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ORIGINAL ARTICLE

Determinants of telomere attrition over 1 year in healthy older women: stress and health behaviors matter

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Telomere length, a reliable predictor of disease pathogenesis, can be affected by genetics, chronic stress and health behaviors. Cross-sectionally, highly stressed postmenopausal women have shorter telomeres, but only if they are inactive. However, no studies have prospectively examined telomere length change over a short period, and if rate of attrition is affected by naturalistic factors such as stress and engagement in healthy behaviors, including diet, exercise, and sleep. Here we followed healthy women over 1 year to test if major stressors that occurred over the year predicted telomere shortening, and whether engaging in healthy behaviors during this period mitigates this effect. In 239 postmenopausal, non-smoking, disease-free women, accumulation of major life stressors across a 1-year period predicted telomere attrition over the same period—for every major life stressor that occurred during the year, there was a significantly greater decline in telomere length over the year of 35 bp ($P < 0.05$). Yet, these effects were moderated by health behaviors (*interaction* $B = 0.19$, $P = 0.04$). Women who maintained relatively higher levels of health behaviors (1 s.d. above the mean) appeared to be protected when exposed to stress. This finding has implications for understanding malleability of telomere length, as well as expectations for possible intervention effects. This is the first study to identify predictors of telomere length change over the short period of a year.

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INTRODUCTION

An aging population brings with it ever-increasing risks and prevalence of diseases of aging such as cardiovascular disease, type 2 diabetes mellitus, Alzheimer's disease and autoimmune disorders.¹ Chronic stress is associated with many of these diseases² and is rooted in both the exposure to stressors experienced from birth through adulthood and in temperaments formed early in life through gene–environment interactions that predispose individuals to high threat perceptions.^{2,3} Stressful events across the lifespan, ranging from abuse and neglect as a child to experiences of financial difficulties and relationship breakdown during adulthood, cumulatively wear down physiologic systems that accelerate individuals' trajectories toward disease.^{3–5}

An impaired and aging immune system partly mediates the deleterious effects of chronic stress that drive diseases of aging.⁶ Adults experiencing chronic stress have impaired wound healing, weaker control of latent viruses, poorer vaccination responses and elevated states of chronic inflammation.⁷ For example, caregivers of family dementia patients have a significant increase in circulating levels of interleukin-6 over a 6-year period that is four times the increase of matched, non-caregiving controls.⁸ In a sample of healthy men and women, those who were exposed to any recent stressful life event over the previous 12 months were more likely to develop a cold following experimental exposure to a rhinovirus.⁹

Several cellular markers indicate aging of immune cells, with significant attention paid to telomere length.^{10,11} Short telomeres have been linked to numerous diseases of aging and, in many but not all cases, to all-cause and disease-specific mortality.^{12–17}

In humans, telomeres consist of repeated sequences (TTAGGG repeats) of DNAs that are thousands of nucleotides long, encapsulated and stabilized by associated proteins. Telomeres cap chromosomes in all eukaryotic cells, protecting DNA from degradation resulting from incomplete replication, exogenous and endogenous damage and detrimental fusion during DNA repair processes. With each cell cycle, the 3'-end replication problem causes telomere shortening, and when telomeres shorten to a critical length, cells typically enter senescence and undergo changes that can be harmful at the organismic level.¹²

The protein–RNA complex reverse transcriptase enzyme, telomerase, significantly delays shortening to a critical length by adding repeated TTAGGG sequences onto chromosomal ends.¹⁸ Mutations in telomere maintenance genes in humans and genetic knockout of either telomerase protein ($TERT^{-/-}$) or telomerase RNA ($TERC^{-/-}$) components in rodent models cause a spectrum of diseases called the telomere syndromes.¹² These reveal the prime mechanistic role of telomere maintenance in tissue degradation associated with early aging and disease pathogenesis.^{19,20} Population-based genome-wide association studies also show that common sequence variants of genes known to function directly in telomere maintenance cause increased risks for cardiovascular and pulmonary disease.²¹

Chronic psychologic stress has been associated with shorter telomeres during childhood and adulthood, although not consistently. Children and adults with adverse and disadvantaged early life experiences^{22–25} (see Glass *et al.*²⁶ for exception), women who provide care for a family member with a chronic health condition^{27,28} (see O'Donovan *et al.*²⁹ for exception), those who

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report high perceived stress^{28,30} and women exposed to domestic violence³¹ have shorter telomeres in leukocytes and varying subtypes of immune cells compared with those who have not experienced such stressors. Severity and chronicity of depression are also related to shorter telomeres.^{32–34} To date, no studies have prospectively examined whether the emergence of new life stressors is related to *shortening* of telomeres over time. It is unknown whether a combination of life stressors is potent enough to lead to greater telomere shortening in a short period of time.

The current study also sought to examine whether health behaviors moderate any effects of major life stressors on telomere shortening. In this study, we examined three specific health behaviors: physical activity, dietary intake and sleep quality. Each behavior has been associated with telomere length cross-sectionally.^{35–39} Importantly, these behaviors have been independently shown to alter the relationship between stress and biologic outcomes, including neural functioning and hypothalamic-pituitary-adrenal axis activation.^{40–43} Health behaviors, however, naturally cluster^{44,45} and a combination of healthy behaviors seem to be a stronger predictor of telomere length than each individual behavior alone.⁴⁶ We recently proposed that healthy lifestyle factors, alone or in combination, might mitigate the impact of chronic psychologic stress on telomere length,^{47,48} and documented this effect cross-sectionally in chronically stressed adults and in depressed adults.^{30,49} Whether each behavior alone or in combination can attenuate the biologic burden of stress over time remains unexamined.

The first question we asked, then, is whether major life stressors over the course of 1 year significantly predict leukocyte telomere shortening over the same time frame. We expected that the accumulation of major life events during the year would significantly predict accelerated shortening of telomeres. The current study also examined whether engaging in higher levels of these health behaviors, either individually or combined, can mitigate any prospective association between life stressors and telomere shortening over the course of 1 year.

MATERIALS AND METHODS

Study design

We recruited 263 healthy midlife women (range 50–65 years) from the San Francisco Bay Area between February and May 2010, with online and paper advertisements for the purpose of tracking women's health behaviors and stress over the course of 1 year and telomere length at the beginning and end of that year. The research objectives of the study were (1) to determine whether changes in major life stressors and health behaviors over the course of 1 year predicts telomere length changes and (2) to examine the psychologic impact of revealing to participants the length of their telomeres. Here, we report the findings related to the first objective of the study. The exclusion criteria included any history of cancer within the previous 10 years, any autoimmune disease and current smoking status.

Settings

Certified phlebotomists and nurses drew blood in the summer of 2010 at the University of California, San Francisco's (UCSF) Department of Psychiatry, and again at 1-year follow-up in the summer of 2011 at UCSF's Clinical and Translational Science Institute Clinical Research Center. Ninety-one percent ($N=239$) of the women returned for follow-up blood draw. Health behaviors, including physical activity, typical food consumption and sleep quality, were self-reported at baseline, 4 months, 8 months and 1-year follow-up. Major life stressors over the previous year were self-reported at 1-year follow-up in the summer of 2011. Complete data with main study variables were available for 231 women. Study design was approved by UCSF's Institutional Review Board and informed consent was obtained from all participants.

Measures

Telomere length. We measured leukocyte telomere length (LTL) in all women. Genomic DNA was purified from whole blood stored at -80°C with the QIAamp mini DNA kit (Qiagen, Hilden, Germany; cat. no. 51106). Mean LTL was analyzed using a quantitative PCR method in Blackburn's laboratory at UCSF.⁵⁰ Quantitative PCR is the most common method of LTL measurement used in the majority of current clinical and population-based studies. This method was validated for mean telomere length measurement and shown to be significantly associated with the Southern blot method.⁵¹ For the quantitative PCR reactions to determine mean telomere length, the telomere thermal cycling profile consists of: (1) cycling for T (telomic) PCR—denature at 96°C for 1 minute, one cycle; denature at 96°C for 1 s, anneal/extend at 54°C for 60 s, with fluorescence data collection, 30 cycles; and (2) cycling for S (single-copy gene) PCR—denature at 96°C for 1 minute, one cycle; denature at 95°C for 15 s, anneal at 58°C for 1 s, extend at 72°C for 20 s, 8 cycles, denature at 96°C for 1 s, anneal at 58°C for 1 s, extend at 72°C for 20 s, hold at 83°C for 5 s with data collection, 35 cycles.

The primers for the telomere PCR are *tel1b* (5'-CGGTTT(GTTTGG)₅GTT-3'), used at a final concentration of 100 nM, and *tel2b* (5'-GGCTTG(CCTTAC)₅CCT-3'), used at a final concentration of 900 nM. The primers for the single-copy gene (human β -globin) PCR are *hbg1* (5'-GCTTCTGACACAACCTGTGTTCACT AGC-3'), used at a final concentration of 300 nM, and *hbg2* (5'-CACCAA CTTCATCCACGTTCCACC-3'), used at a final concentration of 700 nM. The final reaction mix contains 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 200 μM each dNTP, 1% dimethylsulfoxide, 0.4 \times Syber Green I, 22 ng *Escherichia coli* DNA per reaction, 0.4 U of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA) per 11 μl reaction and 0.5–10 ng of genomic DNA. Tubes containing 26, 8.75, 2.9, 0.97, 0.324 and 0.108 ng of a reference DNA (from HeLa cancer cells) are included in each PCR run so that the quantity of targeted templates in each sample can be determined relative to the reference DNA sample by the standard curve method. Each concentration of the reference DNA is run as quadruplets and samples are run as triplicate.

To control for interassay variability, eight control DNA samples from cancer cell lines (including 293T, H1299, UMUC3 and UMUC3 cells infected with a lentiviral construct containing the telomerase RNA gene to extent telomeres, harvested at various population doublings after infection) are included in each run. In each batch, the T/S ratio of each control DNA is divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. This is done for all eight samples, and the average normalizing factor for all eight samples is used to correct the participant DNA samples to get the final T/S ratio. The T/S ratio for each sample is measured two times, each time in triplicate wells. When the duplicate T/S value and the initial value vary by more than 7%, the sample is run the third time and the two closest values will be reported. The interassay coefficient of variation for telomere length measurement was 4.3% for this study. To convert T/S ratios to base pairs, the mean telomere length of a set of genomic DNA samples from the human fibroblast primary cell line IMR90 at different population doublings, as well as with the telomerase protein subunit gene (*hTERT*) transfected into a lentiviral construct was measured using the above method. The mean telomeric restriction fragment length from these DNA samples was determined using Southern blot analysis. The slope of the plot of mean telomeric restriction fragment length versus T/S for these samples served as the conversion factor for calculation of telomere length in base pairs from the T/S ratio. The equation for conversion from T/S ratio to base pairs for this study was: base pairs (bp) = $3274 + 2413 \times (T/S)$. The Y intercept in this equation represents the length of the subtelomeric region.

Major life stressors

At follow-up, participants completed a self-reported 15-item checklist for major adverse life stressors of considerable high threat that may have happened during the previous year. Two items of occurrence of health-related stressors that are confounded with disease and can affect physiology (any major health problem, experiences of drug/alcohol dependence or addiction) were excluded, for a tally of 13. The final 13 items included (1) loss of household, (2) major financial difficulties, (3) self or (4) family member employment loss, (5) continued unemployment and job searching, major interpersonal problems including (6) divorce, (7) ongoing arguments with a spouse or (8) close family member or friend, (9) caregiving for an adult or (10) child with a serious illness, death of a (11) family member or (12) friend and (13) sexual harassment. Items endorsed were counted as 1 and all items were summed.

Cumulative health behaviors

At three time points between baseline and 1-year follow-up (at 4, 8 and 12 months), we assessed (1) leisure time physical activity, (2) typical dietary practices and (3) sleep quality.

(1) *Leisure time physical activity* was assessed with the leisure activity subsection of the Stanford Brief Activity Scale, shown to successfully discriminate different activity levels, body weight and key metabolic markers.⁵² The Stanford Brief Activity Scale is a self-administered questionnaire, developed for quick assessment of frequency, intensity and type of physical activity at work and during leisure activities over the past week. Participants are provided five scenarios that provide a global statement of activity level and descriptors of frequencies, intensities and type of activity. For example, the least active scenario (score of 0) is described as, 'Most of my leisure time was spent without very much physical activity. I mostly did things like watching television, reading, or playing cards. If I did anything else, it was likely to be light chores around the house or yard or some easy-going game like bowling or catch. Only occasionally, no more than once or twice a month, did I do anything more vigorous, like jogging, playing tennis, or active gardening'. Each rating appropriately progresses in description of intensity, frequency and type. Participants selected one statement from the five provided activities from 0=little activity, mostly sedentary lifestyle to 4=engaging in a regular physical fitness program at least 5 days a week. The Stanford Brief Activity Scale was adjusted to ask about previous 3 months of activity. The Stanford Brief Activity Scale and its revised L-CAT are well validated and reliable, as seen in studies demonstrating matching results to the Seven Day Physical Activity Recall and to Centers for Disease Control and Prevention recommendations for physical activity.^{53,54} Scores from each assessment were summed for a total activity score for the year and then standardized.

(2) *Typical dietary practices* were self-reported with diet questions developed based on the Food Frequency Questionnaire used in the Multi-Ethnic Study of Atherosclerosis (MESA).⁵⁵ These questions measure how often participants eat foods from each of 13 food categories, including typical healthy foods, such as whole grains, fruits, vegetables, nuts, seeds, low fat dairy and fish, and typical unhealthy foods, including starches such as white potatoes and refined grains, red meat, high-fat dairy, fried foods and sodas. Each food category was rated on a 9-point scale, including 1 'rarely or never'; 2 'once per month'; 3 'two to three times per month'; 4 'once per week'; 5 'twice per week'; 6 'three to four times per week'; 7 'five, six times per week'; 8 'once per day'; and 9 'twice a day or more'. Healthy and unhealthy foods (reverse scored) were summed across all time points and standardized for a total healthy foods score.

(3) Self-reported sleep quality was obtained using one question from the Pittsburgh Sleep Quality Index,⁵⁶ 'How would you rate your sleep quality overall?' rated (1) very bad, (2) fairly bad, (3) fair, (4) fairly good and (5) very good. Again, responses were summed across the three assessments and standardized.

Cumulative health behaviors were computed as the sum of the three scores for leisure time physical activity, dietary practices and sleep quality. Higher *cumulative health behaviors* designate greater engagement in healthier behaviors.

Statistical approach

Bivariate correlations between telomere length, lifestyle behaviors and major life stressors were performed. To test our hypotheses, LTL change (LTL from baseline subtracted from 1 year follow-up) was first regressed on major life stressors. Covariates included baseline LTL, sociodemographic factors (age, income, education (less than Bachelor's degree/Bachelor's or higher), ethnicity (Caucasian/other)), medication use at baseline (hypertension reducing medication (Yes/No), cholesterol reducing medication (Yes/No), hormone replacement therapy (Yes/No), antidepressants (Yes/No)), body mass index (BMI) at baseline and health behaviors over the previous year. Next, a series of regression analyses were completed with (1) the interaction between major life stressors and each health behavior alone (while covarying the two other behaviors) and (2) cumulative health behaviors as a combined factor. Briefly, a significant interaction between two continuous variables, such as major life stressors and cumulative health behaviors, suggests that the relationship between major stressors and telomere length change is significantly different at varying levels of health behaviors. A significant interaction is followed up with simple slope analyses, whereby the relationship between major stressors and LTL change is tested in two different analyses: (1) at 1 s.d. above and (2) 1 s.d. below the mean of cumulative health behaviors. This approach is the

typical, standard approach for testing significant interactions.⁵⁷ We also conducted analyses for each health behavior individually.

RESULTS

Participants

Women were highly educated (84% with a college degree or higher), had relatively high income (54% earned >US\$100 000 per year) and were primarily (84%) Caucasian. Average baseline BMI for the sample was in the normal weight range at 24.19 (s.d.=4.59) and minorities of women were using medications (15% hypertension-reducing medication; 10% statins; 17% antidepressants; 42% hormone replacement therapy).

Descriptive statistics

At baseline, mean LTL was 5548 bp (s.d.=328.9) and mean 12-month follow-up LTL was similar, 5584 bp (s.d.=354.9). Baseline and follow-up telomere length were significantly related ($r(229)=0.74$, $P<0.001$), indicating considerable stability over time, and the average percent change was minimal (0.65%) with a standard deviation for percent change in telomere length of 4.86%. Thus, as would be expected for a short period of 1 year, the majority of people (68%) did not show a large increase or decrease greater than approximately 5% of their baseline telomere length. As consistently reported in other longitudinal studies of telomere length change, longer telomeres at baseline were more likely to shorten over time ($B=-0.31$, $P<0.001$), such that for every 100 bp above the average of the sample at baseline, telomeres were likely to significantly shorten by an average 24 (s.e.=0.5, 95% confidence interval (CI)=-33.9, -12.8) bp over the year.

At 1 year follow-up, the distribution of stressors was as follows: 32% experienced the death of a family member or close friend, 26% reported that they experienced relationship difficulties, 20% were involved in caregiving, 17% became unemployed or experienced financial strain, 4% experienced sexual harassment and 3% experienced loss of their house. Thirty-seven percent of women did not have any major life events over the previous year, 47% had 1 or 2 and 16% experienced 3 or more.

Women reported an average 2.29 (s.d.=1.13) leisure time physical activity over the course of the year, corresponding to a moderately active lifestyle. Furthermore, women reported eating fairly healthy diets, with a mean food frequency of 6.77 (s.d.=0.67), corresponding to 2-3 times a week of eating healthy foods, and 2-3 times a month of eating less healthy foods. Finally, on average, participants reported fairly good sleep over the year (mean=3.56, s.d.=0.69). Cumulative leisure time physical activity over the year was significantly related to typical foods eaten over the year ($r(229)=0.34$, $P<0.001$) and to sleep quality during the year ($r(229)=0.14$, $P=0.04$). Typical foods and sleep quality were not significantly related to each other ($r(229)=0.10$, $P=0.11$) but the trend was in a positive direction.

Healthy behaviors were not significantly related to number of life events, although a marginal trend emerged for leisure activity, such that those who had more events during the year were less likely to be active ($r(229)=-0.12$, $P=0.08$).

Multivariate analyses main results

Major stressors and telomere shortening: Major stressors during the year significantly predicted accelerated telomere shortening over the same time frame ($B=-0.18$, $P=0.01$). These results suggest that for every one event, there was a significantly greater decline in telomere length over the year of 34.7 bp (s.e.=14.04, 95% CI=-62.3, -6.9). Table 1 presents the complete model with covariates, individual health behaviors and major life stressors.

Moderating roles of each health behavior: Three separate regression equations were completed that included the

Table 1. Multivariate regression analyses, including covariates, health behaviors and major life events

	<i>b</i>	95% <i>CI</i>	<i>B</i>	<i>P</i> -value
<i>Covariates</i>				
TL at baseline (bp)	-0.24	-0.35, -0.13	-0.31	< 0.000
Age (years)	-7.86	-16.67, 0.95	-0.13	0.08
Household income	7.20	-16.31, 30.71	0.04	0.55
Education (completed bachelor's or more = 1; bachelor's or lower = 0)	52.41	-49.17, 153.98	0.07	0.31
Ethnicity (Caucasian = 1; other = 0)	11.79	-91.97, 115.54	0.02	0.82
BMI at baseline (kg/m ²)	0.01	-7.95, 7.96	0.00	0.99
Antidepressant use at baseline (yes = 1; no = 0)	-11.15	-109.72, 87.42	-0.02	0.82
CVD medication use at baseline (yes = 1; no = 0)	0.92	-100.91, 102.74	0.02	0.98
Statins use at baseline (yes = 1; no = 0)	-103.70	-226.10, 18.71	-0.12	0.09
Hormone replacement therapy at baseline (yes = 1; no = 0)	61.38	-17.78, 140.55	0.12	0.13
Cumulative physical activity	-10.96	-57.03, 35.12	-0.04	0.64
Cumulative typical diet	33.03	-10.44, 76.51	0.12	0.14
Cumulative sleep quality	6.94	-36.09, 49.97	0.02	0.75
Major life events	-34.65	-62.35, -6.95	-0.18	0.01

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; TL, telomere length. Note: *b* is the unstandardized β and *B* is the standardized β .

Table 2. Simple slopes for association between life stressors and telomere shortening over 1 year at -1 s.d. below the mean, at the mean and +1 s.d. above the mean for each health behavior independently

	<i>Physical activity</i>		<i>Diet</i>		<i>Sleep quality</i>	
	<i>b</i> (s.e.)	<i>P</i> -value	<i>b</i> (s.e.)	<i>P</i> -value	<i>b</i> (s.e.)	<i>P</i> -value
-1 s.d.	-48.1 (17.9)	0.008	-46.5 (18.4)	0.01	-46.2 (16.8)	0.007
Mean	-31.5 (14.2)	0.03	-33.5 (14.1)	0.02	-31.7 (14.2)	0.03
+1 s.d.	-14.9 (21.1)	0.48	-20.5 (19.6)	0.30	-17.2 (19.5)	0.38

Note: Values indicate the unstandardized β -coefficient (*b*), standard errors (s.e.) and *P*-values for the associations between life stressors and telomere shortening over the course of the year at varying levels of each behavior.

covariates, major life stressors, each of three health behaviors (physical activity, sleep quality or diet) and tested whether each behavior alone moderated the relationship between major life stressors and telomere shortening. As seen in Table 2, results indicated that at 1 s.d. below the mean *and* at the mean of each behavior separately, major life stressors significantly predicted telomere shortening. At 1 s.d. above the mean of each health behavior, life stressors were unrelated to telomere shortening. However, the interaction effects for the three moderation analyses were not statistically significant (all interaction *P*'s > 0.10).

Moderating role of healthy behaviors: Results testing an interaction between major stressors and cumulative health behaviors (i.e. sum score of all three health behaviors over the year) support the hypothesis that a constellation of health behaviors during the 12 months from baseline to follow-up significantly moderated the relationship between major life stressors and telomere shortening during the same time frame (interaction *B* = 0.19, *P* = 0.041). For women who engaged in lower levels of health behaviors (1 s.d. below the average sample mean of cumulative health behaviors), every additional stressor was related to accelerated telomere shortening over the year by an additional mean 76.5 bp (95% CI = -123.1, -30.0 bp, *B* = -0.29, *P* = 0.001) compared with women with no adverse stressors. At average levels of health behaviors, accelerated telomere shortening over the year occurred by an additional 39.6 bp for every major life stressor that occurred, on average (95% CI = -76.4, -2.7 bp, *B* = -0.15, *P* = 0.04). For those women at higher levels of health behaviors (1 s.d. above the average sample mean of

health behaviors), life stressors were unrelated to telomere shortening (unstandardized *b* = -2.6, 95% CI = -58.0, 52.7 bp, *B* = -0.01, *P* > 0.50). Figure 1 illustrates the simple slopes for major life stressors on telomere shortening at mean cumulative health behaviors, and 1 s.d. above and one below the mean.

DISCUSSION

Healthy aging is a complex interplay of genetics, lifespan stressors from the social and physical environments, and behaviors.^{58,59} We examined whether the interaction between life stressors from the social environment and behaviors during a 1-year period shapes cell aging, indexed by telomere attrition over time. It has been an open question about whether telomere length might change in as short of a period as 1 year. This is the first observational study to examine short-term changes in telomere length. We found that while a majority of women remained within 5% of their original telomere length, there was still a significant amount of change, and this change was predictable based on life stressors over the previous year and modifiable lifestyle behaviors. These findings are supportive of previous models suggesting that the accumulation of varied stressful events across the lifespan promote wear and tear on physiologic systems that ultimately shape cellular aging processes.^{3,60-62} These findings, however, are suggestive of the hopeful message that engagement in healthy behaviors during periods of high stress can perhaps attenuate immune cell aging.

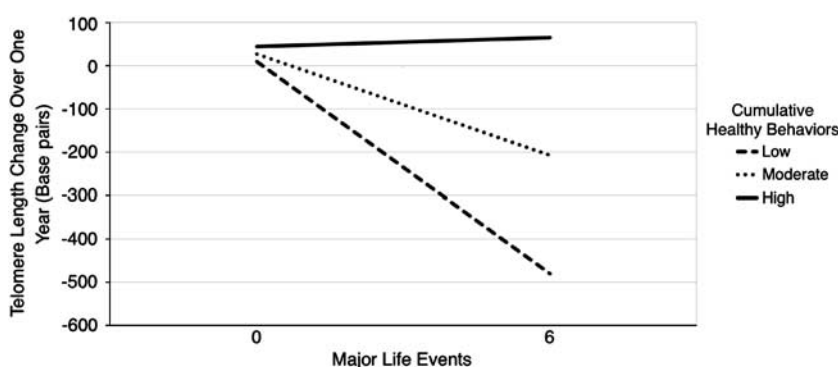


Figure 1. Predicted telomere length change over 1 year as a function of major life stressors and cumulative health behaviors. *Note:* Lines are predicted relationships between major life stressors and change in telomere length over the 1 year of study at three levels of cumulative health behaviors: low (−1 s.d.), moderate (mean) and high (+1 s.d.).

Recent reviews in the literature identify strong relationships between psychological stress and physical activity,⁶³ stress and eating⁶⁴ and stress and sleep.^{65,66} These three behaviors are especially important to the biologic pathogenesis of depression.⁶⁷ In the present study, each behavior did not independently attenuate the association of life stress on telomere attrition over time. However, the relationships between life stressors and telomere attrition that were evidenced at low, moderate and higher levels of each individual behavior patterned similarly to those with the cumulative health behaviors score. A composite of health behaviors has previously been evidenced to be a stronger cross-sectional predictor of telomere length than each individual behavior alone.⁴⁶ It is not surprising, then, that only cumulative health behaviors were a potent moderator of the life stressors–telomere attrition relationship over time.

Several pathways are suggested for the protective effects of these behaviors. Physical activity promotes neurogenesis, cognitive flexibility and memory formation in both humans and other animals alike,⁶⁸ perhaps shaping biologic stress reactivity, and emotional and cognitive responses to stress.^{3,68} Previous studies have shown that fit younger and older adults have quicker biologic stress recoveries than unfit adults.^{69–71} Physical activity also bolsters mediating immune proteins and gene expression^{72,73} and more distal biologic aging end points, such as mitochondrial health and telomerase levels,^{74–77} which are closely related to telomere maintenance.^{19,20,78} There are suggestions of quite similar mechanistic benefits of sleep^{43,79,80} and nutrition^{42,81} on stress-responsive systems.

Randomized controlled trials to improve these behaviors in chronically stressed individuals can help us understand the extent to which the biologic damage from life stress is reversible or preventable. Ornish *et al.*⁸² recently demonstrated that men with low-risk prostate cancer who adhered to recommended lifestyle changes consisting of diet, activity, stress management and social connections had longer 5-year follow-up telomere length compared with those who did not adhere to recommendations for lifestyle change. Furthermore, across the control and intervention groups, those with greater health behaviors tended to show telomere lengthening over the 5 years. A next logical step is to examine lifestyle effects on cell aging in a randomized design targeting people living with high levels of chronic stress. There is existing evidence of success in combining these factors for the treatment of depression in a randomized trial setting.⁸³

Several key chromosomal genetic single-nucleotide polymorphisms in genes regulating telomere length (i.e. rs10936599 in the *TERC* gene, rs2736100 in the *TERT* gene, rs7675998, in the *NAF1* gene, etc.) place individuals at increasing risk for disease.²¹ Unknown, however, is the extent to which lifestyle interventions

may succeed or fail in decelerating cell aging within the context of genetic polymorphisms in telomerase-regulating genes. Examining the cellular benefits of a lifestyle intervention within the context of known vulnerability single-nucleotide polymorphism precursors of disease and/or varied contexts of chronic stress is important to help determine for whom an exercise, sleep and diet lifestyle intervention will most benefit.

Strengths of the study are the prospective measurement of lifestyle throughout the year, at three time points, and the ability to examine short-term changes in telomere length over 1 year. The stability of telomere length ($r=0.74$) suggests that telomere length tends to be stable over a short period, but is far from fixed, and many people showed changes. The current findings are perhaps limited to postmenopausal and non-smoking women. The women in the current study were also primarily Caucasian, highly educated, healthy (lower BMI, low medication use) and had lower levels of stress than national averages.⁸⁴ The extent to which the effects of stress on telomere biology are reversible may depend on how long the stressor remains. For example, years of providing care significantly predicts shorter telomeres,²⁸ thus possibly limiting the extent to which we can quickly reverse these effects with behavioral intervention. Lifespan studies that combine factors from early life and adulthood experiences together with health behaviors are needed to examine adequate doses for reversing the damage of accumulated stress across the lifespan. Finally, our behavioral measures were self-reported, allowing for reporting bias to influence our results. Future studies should use objective monitoring devices including accelerometers for objective activity and sleep measurement and daily food diaries using computer-aided technologies to advance our understanding of the potential for healthy behaviors to impact directly aging or moderate the impact of stress on aging processes.

Currently, levels of general stress are reported at an all-time high in adult United States residents⁸⁵ and stressed adults are less likely to engage in healthy behaviors.^{63–66,86} Although stress varies between individuals, it is not an individual problem. It is imperative that the health care system and policy makers find creative and powerful ways to promote engagement and maintenance of health behaviors, which in turn can help allay the destructive effects of the increasing levels of societal stress.

CONFLICT OF INTEREST

Drs Jue Lin, Elissa Epel and Elizabeth Blackburn are cofounders of Telome Health, a diagnostic company measuring telomere biology.

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