	25.17
High	4.38	4.49	4.44	4.37	4.47
Neighborhood stress, %					
Low	24.18	25.02	24.61	24	24.39
Moderate	51.37	50.75	51.05	51.02	51.12
High	24.45	24.23	24.34	24.98	24.49
Stress level changed since baseline, %					
Total stress					26.14
Individual-level stress					31.97
Neighborhood stress					3.30
Demographics					
Age (years), mean	60.77	69.96	65.44	60.62	70.07
Female	52.83	55.24	54.06	52.96	52.96
Married/living with partner, %	58.12	58.94	58.54	58.7	58.9
Race/Ethnicity, %					
Non-Hispanic White	27.01	27.75	27.39	27.41	27.41
Non-Hispanic Black	29.29	29.43	29.36	28.09	28.09
Hispanic	43.7	42.82	43.25	44.51	44.51
Education, %					
≤ College	40.78	39.74	40.25	40.43	40.43
Some College	29.93	30.31	30.12	30.03	30.03
≥ Bachelor's Degree	29.29	29.96	29.63	29.54	29.54
Income-wealth index, %					
Low (0-2)	27.28	27.22	27.25	27.11	27.69
Middle (3-5)	37.59	37	37.29	37.71	36.11
High (6-8)	35.13	35.77	35.45	35.18	36.2
Behavioral & Biomedical Covariates					
Alcohol: currently using, %	57.12	49.43	53.2	57.73	50.24
Pack-years of cigarettes smoked, mean	8.51	8.89	8.71	8.27	8.55
Processed meat (daily servings), mean	0.18	0.19	0.19	0.18	0.2
Body mass index, mean	29.08	29.12	29.1	29.04	29.1
Moderate/vigorous physical activity (tertiles of MET/min/week), %					
Low	31.39	35.24	33.35	31.39	35.08
Middle	34.22	33.83	34.02	34.11	33.92
High	34.4	30.93	32.63	34.5	31
Spielberger anxiety score, mean	15.8	15.54	15.67	15.82	15.53
Depressive symptoms (CESD score), mean	7.86	8.35	8.11	7.85	8.31
Systolic blood pressure, mean	124.33	121.86	123.07	124.07	121.89
Diastolic blood pressure, mean	71.88	69.72	70.78	71.78	69.83
Hypertension (at or before this point), %	43.98	53.48	48.81	43.15	53.55
Diabetes (at or before this point), %	12.68	17	14.88	12.63	17.49
Cardiovascular event[d] (at or before this point), %	0	1.32	0.67	0	1.46
Cancer diagnosis (at or before this point), %	6.11	8.63	7.4	6.03	8.75

CES-D: Center for Epidemiologic Studies 20-item Depression score; MET: metabolic equivalents.

[a] Baseline is Exam 1 for all measures. Follow-up period is: Exam 5 for telomere length, age, and processed meat consumption data; Exam 4 for cigarettes pack-years; and Exam 3 for all other measures.

[b] We used a 15% cutoff to classify percent changes in T/S ratio as attrition/lengthening, following van Ockenberg et al 2015.

[c] See text for description of construction of stress measures.

[d] Cardiovascular event refers to myocardial infarction or stroke from clinical event data.

Table 2
Adjusted cross-sectional associations of chronic stress with telomere length in the Multi-Ethnic Study of Atherosclerosis.

	Model 1	Model 2	Model 3	Model 4 *Main Model*
Among participants with …. stress, relative to those at low stress,	*T/S ratio was different (shorter/longer) by a mean of … units*			
Total Stress				
Low	Ref	Ref	Ref	Ref
Moderate	−0.019†	−0.024‡	−0.026‡	−0.022‡
	[−0.035, −0.003]	[−0.040, −0.008]	[−0.042, −0.010]	[−0.038, −0.007]
High	−0.005	−0.031‡	−0.033‡	−0.036‡
	[−0.028,0.019]	[−0.054, −0.008]	[−0.057, −0.009]	[−0.059, −0.012]
Individual Stress				
Low	Ref	Ref	Ref	Ref
Moderate	0.005	−0.010	−0.011	−0.010
	[−0.011,0.022]	[−0.026,0.006]	[−0.027,0.006]	[−0.026,0.006]
High	0.005	−0.027	−0.027	−0.027
	[−0.030,0.039]	[−0.060,0.005]	[−0.062,0.007]	[−0.060,0.006]
Neighborhood Stress				
Low	Ref	Ref	Ref	Ref
Moderate	0.000	0.009	0.010	0.005
	[−0.018,0.017]	[−0.009,0.027]	[−0.008,0.028]	[−0.012,0.022]
High	−0.025†	−0.026†	−0.027†	−0.028‡
	[−0.045, −0.005]	[−0.048, −0.005]	[−0.049, −0.006]	[−0.049, −0.007]
N Observations	2231			
N Persons	1242			

Listed estimates are averaged over modeled interactions. Each stress exposure type (individual, neighborhood, total) was studied in separate model sets.
Model 1: Stress exposure, MESA Exam indicator, and their interaction.
Model 2: Model 1 + Demographics (age, gender, race/ethnicity, education, income-wealth, marital status).
Model 3: Model 2 + Behaviors, depressive symptoms, anxiety, and incidence of cancer, diabetes, hypertension, or CVD up until exam.
Model 4: Model 3 + specimen storage duration (linear and quadratic terms).
* $p < 0.10$, † $p < 0.05$, ‡ $p < 0.01$. 95% confidence interval in brackets.

3.4. Associations by gender

Cross-sectional associations were generally similar by gender, with the exception of high neighborhood-level stress, which was associated with shorter LTL in men than in women, −0.046 [−0.075, −0.016] vs. −0.014 [−0.041, 0.013], respectively (Table 4). This latter pattern was also apparent in within-person longitudinal analyses (Table 5). On the other hand, within-person analyses also revealed that increases in individual-level stress over time were associated with a stronger magnitude of telomere attrition among women than among men. For example, change from low to high stress was associated with LTL shortening by 0.077 units [−0.128, −0.025] in women vs. only by −0.015

Table 3
Adjusted 10-year, within-person associations of change in chronic stress with change in telomere length in the Multi-Ethnic Study of Atherosclerosis.

	Model 0	Model 1	Model 2	Model 3	Model 4 *Main Model*
Within a given person, change from low stress to …	*Is associated with a mean change in the T/S ratio of … units*				
Total Stress					
Low	Ref	Ref	Ref	Ref	Ref
Moderate	−0.030	−0.015	−0.015	−0.017	−0.010
	[-0.073,0.013]	[-0.041,0.010]	[-0.041,0.010]	[-0.044,0.009]	[-0.035,0.016]
High	−0.002	−0.051†	−0.051†	−0.050†	−0.054†
	[-0.067,0.064]	[-0.093,-0.009]	[-0.093,-0.009]	[-0.094,-0.007]	[-0.095,-0.013]
Individual Stress					
Low	Ref	Ref	Ref	Ref	Ref
Moderate	0.011	−0.018*	−0.017	−0.017	−0.017
	[-0.022,0.044]	[-0.038,0.003]	[-0.038,0.004]	[-0.038,0.005]	[-0.037,0.004]
High	−0.048	−0.053†	−0.054†	−0.050†	−0.055†
	[-0.120,0.025]	[-0.098,-0.007]	[-0.100,-0.007]	[-0.099,-0.002]	[-0.101,-0.010]
Neighborhood Stress					
Low	Ref	Ref	Ref	Ref	Ref
Moderate	0.097	0.054	0.059	0.033	0.015
	[-0.046,0.240]	[-0.032,0.140]	[-0.031,0.149]	[-0.058,0.124]	[-0.073,0.103]
High	0.094	0.001	0.005	−0.018	−0.021
	[-0.063,0.251]	[-0.093,0.095]	[-0.090,0.101]	[-0.114,0.079]	[-0.115,0.073]
N Observations	**2,058**				
N Persons	1029				

Listed estimates are averaged over modeled interactions. Each stress exposure (individual, neighborhood, total) was analyzed in separate model sets.
Model 0: Time-varying stress exposure (Exams 1/3), with participant fixed effects.
Model 1: Model 0 with follow-up T/S corrected for regression to the mean.
Model 2: Model 1 + Time-varying (Exams 1/3) demographics (income-wealth, marital status).
Model 3: Model 2 + Time-varying (Exams 1/3) behaviors, depressive symptoms, anxiety, and incidence of cancer, diabetes, hypertension, or CVD.
Model 4: Model 3 + specimen storage duration (linear and quadratic terms).
* $p < 0.10$, † $p < 0.05$, ‡ $p < 0.01$. 95% confidence interval in brackets.

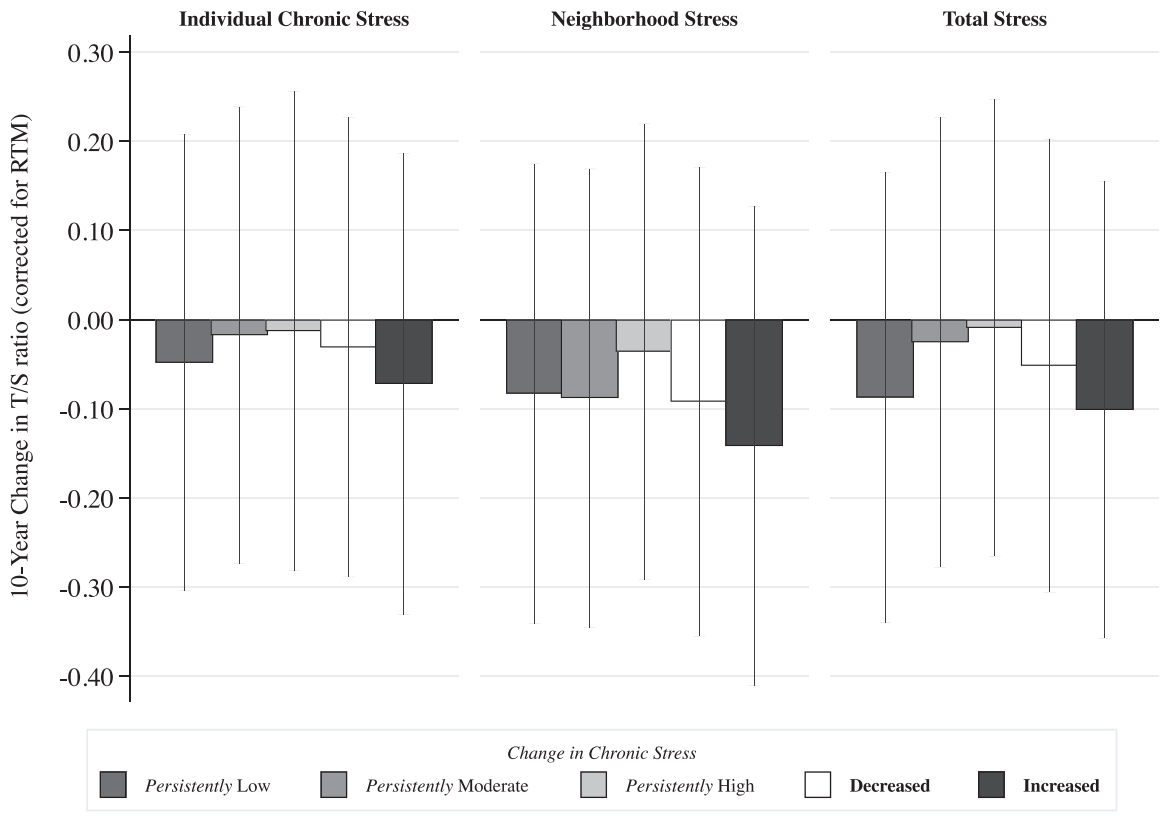

Fig. 2. 10-year change in telomere length (T/S ratio) by *change* in chronic stress in the Multi-Ethnic Study of Atherosclerosis. Change in T/S was corrected for regression to the mean (RTM). Plotted estimates were recovered from a specification similar to fixed-effects Model 4 (Table 3) using directional change in stress interacted with time since baseline, instead of time-varying stress. The model further adjusted for time-varying demographics (income-wealth and marital status), time-varying behaviors, depressive symptoms, anxiety, and incidence of cancer, diabetes, hypertension, or CVD, and specimen storage duration (linear and quadratic terms).

units [−0.084, 0.053] in men (Table 5). These gendered patterns, however, are exploratory and should be interpreted with caution given the low power in our data.

4. Discussion

The inverse relationship between stress and LTL is supported by a wide body of research, yet longitudinal evidence remains limited. In this study, we examined the cross-sectional associations as well as the

Table 4
Adjusted cross-sectional associations of chronic stress with telomere length by gender in the Multi-Ethnic Study of Atherosclerosis.

	All	Men	Women	Difference
Total Stress				
Low	Ref	Ref	Ref	Ref
Moderate	−0.022‡	−0.022°	−0.022†	0.000
	[-0.038,-0.007]	[-0.044,0.000]	[-0.043,-0.001]	[-0.030,0.029]
High	−0.036‡	−0.046†	−0.030†	0.016
	[-0.059,-0.012]	[-0.085,-0.007]	[-0.058,-0.002]	[-0.031,0.063]
Individual Stress				
Low	Ref	Ref	Ref	Ref
Moderate	−0.010	−0.002	−0.016	−0.014
	[-0.026,0.006]	[-0.026,0.022]	[-0.037,0.004]	[-0.045,0.017]
High	−0.027	−0.024	−0.029	−0.006
	[-0.060,0.006]	[-0.077,0.029]	[-0.071,0.012]	[-0.071,0.060]
Neighborhood Stress				
Low	Ref	Ref	Ref	Ref
Moderate	0.005	0.001	0.009	0.008
	[-0.012,0.022]	[-0.023,0.026]	[-0.014,0.032]	[-0.025,0.041]
High	−0.028‡	−0.046‡	−0.014	0.032°
	[-0.049,-0.007]	[-0.075,-0.016]	[-0.041,0.013]	[-0.006,0.070]
N Observations	2231	1,025	1,206	2231
N Persons	1242	563	679	1242

Associations by gender were estimated from pooled cross-sectional Model 4 (Table 2), with stress measure interacted with gender.
Listed estimates are averaged over modeled interactions. Each stress exposure type (individual, neighborhood, total) was analyzed in separate model sets.
° $p < 0.10$, † $p < 0.05$, ‡ $p < 0.01$. 95% confidence interval in brackets.

Table 5
Adjusted 10-year within-person associations of change in chronic stress with change in telomere length by gender in the Multi-Ethnic Study of Atherosclerosis.

	All	Men	Women	Difference
Total Stress				
Low	Ref	Ref	Ref	Ref
Moderate	−0.010	0.010	−0.025	−0.035
	[-0.035,0.016]	[-0.033,0.052]	[-0.060,0.010]	[-0.090,0.020]
High	−0.054†	−0.015	−0.077‡	−0.062
	[-0.095,-0.013]	[-0.084,0.053]	[-0.128,-0.025]	[-0.146,0.022]
Individual Stress				
Low	Ref	Ref	Ref	Ref
Moderate	−0.017	0.002	−0.026*	−0.028
	[-0.037,0.004]	[-0.031,0.035]	[-0.053,0.001]	[-0.071,0.015]
High	−0.055†	0.015	−0.098‡	−0.113†
	[-0.101,-0.010]	[-0.069,0.099]	[-0.158,-0.038]	[-0.214,-0.012]
Neighborhood Stress				
Low	Ref	Ref	Ref	Ref
Moderate	0.015	−0.014	0.017	0.031
	[-0.073,0.103]	[-0.140,0.111]	[-0.087,0.121]	[-0.131,0.193]
High	−0.021	−0.159	0.005	0.164
	[-0.115,0.073]	[-0.360,0.042]	[-0.132,0.141]	[-0.076,0.404]
N Observations	2,058	946	1,072	2,018
N Persons	1029	484	545	1029

Associations by gender were estimated from fixed-effects Model 4 (Table 3), with stress measure interacted with gender.
Listed estimates are averaged over modeled interactions. Each stress exposure type (individual, neighborhood, total) was analyzed in separate model sets.
* $p < 0.10$, † $p < 0.05$, ‡ $p < 0.01$. 95% confidence interval in brackets.

effects of within-person changes in chronic psychosocial stress on changes in telomere length over 10 years. We hypothesized that having greater chronic stress would be associated with shorter telomeres and greater telomere attrition over 10 years. In the cross-sectional analyses, higher chronic stress was associated with shorter LTL, consistent with the literature and previous research in MESA (Needham et al., 2014a; Oliveira et al., 2016).

In the longitudinal fixed-effects analyses, a more nuanced picture of the relationship between change in stress and change in telomere length emerged. First, consistent with our hypothesis and the literature, within-person *change* from low to high stress was associated with sizable telomere shortening, by about 6% relative to baseline. This was primarily driven by changes in individual-level chronic stress and robust to covariate adjustment. Second, when we attempt to unpack these effects by the direction of change in stress, separating out those who remained exposed to the same level of stress as at baseline from those whose stress levels improved or worsened, we found the greatest shortening among those who had experienced worsening stress, once again consistent with our main findings. However, we also found suggestive evidence that individuals with *persistently* high stress over 10 years experienced less LTL attrition than individuals with persistently low stress, which seems counter to our hypothesis, main findings, and the literature more broadly. Nonetheless, there are several possible explanations for this observation. This observation may be due to the fact that individuals with higher baseline chronic stress have shorter telomeres at baseline to begin with than those with less severe or no chronic stress, leaving little room for further shortening (Nordfjall et al., 2009; Verhulst et al., 2013). Indeed, our cross-sectional findings and the literature indicate exactly that: those at higher stress have shorter telomeres. Further, LTL attrition has been found to be associated with baseline LTL in longitudinal studies even after accounting for regression to the mean effect, potentially because longer telomeres are a target for damaging agents, (Aviv et al., 2009; Revesz et al., 2016; Shalev et al., 2013; Verhulst et al., 2013). Another possibility is that those with persistently high stress have activated cellular coping mechanisms, such as telomerase activity, to maintain telomere length and healthy cell function. Studies have demonstrated that stress is associated with increased telomerase activity in both animal models and humans (de Punder et al., 2016; Beery et al., 2012; Epel et al., 2010). Thus, individuals with higher persistent stress may present with less telomere attrition over time due to increased telomerase activity compared to their less stressed peers. Lastly, due to availability of data, this study has measured stress trajectories from MESA exams 1 and 3 and telomere trajectories from exams 1 and 5. Stress measurements were not available at exam 5. The imperfect correlation of stress and telomere change measurements may mean that stress trajectories examined in this study may have misclassified individuals relative to their true stress levels at exam 5. Additional longitudinal studies of telomere attrition in relation to stress and other environmental exposures are necessary to confirm our observations and lend greater insight into drivers of telomere shortening and maintenance.

Previous studies, including in MESA, have found gender differences in adult telomere length (Diez Roux et al., 2009; Needham et al., 2014c; Sanders and Newman, 2013). Less well understood is whether gender modifies the association between chronic stress and LTL. Indeed existing studies report conflicting result with no difference in the stress-LTL association by gender (Mathur et al., 2016), a greater effect in men (Li et al., 2017) and a greater effect in women (Needham et al., 2015). In exploring whether the association of chronic stress and LTL associations differ by gender, we found suggestive evidence that increasing individual-level stress was associated with greater telomere attrition among women, whereas increasing neighborhood stress was associated with greater telomere attrition among men. While these patterns need to be interpreted with caution due to the limited power in our analysis (Gelman and Carlin, 2014), they are worthy of further exploration. Though the current literature is varied, it is possible that the influence of gender on the association between stress and telomere length depends upon the nature and measurement of the stress exposure (i.e., acute, chronic, perceived stress, financial stress, social stress, trauma, etc.), the timing of the stress exposure during the lifecourse, and may be buffered health behaviors (Shalev et al., 2013). Indeed, women may be more susceptible to certain stress exposures than men (Koch et al., 2017), as we found in our analysis. Evidence supports differences by gender in activation of the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system, and behavioral outcomes in response to stress (Bekhbat and Neigh, 2018). In addition, the influence of chronic stress on inflammatory, oxidative, and cortisol responses may vary by gender (Bekhbat and Neigh, 2018), producing differences in telomere attrition between men and women. Unfortunately, many studies with large sample sizes do not report their findings on stress and telomere attrition by gender (Puterman et al., 2016). Further research on gender differences in telomere attrition associated with stress exposures are

needed to better understand these complex interactions.

Interestingly, our study may provide a preliminary indication that reducing chronic stress levels over time lessens the LTL aging trajectory relative to increasing stress levels over time. Over 10 years, individuals who have *decreased* stress have telomere aging rates that are smaller than their counterparts with increasing chronic stress and comparable to those with persistently low or no chronic stress. Though our analysis is limited by small numbers of highly stressed individuals, stress reduction is a common intervention to improve health and further research into the effect of stress reduction on LTL aging trajectory is needed (Epel, 2012; Ornish et al., 2013).

While associations of individual and neighborhood stress are comparable in cross-sectional models, individual-level stress seems to be driving longitudinal associations, likely reflecting the little within-person changes over time in neighborhood stress. This latter point also explains the imprecision of neighborhood estimates in the longitudinal models.

The measurement of chronic stress has been noted as a limitation to previous studies of chronic stress and LTL. Caregiving, poverty and violence, among others, have all been used as proxies for chronic stress exposure (Oliveira et al., 2016; Quinlan et al., 2014). However, the variability across studies in the timing, duration, or detailed measurement of chronic stress exposure does not capture the full range of stressors operating at different levels and limits inference from the collective literature. We assess stress at both the individual and neighborhood levels, as well as create a total chronic stress burden measure to more fully capture stress exposure. Previous work in the MESA Stress Study has shown that these stress measures have good internal consistency and test-retest reliability, therefore, we hypothesize minimal bias of stress measures in the present study (Mujahid et al., 2011).

This study has many strengths. We used a large, multi-ethnic, population based sample (as opposed to a caregiver sample) to conduct a longitudinal study of moderate, chronic psychosocial stress and telomere attrition. The ten-year follow-up duration allows us to determine that the estimated telomere attrition rate has negligible error due to measurement variability and is not an artifact of short-term dynamics in telomere length (De Meyer et al., 2008). LTL was measured in a laboratory with extensive telomere length measurement experience in both clinical and population studies. Further, fixed-effects models estimated the association between within-person change in stress level and telomere length change while inherently controlling for all time-invariant confounding. Last, the rich MESA data allowed us to examine and control for morbidities, such as depression, to isolate the association between stress and LTL independent of these factors.

This study also has limitations. Inherent in any longitudinal study is the differential storage time of biological samples. Baseline samples in our study had an average of 13 years in freezer storage while follow-up samples were stored an average of 3 years. Measured apparent TL has been found to lengthen over time in freezer storage (Reichert et al., 2017; Tolios et al., 2015), and the additional years of storage for baseline samples compared to follow-up samples could cause an underestimation of the overall LTL aging effect (within-person attrition) and between-person differences. To address this potential source of bias, our modeling strategy adjusts for storage time using linear and quadratic terms. While our estimates are only slightly affected by adjustment for storage time, more research is needed to better understand the complex nature of telomere erosion to ensure longitudinal change is not an artifact of laboratory processes.

Our study evaluated longitudinal chronic stress exposure in middle age and previous studies have linked childhood stressful exposures to adult telomere length, warranting future work connecting stressful exposures over the lifecourse to telomere attrition in adulthood. Further, future work should examine the role of early life stress exposure as a modifier of the biological consequences (telomere attrition) of mid-life stress exposure. Lastly, there is a need to evaluate the potential protective factors that might buffer the effect of chronic stress on LTL, such as wealth, income equality and social networks, in longitudinal studies (Oliveira et al., 2016).

5. Conclusion

We examined whether chronic psychosocial stress was longitudinally associated with LTL changes over time in a diverse, well-characterized cohort of middle-aged individuals. Our findings corroborate existing evidence that those exposed to higher stress have shorter telomeres, and provide strong longitudinal evidence that worsening stress exposure is associated with shortening of telomeres, independent of all time-invariant confounding. Changes in chronic stress acting on telomeres may be one way in which our exposome gets "under the skin" to influence our health, though further longitudinal research is necessary to better understand telomere dynamics in response to stress over time and across genders and population subgroups.

Funding sources

This work was supported by the National Institutes of Health [MESA grant]. This research was supported by contracts N01-HC-95159 through N01-HC-95169 from the National Heart, Lung, and Blood Institute and by grants UL1-RR-024156 and UL1-RR-025005 from NCRR and R01 HL071759 from National Heart, Lung, and Blood Institute at the National Institutes of Health. The Telomere study was supported by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the National Heart, Lung, and Blood Institute, and by grants UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420 from NCATS. The MESA Stress Study was supported by R01HL076831 and R01HL101161 (PI: Ana Diez Roux). Work was also partially supported by funding from the UCLA Older Americans Independence Center, NIH/NIA Grant P30AG028748, and the USC/UCLA Biodemography Center through a P30 grant from the NIA (P30AG017265). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author contributions

All authors have approved the final article. MH designed the study, acquired, analyzed and interpreted the data, and critically revised the manuscript. HM contributed to the analysis and interpretation of the data and drafted the manuscript. BN contributed to the interpretation of the data and critically revised the manuscript. JL collected data and critically revised the manuscript. ADR conceptualized the study, collected the data, contributed to the interpretation of the data and critically revised the manuscript. SB critically revised the manuscript.

Conflict of interest

The authors have no conflicts of interest to report.

Acknowledgements

We thank Dr. Elizabeth Blackburn and her laboratory for conducting the telomere length assays and Dr. Elissa Epel for her contributions to earlier stages of this project. We also wish to thank MESA investigators and staff, as well as the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.04.018.

References

Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E.V., Futcher, A.B., Greider, C.W., Harley, C.B., 1992. Telomere length predicts replicative capacity of human fibroblasts. PNAS 89, 10114–10118.

Angrist, J.D., Pischke, J.-S., 2009b. Parallel worlds: fixed effects, difference-in-differences, and panel data. In: Angrist, J.D., Pischke, J.-S. (Eds.), Mostly Harmless Econometrics, An Empiricist's Companion. Princeton University Press, Princeton, NJ, pp. 221–248.

Angrist, J.D., Pischke, J.-S., 2009a. Parallel worlds: fixed effects, difference-in-differences, and panel data. In: Angrist, J.D., Pischke, J.-S. (Eds.), Mostly Harmless Econometrics, An Empiricist's Companion. Princeton University Press, Princeton, NJ, pp. 244–246.

Armanios, M., Blackburn, E.H., 2012. The telomere syndromes. Nat. Rev. Genet. 13, 693–704.

Aubert, G., Lansdorp, P.M., 2008. Telomeres and aging. Physiol. Rev. 88, 557–579.

Aviv, A., Chen, W., Gardner, J.P., Kimura, M., Brimacombe, M., Cao, X., Srinivasan, S.R., Berenson, G.S., 2009. Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. Am. J. Epidemiol. 169, 323–329.

Barber, S., Hickson, D.A., Wang, X., Sims, M., Nelson, C., Diez-Roux, A.V., 2016. Neighborhood disadvantage, poor social conditions, and cardiovascular disease incidence among African American adults in the Jackson Heart Study. Am. J. Public Health 106, 2219–2226.

Barnett, A.G., van der Pols, J.C., Dobson, A.J., 2005. Regression to the mean: what it is and how to deal with it. Int. J. Epidemiol. 34, 215–220.

Bateson, M., Eisenberg, D.T.A., Nettle, D., 2018. Controlling for Baseline Telomere Length Biases Estimates of the Rate of Telomere Attrition (Version 3). Zenodohttps://doi.org/10.5281/zenodo.2458376.

Baum, A., Garofalo, J.P., Yali, A.M., 1999. Socioeconomic status and chronic stress: does stress account for SES effects on health? Ann. N.Y. Acad. Sci. 896, 131–144.

Beery, A.K., Lin, J., Biddle, J.S., Francis, D.D., Blackburn, E.H., Epel, E.S., 2012. Chronic stress elevates telomerase activity in rats. Biol. Lett. 8, 1063–1066.

Bekhbat, M., Neigh, G.N., 2018. Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. Brain Behav. Immun. 67, 1–12.

Benetos, A., Kark, J.D., Susser, E., Kimura, M., Sinnreich, R., Chen, W., Steenstrup, T., Christensen, K., Herbig, U., von Bornemann Hjelmborg, J., Srinivasan, S.R., Berenson, G.S., Labat, C., Aviv, A., 2013. Tracking and fixed ranking of leukocyte telomere length across the adult life course. Aging Cell 12, 615–621.

Bild, D.E., Bluemke, D.A., Burke, G.L., Detrano, R., Diez Roux, A.V., Folsom, A.R., Greenland, P., Jacob Jr., D.R., Kronmal, R., Liu, K., Nelson, J.C., O'Leary, D., Saad, M.F., Shea, S., Szklo, M., Tracy, R.P., 2002. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol . 156, 871–881.

Blackburn, E.H., Greider, C.W., Szostak, J.W., 2006. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat. Med. 12, 1133–1138.

Bromberger, J.T., Matthews, K.A., 1996. A longitudinal study of the effects of pessimism, trait anxiety, and life stress on depressive symptoms in middle-aged women. Psychol. Aging 11, 207–213.

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30, e47.

Chen, W., Gardner, J.P., Kimura, M., Brimacombe, M., Cao, X., Srinivasan, S.R., Berenson, G.S., Aviv, A., 2009. Leukocyte telomere length is associated with HDL cholesterol levels: the Bogalusa heart study. Atherosclerosis 205, 620–625.

D'Mello, M.J., Ross, S.A., Briel, M., Anand, S.S., Gerstein, H., Pare, G., 2015. Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. Circ. Cardiovasc. Genet. 8, 82–90.

De Meyer, T., Rietzschel, E.R., De Buyzere, M.L., Van Criekinge, W., Bekaert, S., 2008. Studying telomeres in a longitudinal population based study. Front. Biosci. 13, 2960–2970.

de Punder, K., Heim, C., Wadhwa, P.D., Entringer, S., 2016. In vitro stimulated leukocyte telomerase activity is associated with chronic stress exposure. Psychoneuroendocrinology 71, 60–61.

Diez Roux, A.V., Ranjit, N., Jenny, N.S., Shea, S., Cushman, M., Fitzpatrick, A., Seeman, T., 2009. Race/ethnicity and telomere length in the multi-ethnic study of atherosclerosis. Aging Cell 8, 251–257.

Epel, E., 2012. How "reversible" is telomeric aging? Cancer Prev. Res. (Phila.) 5, 1163–1168.

Epel, E.S., Prather, A.A., 2018. Stress, telomeres, and psychopathology: toward a deeper understanding of a triad of early aging. Annu. Rev. Clin. Psychol. 14, 371–397.

Epel, E.S., Lin, J., Dhabhar, F.S., Wolkowitz, O.M., Puterman, E., Karan, L., Blackburn, E.H., 2010. Dynamics of telomerase activity in response to acute psychological stress. Brain Behav. Immun. 24, 531–539.

Fitzpatrick, A.L., Kronmal, R.A., Gardner, J.P., Psaty, B.M., Jenny, N.S., Tracy, R.P., Walston, J., Kimura, M., Aviv, A., 2007. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am. J. Epidemiol. 165, 14–21.

Gelman, A., Carlin, J., 2014. Beyond power calculations:assessing type S (sign) and type M (magnitude) errors. Perspect. Psychol. Sci. 9, 641–651.

Geronimus, A.T., Pearson, J.A., Linnenbringer, E., Schulz, A.J., Reyes, A.G., Epel, E.S., Lin, J., Blackburn, E.H., 2015. Race-ethnicity, poverty, urban stressors, and telomere length in a detroit community-based sample. J. Health Soc. Behav. 56, 199–224.

Glymour, M.M., Weuve, J., Berkman, L.F., Kawachi, I., Robins, J.M., 2005. When is baseline adjustment useful in analyses of change? An example with education and cognitive change. Am. J. Epidemiol. 162, 267–278.

Gunasekara, F.I., Richardson, K., Carter, K., Blakely, T., 2013. Fixed effects analysis of repeated measures data. Int. J. Epidemiol. 43, 264–269.

Hajat, A., Diez-Roux, A., Franklin, T.G., Seeman, T., Shrager, S., Ranjit, N., Castro, C., Watson, K., Sanchez, B., Kirschbaum, C., 2010. Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: the multi-ethnic study of atherosclerosis. Psychoneuroendocrinology 35, 932–943.

Halaby, C.N., 2004. Panel models in sociological research: theory into practice. Annu. Rev. Soc. 30, 507–544.

Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.

Jeanclos, E., Schork, N.J., Kyvik, K.O., Kimura, M., Skurnick, J.H., Aviv, A., 2000. Telomere length inversely correlates with pulse pressure and is highly familial. Hypertension 36, 195–200.

Jodczyk, S., Fergusson, D.M., Horwood, L.J., Pearson, J.F., Kennedy, M.A., 2014. No association between mean telomere length and life stress observed in a 30 year birth cohort. PloS one 9, e97102.

Kershaw, K.N., Diez Roux, A.V., Bertoni, A., Carnethon, M.R., Everson-Rose, S.A., Liu, K., 2015. Associations of chronic individual-level and neighbourhood-level stressors with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis. J. Epidemiol. Commun. Health 69, 136–141.

Koch, C.E., Leinweber, B., Drengberg, B.C., Blaum, C., Oster, H., 2017. Interaction between circadian rhythms and stress. Neurobiol. Stress 6, 57–67.

Kong, C.M., Lee, X.W., Wang, X., 2013. Telomere shortening in human diseases. FEBS J. 280, 3180–3193.

Li, X., Wang, J., Zhou, J., Huang, P., Li, J., 2017. The association between post-traumatic stress disorder and shorter telomere length: a systematic review and meta-analysis. J. Affect. Disord. 218, 322–326.

Linden, A., 2013. Assessing regression to the mean effects in health care initiatives. BMC Med. Res. Methodol. 13, 119.

Ludlow, A.T., Roth, S.M., 2011. Physical activity and telomere biology: exploring the link with aging-related disease prevention. J. Aging Res. 2011, 790378.

Mathur, M.B., Epel, E., Kind, S., Desai, M., Parks, C.G., Sandler, D.P., Khazeni, N., 2016. Perceived stress and telomere length: a systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain Behav. Immun. 54, 158–169.

Matthews, K.A., Gallo, L.C., Taylor, S.E., 2010. Are psychosocial factors mediators of socioeconomic status and health connections? Ann. N.Y. Acad. Sci. 1186, 146–173.

McEwen, B.S., 2004. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann. N.Y. Acad. Sci. 1032, 1–7.

Mujahid, M.S., Diez Roux, A.V., Morenoff, J.D., Raghunathan, T., 2007. Assessing the measurement properties of neighborhood scales: from psychometrics to ecometrics. Am. J. Epidemiol. 165, 858–867.

Mujahid, M.S., Diez Roux, A.V., Cooper, R.C., Shea, S., Williams, D.R., 2011. Neighborhood stressors and race/ethnic differences in hypertension prevalence (the multi-ethnic study of atherosclerosis). Am. J. Hypertens. 24, 187–193.

Needham, B.L., Carroll, J.E., Diez Roux, A.V., Fitzpatrick, A.L., Moore, K., Seeman, T.E., 2014a. Neighborhood characteristics and leukocyte telomere length: the Multi-Ethnic Study of Atherosclerosis. Health Place 28, 167–172.

Needham, B.L., Carroll, J.E., Roux, A.V.D., Fitzpatrick, A.L., Moore, K., Seeman, T.E., 2014b. Neighborhood characteristics and leukocyte telomere length: the Multi-Ethnic Study of Atherosclerosis. Health Place 28, 167–172.

Needham, B.L., Diez Roux, A.V., Bird, C.E., Bradley, R., Fitzpatrick, A.L., Jacobs, D.R., Ouyang, P., Seeman, T.E., Thurston, R.C., Vaidya, D., Wang, S., 2014c. A test of biological and behavioral explanations for gender differences in telomere length: the multi-ethnic study of atherosclerosis. Biodemogr. Soc. Biol. 60, 156–173.

Needham, B.L., Mezuk, B., Bareis, N., Lin, J., Blackburn, E.H., Epel, E.S., 2015. Depression, anxiety and telomere length in young adults: evidence from the National Health and Nutrition Examination Survey. Mol. Psychiatry 20, 520–528.

Nettleton, J.A., Diez-Roux, A., Jenny, N.S., Fitzpatrick, A.L., Jacobs Jr, D.R., 2008. Dietary patterns, food groups, and telomere length in the Multi-Ethnic Study of Atherosclerosis (MESA). Am. J. Clin. Nutr. 88, 1405–1412.

Nordfjall, K., Svenson, U., Norrback, K.F., Adolfsson, R., Lenner, P., Roos, G., 2009. The individual blood cell telomere attrition rate is telomere length dependent. PLos Genet. 5, e1000375.

Oliveira, B.S., Zunzunegui, M.V., Quinlan, J., Fahmi, H., Tu, M.T., Guerra, R.O., 2016. Systematic review of the association between chronic social stress and telomere length: a life course perspective. Ageing Res. Rev. 26, 37–52.

Ornish, D., Lin, J., Chan, J.M., Epel, E., Kemp, C., Weidner, G., Marlin, R., Frenda, S.J., Magbanua, M.J.M., Daubenmier, J., Estay, I., Hills, N.K., Chainani-Wu, N., Carroll, P.R., Blackburn, E.H., 2013. Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. Lancet Oncol. 14, 1112–1120.

Pilkonis, P.A., Imber, S.D., Rubinsky, P., 1985. Dimensions of life stress in psychiatric patients. J. Human Stress 11, 5–10.

Puterman, E., Gemmill, A., Karasek, D., Weir, D., Adler, N.E., Prather, A.A., Epel, E.S., 2016. Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. PNAS 113, E6335–E6342.

Quinlan, J., Tu, M.T., Langlois, E.V., Kapoor, M., Ziegler, D., Fahmi, H., Zunzunegui, M.V., 2014. Protocol for a systematic review of the association between chronic stress during the life course and telomere length. Syst. Rev. 3, 40.

Rabe-Hesketh, S., Skrondal, A., 2012. Chapter 5 subject-specific effects and dynamic models. In: Rabe-Hesketh, S., Skrondal, A. (Eds.), Multilevel and Longitudinal Modeling Using Stata, 3rd ed. Stata Press, College Station, TX, pp. 262–264.
Reichert, S., Froy, H., Boner, W., Burg, T.M., Daunt, F., Gillespie, R., Griffiths, K., Lewis, S., Phillips, R.A., Nussey, D.H., Monaghan, P., 2017. Telomere length measurement by qPCR in birds is affected by storage method of blood samples. Oecologia 184, 341–350.
Revesz, D., Verhoeven, J.E., Milaneschi, Y., Penninx, B.W., 2016. Depressive and anxiety disorders and short leukocyte telomere length: mediating effects of metabolic stress and lifestyle factors. Psychol. Med. 46, 2337–2349.
Rode, L., Nordestgaard, B.G., Bojesen, S.E., 2015. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. J. Natl. Cancer Inst. 107 djv074.
Sampson, M.J., Winterbone, M.S., Hughes, J.C., Dozio, N., Hughes, D.A., 2006. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. Diabetes care 29, 283–289.
Sanders, J.L., Newman, A.B., 2013. Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? Epidemiol. Rev. 35, 112–131.
Schaakxs, R., Wielaard, I., Verhoeven, J.E., Beekman, A.T.F., Penninx, B.W.J.H., Comijs, H.C., 2015. Early and recent psychosocial stress and telomere length in older adults. Int. Psychogeriatr. 1–9.
Schempf, A.H., Kaufman, J.S., 2012. Accounting for context in studies of health inequalities: a review and comparison of analytic approaches. Ann. Epidemiol. 22, 683–690.
Schutte, N.S., Malouff, J.M., 2014. The relationship between perceived stress and telomere length: a meta-analysis. Stress Health n/a-n/a.
Seeman, T.E., Epel, E., Gruenewald, T., Karlamangla, A., McEwen, B.S., 2010. Socio-economic differentials in peripheral biology: cumulative allostatic load. Ann. N.Y. Acad. Sci. 1186, 223–239.
Shalev, I., Entringer, S., Wadhwa, P.D., Wolkowitz, O.M., Puterman, E., Lin, J., Epel, E.S., 2013. Stress and telomere biology: a lifespan perspective. Psychoneuroendocrinology 38, 1835–1842.
Starkweather, A.R., Alhaeeri, A.A., Montpetit, A., Brumelle, J., Filler, K., Montpetit, M., Mohanraj, L., Lyon, D.E., Jackson-Cook, C.K., 2014. An integrative review of factors associated with telomere length and implications for biobehavioral research. Nurs. Res. 63, 36–50.
Steptoe, A., Feldman, P.J., 2001. Neighborhood problems as sources of chronic stress: development of a measure of neighborhood problems, and associations with socio-economic status and health. Ann. Behav. Med. 23, 177–185.
Strandberg, T.E., Saijonmaa, O., Tilvis, R.S., Pitkala, K.H., Strandberg, A.Y., Miettinen, T.A., Fyhrquist, F., 2011. Association of telomere length in older men with mortality and midlife body mass index and smoking. J. Gerontol. Ser. A, Biol. Sci. Med. Sci. 66, 815–820.
Surtees, P.G., Wainwright, N.W.J., Pooley, K.A., Luben, R.N., Khaw, K.-T., Easton, D.F., Dunning, A.M., 2012. Educational attainment and mean leukocyte telomere length in women in the European Prospective Investigation into Cancer (EPIC)-Norfolk population study. Brain Behav. Immun. 26, 414–418.
Theall, K.P., Brett, Z.H., Shirtcliff, E.A., Dunn, E.C., Drury, S.S., 2013. Neighborhood disorder and telomeres: connecting children's exposure to community level stress and cellular response. Soc. Sci. Med. 85, 50–58.
Tolios, A., Teupser, D., Holdt, L.M., 2015. Preanalytical conditions and DNA isolation methods affect telomere length quantification in whole blood. PloS one 10, e0143889.
Valdes, A.M., Andrew, T., Gardner, J.P., Kimura, M., Oelsner, E., Cherkas, L.F., Aviv, A., Spector, T.D., 2005. Obesity, cigarette smoking, and telomere length in women. Lancet 366, 662–664.
van Ockenburg, S.L., Bos, E.H., de Jonge, P., van der Harst, P., Gans, R.O., Rosmalen, J.G., 2015. Stressful life events and leukocyte telomere attrition in adulthood: a prospective population-based cohort study. Psychol. Med. 45, 2975–2984.
Verhulst, S., Aviv, A., Benetos, A., Berenson, G.S., Kark, J.D., 2013. Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for' regression to the mean'. Eur. J. Epidemiol. 28, 859–866.