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Pessimistic orientation in relation to telomere length in older men: the VA Normative Aging Study

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Abstract

Background—Recent research suggests pessimistic orientation is associated with shorter leukocyte telomere length (LTL). However, this is the first study to look not only at effects of pessimistic orientation on average LTL at multiple time points, but also at effects on the rate of change in LTL over time.

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Methods—Participants were older men from the VA Normative Aging Study (n=490). The Life Orientation Test (LOT) was used to measure optimistic and pessimistic orientations at study baseline, and relative LTL by telomere to single copy gene ratio (T:S ratio) was obtained repeatedly over the course of the study (1999-2008). A total of 1,010 observations were included in the analysis. Linear mixed effect models with a random subject intercept were used to estimate associations.

Results—Higher pessimistic orientation scores were associated with shorter average LTL (percent difference by 1-SD increase in pessimistic orientation (95% CI): -3.08 (-5.62, -0.46)), and the finding was maintained after adjusting for the higher likelihood that healthier individuals return for follow-up visits (-3.44 (-5.95,-0.86)). However, pessimistic orientation scores were not associated with rate of change in LTL over time. No associations were found between overall optimism and optimistic orientation subscale scores and LTL.

Conclusion—Higher pessimistic orientation scores were associated with shorter LTL in older men. While there was no evidence that pessimistic orientation was associated with rate of change in LTL over time, higher levels of pessimistic orientation were associated with shorter LTL at baseline and this association persisted over time.

Keywords

Optimism; Pessimism; Telomere length

INTRODUCTION

Telomeres are repetitive structures at the end of eukaryotic chromosomes which protect chromosome ends from deterioration (Wong and Collins, 2001).¹ Numerous studies have documented the gradual shortening of telomere length with increasing age (Slagboom et al., 1994). Additional work has also found shorter telomere length in patients suffering from coronary heart disease compared to controls (Brouillette et al., 2007), in diabetic patients (Sampson et al., 2006) and in individuals with cardiovascular risk factors such as obesity, smoking (Nordfjäll et al., 2008), as well as psychological stress (Epel et al., 2004). Thus, investigators have suggested that telomere shortening is a marker of cellular aging that may be accelerated by chronic stress and may indicate risk of chronic disease development and premature mortality. A growing body of research has identified links between psychological factors (e.g., stress, pessimistic orientation, depression) and incident disease, disease progression, and mortality, but the mechanisms underlying these associations remain to be determined. Building on the rapidly emerging science on telomeres, recent work has begun to consider whether psychological functioning is associated with rate of cellular aging. For example, one cross-sectional study found higher levels of pessimistic orientation were associated with shorter telomere length (O'Donovan et al., 2009).

Pessimism is a cognitive orientation (personality trait) characterized by the general expectation that outcomes will be negative (Chang et al., 1997; Roy et al., 2010). High levels of pessimistic orientation are associated with greater psychological distress; for example, pessimistic orientation is a strong predictor of depressive symptoms even after accounting for other psychological factors (Chang et al., 1997). More recent work has found

pessimistic orientation is also associated with physical health outcomes, with studies demonstrating higher levels of pessimistic orientation to be associated with increased risk of heart disease and stroke, and reduced cancer survival (Kubzansky et al., 2001; Nabi et al., 2010; Novotny et al., 2010).

A number of mechanisms might explain the recently observed association between pessimistic orientation and telomere shortening. Pessimistic orientation may induce negative reactions to stressful events thereby increasing the potentially toxic effects of stress-related neuroendocrine activation. Chronic neuroendocrine activation can increase oxidative stress, which induces telomeric DNA damage in the telomere sequence (TTAGGG) (Kawanishi & Oikawa, 2004). Telomeric DNA is synthesized by the enzyme telomerase. Thus, this DNA damage may induce the slowing down of existing telomerase enzymatic activity needed to synthesize telomeric DNA and thus accelerate telomere shortening (von Zglinicki et al., 2002).

Pessimistic orientation may also affect telomere shortening through behavioral pathways such as increasing the likelihood of smoking or being sedentary. These unhealthy behaviors have been demonstrated to have highly significant effects on biochemical processes (i.e., oxidative stress and inflammation) that can alter telomere dynamics (Nordfjäll et al., 2008). Such damaging effects lead to shorter telomere length and more rapid age-dependent telomere attrition rate.

Only one study to date has examined LTL in relation to pessimistic orientation (O'Donovan et al., 2009); other studies have linked pessimistic orientation to factors associated with telomere length, including increased risk of inflammation (O'Donovan et al., 2009), depression (Isaacowitz and Seligman, 2001), and premature mortality (Brummett et al., 2006). O'Donovan and colleagues (2009) suggested the association with LTL was stronger in relation to the pessimistic orientation subscale as compared with either the optimistic orientation subscale or the overall optimism score (O'Donovan et al., 2009). This finding is consistent with prior work we and others have done showing clearer effects of a pessimistic versus optimistic orientation on measures of inflammation and endothelial function as well as other objective indicators of health (Milam et al., 2004; Ikeda et al., 2011). However, the initial study of pessimistic orientation and telomere length by O'Donovan and colleagues, employed a cross-sectional design, and was conducted among only 36 healthy post-menopausal women (O'Donovan et al., 2009).

To gain greater insight into the relationship between pessimistic orientation and LTL and consider whether and how pessimism may influence rate of LTL change, we examine the association of a pessimistic or optimistic orientation with change in telomere length over time in the VA Normative Aging Study (NAS). The life orientation test (LOT) was used to measure pessimistic or optimistic orientation in the present study. In prior work, it has exhibited significant reliability with a test-retest reliability reported to be 0.79 over a 4-week period and 0.69 over a 3-year period (Scheier et al., 1985), supporting the notion that dispositional optimism is relatively stable across time.

We considered a range of covariates which could be associated with the level of pessimistic or optimistic orientation scores in our sample including baseline (time at first visit when optimistic/pessimistic orientation and LTL were measured) age, the difference in age between baseline and time at which each LTL was measured, body mass index (BMI), education attainment level, cigarette smoking status, alcohol drinking, physical activity, medical conditions (i.e., hypertension, diabetes, cardiovascular disease) and interleukin-6 (IL-6). All covariates were chosen based on prior literature suggesting their potential relevance to both telomere change and pessimistic or optimistic orientations (Nilsson, 2011; Needham et al., 2013). We further considered possible effect modification by age because age at study baseline could be a strong factor associated with LTL while pessimism is not necessary strongly associated with age. Moreover, other studies have suggested the effects of psychological factors on health-related outcomes are often stronger at younger versus older ages (Hamer et al., 2008). Thus, we believed that age might alter finding not because the trait varies by age, but because the effects of trait may vary by age.

METHODS

Study Population

The VA Normative Aging Study (NAS) has been described in detail elsewhere (LoCastro et al., 2000). In brief, the NAS is a longitudinal study of aging established by the Veterans Administration (now the Department of Veterans Affairs) between 1961 and 1963 among 2,280 men (98% Caucasian, 1% African-American, and 1% Hispanic) from the Greater Boston area aged 21 to 80 years at the time of entry, who were free of any known chronic medical conditions. Participants are community-residing men, most of whom were veterans. Many veterans had combat experience but not all; it was not a requirement for the study. Men were asked to return for onsite physical examinations and questionnaires every 3 to 5 years. Study participants provided written informed consent and the study protocol was approved by the Institutional Review Boards of all participating institutions. Eligibility for the present study required continued participation as of the time when telomere measurement began (1999). Drop out has been less than 1% per year in this cohort, and predominantly occurs only if participants move out of the study area. The other major reason for loss to follow-up has been mortality.

Participants come to the clinic for each physical examination and blood is drawn at each visit. Telomere length was measured at each exam between 1999 and 2008 (the present study period). Participants were also administered a series of questionnaires that included items measuring an optimistic or pessimistic orientation and other covariates during the present study period. The present study included a total of 490 men who were active participants, provided at least once telomere measure, and concurrently responded to all optimistic and pessimistic orientation items at the time when the leukocyte telomere length (LTL) was first measured (Figure 1). Of these, 5 (1.0%) had 4 telomere length measurements, 149 (30.4%) had 3 telomere length measurements, 207 (42.2%) had 2 telomere length measurements, and 129 (26.3%) had one telomere length measurement. Thus, a total of 1,010 observations were used in the present analysis.

To assess whether subjects excluded from our study were different from those included, we compared some baseline characteristics such as age, BMI, smoking status, drinking behavior, physical activity, educational level, and health conditions (hypertension, diabetes, and cardiovascular disease) between our included subjects and excluded subjects ($n=327$) who came in during the study period (1998-2008) but did not have LTL measures and/or valid responses to the optimistic and pessimistic orientation measures. There were no differences in the characteristics between excluded and included subjects.

Assessment of Subscales for Optimistic Orientation and Pessimistic

Orientation—The LOT was used to measure optimistic and pessimistic orientations (Ikeda et al., 2011). The LOT is a validated instrument (Scheier et al., 1985) made up of 8 relevant items comprising questions such as “In unclear times, I usually expect the best”; “If something can go wrong for me, it will.” We tested reliability of each scale or subscale (i.e., overall optimism, subscales of optimism and pessimism) by using Cronbach's α (alpha), a coefficient that assesses the internal consistency of the items (intercorrelation within the items). Cronbach alpha coefficient was 0.81 for overall; 0.78 for optimistic orientation subscale; 0.82 for pessimistic subscale. In the analysis, we used the concurrent measurements for optimistic and pessimistic orientations taken at the time when the LTL was first measured.

Telomere length measurements

We collected 7mL of whole blood by venous phlebotomy in EDTA tubes. DNA was extracted from stored frozen buffy coat using the QiAmp DNA blood kits (Qiagen, Germantown, MD, USA) and used to obtain LTL measurement by means of quantitative real-time polymerase chain reaction (qRT-PCR) (Cawthon, 2002). The assay process is described in detail elsewhere (McCracken et al., 2010). In brief, relative LTL was measured on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) by determining the ratio of the telomere (T) repeat copy number to the single-copy gene (S) copy number (T:S ratio) in a given sample. LTL was reported as relative units expressing the ratio between LTL in the test DNA and LTL in a reference DNA pool. We ran all samples in triplicates, and the average of the three T measurements was divided by the average of the three S measurements to calculate the average T:S ratio (Farzaneh-Far et al., 2008). LTL measures were assayed all at once; therefore we did not adjust for potential batch effects.

Measurement of Other Covariates

Every 3-5 years, participants in the NAS are followed up by physical examination, updating of medical history, height, weight, measurement of biomarkers including fasting glucose, and complete a questionnaire including various measures of lifestyle and psychosocial factors (these may vary across exams). Educational attainment level (years), cigarette smoking status (current, former, or never) and alcohol drinking (less than two versus two or more drinks per day) are ascertained by a trained interviewer. Current smokers (yes, no) are defined as men who smoke 1 or more cigarettes per day. Responses to questions about the number of flights of stairs climbed per day, walking pace, and frequency of various sports activities were used to derive a continuous physical activity variable that assessed total

kilocalories expended per week (METs-hr/wk). Interleukin-6 (IL-6) was assayed with the Milliplex Map Human Cytokine/Chemokine Kit, (Millipore Corporation, MO) and detected with a multiplex detection platform (Luminex® 100/200™ System, Austin, TX).

Statistical Analysis

Analysis of covariance and chi-square tests were used to compare mean values and proportions of covariates. LTL was natural log-transformed for the analyses to improve the normality of the data distribution. The present study data consisted of LTL measurements repeated over time, meaning that we have longitudinal data with multiple measurements on each individual obtained over time. Analysis of these repeated measures were performed using mixed effects modeling, the commonly-accepted method for dealing with longitudinal data, which accounts for the correlation existing between measurements taken from the same individual (Cnaan et al., 1997). In the present study, we used the following model:

$$y_{ij} = (\beta_0 + b_{0i}) + \beta_P \cdot \text{Pessimism}_i + \beta_T \cdot \text{Time}_{ij} + \beta_{PT} \cdot (\text{Pessimism} * \text{Time})_{ij} + \varepsilon_{ij}$$

where y_{ij} represents the logarithm of the telomere length for individual i taken at time j ; β_0 and β_T represent respectively the intercept and the slope of the linear relationship between the logarithm of the LTL and time; β_P is the effect of a unit increase in pessimistic orientation on log (telomere length), considered as constant across time (what we called a “pooled” across time); β_{PT} is the effect of a unit increase in pessimistic orientation on the slope describing the linear relationship between log (LTL) and time. Coefficients for this model were estimated by maximization of the likelihood using the SAS procedure MIXED and specifying a compound symmetry structure for the covariance matrix. We also reported LTL means at each time point according to the levels of pessimism (low vs. medium to high) among subjects who had at least three sets of visits ($n=154$). For interpretability, exponentiated regression coefficients from regression models with logged continuous outcomes were reported as percent differences in LTL associated with a 1-standard deviation increase in pessimistic orientation and their 95% confidence intervals (95% CI) after adjustment for age and other potential confounding factors. We also calculated and presented the percent difference in LTL according to quartiles of pessimistic orientation to assess the possibility of a threshold effect on telomere length.

In multivariate analysis, we only adjusted for covariates that were associated with the level of pessimistic orientation score at a significant level of $p < 0.10$ in our sample, including baseline age (years), delta age (the difference in age between baseline and time at which the outcome was measured), body mass index (kg/m^2), glucose category (normal, borderline or diabetes mellitus), education attainment level (<12 , ≥ 12 years). Borderline diabetes mellitus was defined as a fasting glucose level of 6.1-6.9 mmol/l. Diabetes mellitus was defined as a fasting glucose level of >7.0 mmol/l or the use of medication for diabetes. Covariate measurements were assessed at baseline of the study period, which was also when pessimistic orientation was assessed, but if measurements were missing we used available measurements closest in time when pessimistic orientation was assessed. To assess the potential modifying effects of baseline age, and change in age on the relation of pessimistic orientation with LTL over time, we ran regression models that included a cross-product term

for interaction between age and change in age with the pessimistic orientation score along with the main-effects.

To adjust for the possibility that healthier men are more likely to return for subsequent exams (revisit), a propensity score was used to model the probability of multiple exams, and inverse probability weights were used to correct for possible selection bias in the analyses using repeated measures over time. This revisit propensity score was calculated from a logistic regression as the probability of not having two or more study center visits, given all relevant factors at baseline including age; measurement of telomere length; total cholesterol level; statin use; abnormal glucose tolerance; hypertension; body mass index; smoking status; alcohol use, education and attainment. Thus, the primary analysis was repeated after weighting follow-up observations by the inverse probabilities of attaining follow-up response (revisit) (Kurth et al., 2005).

To further address possible concerns about whether other factors influencing likelihood of revisits might also influence association with LTL, we conducted a sensitivity analysis removing subjects who only had one measurement of LTL and the primary analysis was repeated within the remaining participants. Moreover, to address the issue of LTL lengthening, a function of “noise” in the measurement process, we ran an additional analysis excluding subjects whose LTL lengthened from baseline and reanalyzed effects within the remaining participants (n=584).

All analyses were conducted using the SAS statistical package Version 9.1 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Characteristics of the study sample are presented in Table 1 according to quartiles of pessimistic orientation score. At baseline, men with higher pessimistic orientation scores were more likely to be current smokers and have higher BMI and lower levels of educational attainment. In addition, more men with high levels of pessimistic orientation had diabetes suggesting that pessimistic orientation is initially associated with worse health. The mean age of the study population was 72.0 years at baseline (SD=6.7 years; median=71 years; range 55-90 years). The mean pessimistic orientation score was 4.7 (SD=2.6) and was normally distributed in this sample. The average LTL for subjects who only had one visit was 1.17 (SD=1.41) and for those who had more than one visit was 1.17 (SD=1.41). The average follow-up time of participants who had at least two visits was 3.5 (SD=0.97, range=2-8) up to visit 2, 6.25 (SD=0.70, range=4-9) up to visit 3 and 8.00 (SD=0.70, range=7-9) up to visit 4.

Age-adjusted and multivariable-adjusted percent differences in LTL (pooled across all time points) associated with a 1-SD difference in pessimistic orientation subscale score and with quartiles of pessimistic orientation subscale score (lowest as a reference category) are reported in Table 2. In the age-adjusted model higher levels of pessimistic orientation were associated with shorter LTL (main-effect) and this association was maintained after adjusting for all covariates. Thus, a 1-SD increase in pessimistic orientation was associated

with a 3.1 % decrease in LTL. The association became somewhat stronger after including the revisit propensity score such that a 1-SD increase in pessimistic orientation was associated with a 3.4 % decrease in LTL. When we further adjusted for baseline IL-6, the associations were not attenuated. The percent difference by 1-SD increase in pessimistic orientation after adjusting for IL-6 was for IL-6 was -3.44 (95% CI:-5.96, -0.85). We also found that individuals with higher pessimistic orientation scores had shorter LTL compared to those in lowest pessimistic orientation score category. A graded relationship was also found between LTL and the ordinal category of pessimistic orientation, whereby with each increase in level of pessimistic orientation, LTL was shorter. Neither the overall optimism or the optimistic orientation subscale were associated with LTL in any analysis of our sample (Appendix 1). In sensitivity analysis removing participants who had only one measurement of LTL, results were unchanged in the final model which included the revisit propensity score (percent LTL difference by 1-SD increase in pessimistic orientation, -3.63 (95% CI:-6.46,-0.71)).

An overall effect of time in the present study was significantly associated with the loss of telomere length (percent difference in LTL associated with a difference in age between baseline and time (one year increment) when LTL measured: -1.94 (95% CI:-2.91,-0.96). However, no association of pessimistic orientation with change in LTL over time was evident. In addition, no statistically significant interactions between pessimistic orientation and baseline age or delta age were evident for either LTL pooled across time or change in LTL over time. For example, the multivariable-adjusted (including inverse probability of multiple exams) percent difference (95% CI) for the interaction between a 1-SD in pessimistic orientation and baseline age or delta age in relation to LTL was 0.10 (-0.28, 0.47) and -0.25(-1.25, 0.76), respectively. While there was no evidence that pessimistic orientation was associated with rate of change in LTL over time, we found some LTL mean differences by the levels of pessimism at each time point. The LTL mean was lower among subjects with medium to high level of pessimistic orientation at baseline and the differences persisted over time; however, these differences were not statistically significant (Figure 2).

Additional Analyses

After we excluded the subjects who had LTL lengthened since baseline, the effects of pessimism on LTL became weaker and marginally significant likely due to the loss of statistical power, but the tendency of the effect was still evident and consistent (percent difference by 1-SD increase in pessimistic orientation, -2.95 (95% CI:-6.02, 0.23; $p=0.07$)). We also compared baseline characteristics between subjects who had LTL lengthened since baseline and those who did not. There were differences in mean LTL (1.35 vs. 1.04) and baseline age (73 vs. 71 years), but no other differences in characteristics (i.e., BMI, history of hypertension, diabetes, and cardiovascular disease, smoking status, drinking behavior, physical activity, and educational level).

DISCUSSION

In this analysis with repeated measures of telomere length, we found a consistent association between pessimistic orientation and LTL pooled over multiple measures across time in

elderly men, but no relation with LTL rate of change over time. Findings were maintained after adjusting for potential confounders and little attenuation of effect was evident even after adjusting for behaviors that could be on the pathway between pessimistic orientation and accelerated aging. We found no evidence of associations between measures of overall optimism or optimistic orientation and either pooled measures of telomere length or rate of change in the telomere length over time.

Processes that influence the rate of cell replication and the extent of telomere loss during each replication will influence how rapidly telomeres shorten. Chronic inflammation, for example, is associated with an increase in the turnover rate of leukocytes, which may lead to the higher rate of replication of cells. We have previously reported that pessimistic orientation was associated with higher levels of inflammation and endothelial function (Ikeda et al., 2011). Including IL-6 in our model did not significantly alter our findings. However, a full test of mediation requires additional longitudinal models beyond the scope of this present study but may be an important direction for future work.

Our findings were also strongly consistent with that of the prior study, which demonstrated a cross-sectional association between higher pessimistic orientation and shorter LTL among 36 post-menopausal women; optimistic orientation was also not associated with LTL in this study (O'Donovan et al., 2009). Unlike this study, we examined the effect of pessimistic orientation on multiple measures of LTL pooled across time, and also on change in LTL over time. Pooling across multiple measures of LTL over time likely reduces noise that may be present among measures taken at a single point in time, and results in a stronger measure of the true level of the parameter being assessed.

However, we found no relation between pessimistic orientation and the rate of change in the LTL over time. One possible reason for this is the wide temporal variability in patterning of telomere length changes over time (Farzaneh-Far et al., 2010). However, we also observed increases in LTL between measurements among some participants rather than the monotonic decrease in length expected to occur with aging. For example, we found increases in LTL among 32% of subjects (n=155) between baseline and 2nd measurement, and continuing increase in LTL among 10% of them at the 3rd measurement. Sensitivity analyses excluding participants whose LTLs lengthened or comparing these participants with those whose LTLs shortened did not significantly change the interpretation of our findings. Furthermore, despite the small coefficient of variation (8.7%) in the present study among the triplicate measures of the T:S ratios, even relatively low measurement error can result in misclassification (Chen et al., 2011). The appearance of LTL lengthening can be more a function of “noise” in the measurement process than a true effect (Chen et al., 2011). In addition, PCR-based measures are considered somewhat more susceptible to age-related changes in DNA because telomere length is expressed in relation to a reference gene which is presumed to be stable (Aviv et al., 2009). The older age of our cohort at baseline, relatively short follow-up period, and PCR-based measures of telomeres may have limited our ability to assess accurately change in telomere length over time in this cohort.

To our knowledge, the present study is the first to test the hypothesis that pessimistic orientation leads to changes in LTL by looking at the relationship between pessimistic

orientation and repeated measures of LTL over time while adjusting for a range of potential confounders or possible behavioral pathways. Our findings of no association of pessimism with rate of change in LTL over time but a constant effect of unit increase across time are consistent with the explanation that rate of change trajectories are set earlier in life at younger ages or at an earlier stage with regard to telomere dynamics. If that is the case, then maintenance of relative differences in LTL will be evident at older ages, but differences in rates may not be.

We failed to find the associations with optimistic orientation. Previous findings (O'Donovan et al., 2009; Roy et al., 2010; Ikeda et al., 2011) have suggested that physiological effects are more strongly evident with a pessimistic versus optimistic orientation. It is possible that this is due to method artifact, for example, negatively worded items (i.e., pessimistic orientation items) may be answered differently from positively worded items particularly in older adult populations. This may result in differential associations with outcomes and optimistic orientation may be less related in older men than in younger people (Mroczek et al., 1993); this may help to explain why we did not find associations between LTL and optimistic orientation or overall optimism. Moreover, LTL provides only one measure of telomere dynamics. In the present study, we were not able to measure telomerase expression and activity, and thus, could not examine whether telomerase levels were associated with pessimistic or optimistic orientations. Telomerase, is a cellular enzyme which adds telomeric repeat sequences to the telomere regions at the ends of eukaryotic chromosomes (Aviv et al., 2011). The sequence of elongation by telomerase is one of the processes maintaining telomeric intactness and healthy cell function (Aviv et al., 2011; Hohensinner et al., 2011). Other work has suggested that telomerase activity may provide additional insight into telomere dynamics offering some protective effects and thereby perhaps more strongly associated with more positive attributes like an optimistic orientation. Thus, telomerase levels, in which short term changes are measureable (Weng et al., 1996), may provide additional insight into telomere dynamics and perhaps also provide an earlier or more sensitive indicator than LTL of cell aging and of disease processes (e.g., cardiovascular disease) (Epel et al., 2006; Chan et al., 2003). Additional work with multiple measures of telomere length as well as telomerase obtained over time will help to clarify how these dynamics are related to both upstream factors like pessimistic orientation as well as subsequent health (Aviv et al., 2009; Martin-Ruiz et al., 2005).

We note several other limitations of our study. First, the ratio of T and S measurements we used in the present study is often used to represent absolute telomere length; however, this measurement only provides the average telomere length per sample. Second, the observed associations in the present study may be biased towards null since this study was limited to a cohort of elderly who may have relatively long telomeres from early in life (Heidinger et al., 2012). The subjects were not all veterans although most were. Thus, our findings will need to be replicated in other population to assess generalizability. That said, to generalize research findings, the NAS study was originally designed to cover as wide a range as possible of socioeconomic variables (Bell et al., 1966). Third, as in most longitudinal studies, there was imbalance in the number of repeated measures per subject, here largely due to loss to follow-up (i.e., poor baseline health condition). However, concerns about how this might affect the findings are mitigated given the similar results obtained after using

inverse probability of response weighting. Fourth, we also note that our findings are limited to men but were consistent with findings from the prior study in women (O'Donovan et al., 2009). Finally, although we adjusted for various possible confounding factors in the current study, there is the possibility of residual confounding by unmeasured variables such as external stressor (i.e., caregiving or loss of spouse). External stressors might modify the effect of pessimism on telomeres or pessimism might mediate the effect of external stressors on telomere length. We have previously found that both adulthood and childhood socioeconomic status (SES), one form of external stressor, were each independently associated with pessimism in this sample (Peters et al, 2012). Childhood SES may effect on the developmental origin of pessimistic orientation and have lasting effects into adulthood. Socioeconomic achievement during adulthood may also be associated with stability in pessimistic orientation (Ek et al 2004). This may suggested that pessimism can mediate effects of SES on LTL. Our adjustment for education (a marker of SES) as a potential confounder in the multivariate model did not significantly altered findings and moreover education was not directly associated with LTL (data not shown). A recent study found that less education (less than high school) compared to more education (college degree) was inversely associated with LTL (Needham et al., 2013). Compared to the study conducted by Needham et al., our study sample was much older (mean baseline age was 72.0 (SD=6.7)); therefore, any effects of having lower education may have happened, but no longer be apparent during our study period. Furthermore, the proportion of less than high school was smaller (6%) compared to the previous study (16% for white population and 32% for full sample) (Needham et al., 2013). Due to small variation in the presented sample, we may not be able to detect the effect of lower education on LTL in our present study.

Strengths of the study include the use of a well-characterized cohort of elderly men who have been followed over time among whom attrition is low. Pessimistic orientation was measured with a widely used, validated measure (Kubzansky et al., 2004; Scheier et al., 1994). Other work has indicated that pessimistic orientation is a stable trait and remains relatively unchanging over the course of adulthood and even after receipt of bad news (Schou et al., 2005). All measures were obtained at the baseline visit and at least two or more measures of LTL were available for most subjects (73.6%).

In summary, we found that a more pessimistic orientation was associated with lower LTL in community-dwelling older men when pooled across multiple points in time. The association was maintained after adjusting for a standard set of biological and behavioral factors that could potentially confound or mediate the association of pessimistic orientation with LTL, suggesting that other mechanisms should be considered. Efforts at prevention and intervention for healthy aging may benefit from a broader focus on psychological resources that are associated with biological wear and tear.

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Appendix

Appendix 1

Age- and multivariate-adjusted percent difference associated with the level of overall optimism score and optimistic orientation subscale score (the lowest category as reference) and a 1-standard deviation increase in optimism score (95% CI) for log-transformed telomere length.

	Level of overall optimism				1SD change 1SD=4.249
	Low			High	
	19	20-22	23-24	25+	
Crude	0	-0.73(-7.48,6.52)	1.18(-6.25,9.22)	3.96(-3.69,12.2)	1.37(-1.25,4.05)
Age-adjusted	0	-0.76(-7.52,6.51)	1.32(-6.15,9.39)	4.06(-3.64,12.4)	1.39(-1.24,4.09)
Multivariate-adjusted	0	-1.27(-8.11,6.08)	1.18(-6.38,9.35)	3.48(-4.31,11.9)	1.26(-1.40,4.00)
Further weighted for inverse probability of revisits***	0	-0.60(-7.50,6.82)	2.49(-5.08,10.7)	4.61(-3.27,13.1)	1.82(-0.84,4.56)

	Level of optimistic orientation subscale				1SD change 1SD=2.496
	Low			High	
	9	10	11-12	13+	
Crude	0	-1.91(-11.3,0.37)	-4.44(-10.5,1.99)	-1.91(-9.62,6.46)	-1.08(-3.63,1.55)
Age-adjusted	0	-3.69(-11.0,4.23)	-4.22(-10.3,2.26)	-1.73(-9.49,6.69)	-1.01(-3.57,1.62)
Multivariate-adjusted	0	-4.17(-11.5,3.79)	-4.55(-10.7,1.99)	-2.09(-9.89,6.39)	-1.09(-3.67,1.57)
Further weighted for inverse probability of revisits***	0	-3.51(-10.9,4.55)	-3.70(-9.86,2.88)	-1.18(-9.05,7.36)	-0.57(-3.16,2.08)

*Adjusted for age at baseline and ^age (difference in age between baseline and time of outcome).

In multivariate-adjusted model, adjusted for baseline age, body mass index, abnormal glucose tolerance, education, and ^age.

**The revisit propensity score was calculated from a logistic regression as the probability of not having two or more study center visits, given all relevant factors at baseline including age; telomere length; total cholesterol level; statin use; abnormal glucose tolerance; hypertension; alcohol intake; smoking status; and education attainment level.

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Figure 1.
Flow chart of participants.

Figure 2.

Analysis of covariance was used to obtain mean values of telomere length (SD) according to two levels of pessimism (low (<3) vs. medium to high (4+)). We used 154 subjects who had at least 3 sets of visits.

Table 1

Distribution of baseline characteristics in 490 men according to the quartiles of pessimistic orientation subscale score.

Range	Level of pessimistic orientation subscale				P-value
	3	4	5-6	7+	
No. at risk	116	135	127	112	
Visits (SD)	1.72 (0.76)	1.70 (0.77)	1.65 (0.73)	1.63 (0.71)	0.15
Baseline age, year (SD)	71.9 (0.62)	71.3 (0.57)	71.9 (0.59)	73.1 (0.63)	0.22
BMI, kg/m ² (SD)	27.4 (0.38)	28.0 (0.36)	28.5 (0.37)	28.8 (0.39)	0.06
Hypertension, %	62.9	58.5	55.9	68.8	0.19
Diabetes, %	11.2	12.6	18.1	22.3	0.07
Physical activity, METS-hr/wk (SD)	13.7 (1.7)	16.8 (1.6)	13.6 (1.7)	11.7 (1.8)	0.20
History of cardiovascular disease, %	31.0	25.2	23.6	33.0	0.30
Current smoker, %	5.17	3.70	1.57	8.04	0.11
Two or more drinks, %	18.1	18.5	17.3	17.9	0.99
Educational attainment level, year (SD)	15.2 (0.27)	14.4 (0.25)	14.1 (0.25)	14.1 (0.27)	<0.001

Table 2

Age- and multivariate-adjusted percent difference associated with the quartile of pessimistic orientation subscale score (the lowest category as reference) or with a 1-standard deviation increase in pessimistic orientation subscale score (95% CI) for log-transformed telomere length.

		Level of pessimism subscale				1SD change 1SD=2.605
		3	4	5-6	High 7+	
Crude	0		-3.79(-10.5, 3.44)	-5.63(-12.4, 1.62)	-8.26(-15.0, -0.96)	-3.20(-5.68, -0.65)
Age-adjusted	0		-4.17(-10.9, 3.07)	-5.96(-12.7, 1.28)	-8.42(-15.2, -1.10)	-3.18(-5.68, -0.61)
Multivariate-adjusted	0		-3.93(-10.7, 3.37)	-5.73(-12.6, 1.63)	-8.17(-15.1, -0.70)	-3.08(-5.62, -0.46)
Further weighted for inverse probability of revisits ^{***}	0		-3.79(-10.6, 3.49)	-5.86(-12.7, 1.54)	-9.04(-15.8, -1.70)	-3.44(-5.95, -0.86)

**Adjusted for age at baseline and Δ age (difference in age between baseline and time of outcome).

In multivariate-adjusted model, adjusted for baseline age, body mass index, abnormal glucose tolerance, education, and Δ age.

*** The revisit propensity score was calculated from a logistic regression as the probability of not having two or more study center visits, given all relevant factors at baseline including age; telomere length; total cholesterol level; statin use; abnormal glucose tolerance; hypertension; alcohol intake; smoking status; and education attainment level.