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## Leukocyte Telomere Length in Postmenopausal Women

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### Abstract

**Objective**—To compare leukocyte telomere length (LTL) by race and describe demographic, health, and psychosocial factors associated with LTL in postmenopausal women.

**Design**—Descriptive study with comparative analyses and correlations.

**Setting**—Data were collected at the University of California San Francisco, San Francisco Clinical and Translational Science Institute (CTSI).

**Participants**—Thirty-nine African American and White postmenopausal women between 58 and 65 years of age (mean age  $61.3 \pm 1.83$  years).

**Methods**—Measures included demographics, blood pressure, anthropometrics, scores on the Perceived Stress Scale (PSS-10) and the Center for Epidemiologic Studies-Depression (CES-D), and blood samples for LTL.

**Results**—African American women ( $n=14$ ) had higher PSS-10 and CES-D scores, higher blood pressure, and higher body mass index (BMI) than White women ( $n=25$ ) ( $p<0.05$ ), but LTL did not significantly differ between the two groups. Age was inversely related to LTL ( $r = -.355$ ,  $p < .05$ ). After controlling for age and race, fewer children ( $p=0.005$ ) and higher perceived stress ( $p=0.036$ ) were related to shorter LTL.

**Conclusion**—Findings from this small sample support the association between age and LTL.

The association between perceived stress, number of children, and shorter LTL in postmenopausal women requires further research and replication of findings in a larger more diverse sample.

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Disclosure

The authors report no conflict of interest or relevant financial relationships.

## Keywords

psychological stress; post-menopause; telomere shortening; social support; aging

With the aging process come physiological changes and an overall decline in health. While modern medical advances have improved health outcomes and longevity, health disparities persist. Chronic exposure to physical and psychological stressors affects health and increases risk for chronic illness (Cohen et al., 2012; Keene & Geronimus, 2011; O'Doherty et al., 2016). The link between chronic stressors and an individual's increased vulnerability to illness is well established (Folkman, Lazarus, Gruen, & DeLongis, 1986; McEwen, 2012). For example, chronic psychological stress triggers hormone responses that result in inflammation and plaque formation within the cardiac vessels that set the stage for heart disease and other health problems (Oeseburg, de Boer, van Gilst, & van der Harst, 2010; Schutte & Malouff, 2014).

Genetic biomarkers of stress, such as leukocyte telomere length (LTL), can be used to objectively measure the effects of stress on the aging process (Oeseburg et al., 2010). Telomeres are repetitive sequences of nucleotide bases (DNA) that cap the ends of chromosomes to stabilize and protect the cell. Telomeres shorten with each replication of the cell. Premature shortening of telomeres occurs with increasing age, chronic stress, cardiac disease, and other factors such as weight, race, and socioeconomic status (Epel et al., 2004; Oeseburg et al., 2010; Schutte & Malouff, 2014). Physical stressors, such as obesity, have been recognized as risk factors for health problems and have been associated with shorter LTL in adults (Al-Attas et al., 2010). The body's response to chronic stress includes prolonged elevated cortisol levels, central obesity, and hypertension (McEwen, 2012; McEwen & Wingfield, 2003).

In addition to the physiological and psychological responses to stress conceptualized in prior theoretical models of perceived stress (Folkman et al., 1986; McEwen, 2012) and allostatic load (McEwen, 2012), other theoretical underpinnings for this research include the weathering hypothesis, in which exposure to long-term racial discrimination is a stressor associated with premature aging and health disparity (Geronimus et al., 2010). Race-based differences in LTL have been reported, yet findings remain inconclusive. Longer LTL in African American adolescents has been reported in comparison with White adolescents (Zhu et al., 2011), while middle-aged and older African American adults have shorter LTL in comparison with White adults (Diez Roux et al., 2009; von Kanel, Malan, Hamer, & Malan, 2014). These differences have prompted exploration into the effects of certain social and economic factors on LTL (Adler et al., 2013; Carroll, Diez-Roux, Adler, & Seeman, 2013; Carroll, Diez Roux, Fitzpatrick, & Seeman, 2013).

The purpose of this study was to compare LTL in a sample of African American and White postmenopausal women and describe the relationships among LTL and demographic and health characteristics in addition to depression symptoms, perceived stress, and perception of general health status. Women report higher stress levels than men, and African American women report more chronic stress than White women (Barbosa-Leiker et al., 2012; Vines, Ta, Esserman, & Baird, 2009). We hypothesized that age, race, income, clinical and

reproductive factors, perceived stress, depression symptoms, weight, and perceived general health status would be associated with LTL in this sample of postmenopausal women.

## Methods

### Study Design & Sample

This descriptive study was conducted as an ancillary follow up study to the University of California San Francisco (UCSF) Women's Health Study involving a multiethnic sample of community-dwelling women living in the San Francisco Bay Area. They were originally recruited between 40–50 years old while healthy and still experiencing regular menstrual cycles. Details of recruitment and protocols were previously reported (Gilliss et al., 2001) and participants gave permission to be re-contacted for future research. The UCSF Committee on Human Research (CHR) approved both the original study and this follow up study. From the prior convenience sample of over 300 women, 50 Whites and 50 African Americans who agreed to future contact were mailed an information letter inviting them to call for an appointment to participate in this study. Mailings to 30 women were returned due to old contact information, and three former participants were deceased. The final response rate was higher for Whites (25 of 40 = 63%) than African Americans (14 of 27 = 52%). Written informed consent was obtained prior to data collection.

Protocols for the objective measures for this study were identical to prior measures of blood pressure and anthropometrics (height, weight, waist and hip circumferences) and also included a blood sample for LTL. Participants also completed a questionnaire packet that included demographic information, reproductive health history, perceived general health status, depression symptoms, and perceived stress. The time lapse between the original study and this ancillary study was 10 years. To control for age and reproductive factors, participants were in a narrow 8-year age range (58–65 years), and had completed their transition to postmenopausal reproductive status. The sample for this ancillary study consisted of 14 African American and 25 White women who completed the questionnaires and an in-person visit at the UCSF Clinical Research Center where anthropometric measures and whole blood samples for LTL were collected.

### Measures

**Leukocyte Telomere Length**—LTL is quantified as the ratio of telomeric product (T) to single (S) copy gene (T/S) obtained from the blood sample using a quantitative polymerase chain reaction (PCR). Samples were frozen on site until completion of the study, and then thawed and analyzed in a single batch by a trained and authorized technician in the UCSF Blackburn Lab using an adapted quantitative PCR method originally published by Cawthon (Cawthon, Smith, O'Brien, Sivatchenko, & Kerber, 2003; Lin et al., 2010). In this study, LTL was used as an objective measure of aging and chronic stress.

**Perceived General Health Status**—Using the general health perception item from the Short Form Health Survey (SF36), participants were asked to rate their health as 'excellent', 'very good', 'good', 'fair' and 'poor'. This item has been highly validated as a general health measure (Ware & Sherbourne, 1992).

**Depression Symptoms**—The 20-item Center for Epidemiological Studies-Depression (CES-D) scale was used to assess frequency of depression symptoms in the past week (Radloff, 1977). Items are rated on a 0 (none in the past week) to 3 (5 to 7 days in the past week), with a total score ranging from 0 to 60. The CES-D has well-established concurrent and construct validity (Radloff, 1977). In this study, the Cronbach alpha coefficient for the CES-D was 0.897 at the initial assessment and 0.896 in the current sample.

**Stress Perception**—The 10-item Perceived Stress Scale (PSS-10) is a widely used self-report instrument developed to measure the perception of general stress (Cohen, Kamarck, & Mermelstein, 1983). Items are rated on a scale of 0 – 4 with a maximum score of 40 (Cohen & Williamson, 1988). Higher levels of stress correspond with higher scores. The PSS has been validated in a variety of populations and languages. The Cronbach alpha coefficient in the original sample (before menopause) of women was 0.88. The Cronbach alpha was also 0.88 for this current smaller sample of postmenopausal women.

**History of Sexual Harassment or Abuse**—Sexual harassment and abuse were evaluated by asking the women to respond ‘yes’ or ‘no’ to the following questions: 1) During the past year, have you experienced, in your opinion, any sexual harassment either on the job or elsewhere” and, 2) At any time in your past, have you ever experienced any sexual abuse or physical abuse.” Results from these survey questions are reported elsewhere (Humphreys & Lee, 2009)

### Statistical Analysis

To evaluate for measures of central tendency, medians, means, and standard deviations (SD) were computed. Log transformation was sufficient to normalize the LTL data. Demographic, health, and psychosocial factors were compared by race (t-test, Mann-Whitney U, and chi square). LTL bivariate relationships (Pearson correlation and Spearman rho) with demographic, health, and psychosocial variables were examined first. Finally, based on accepted relationships with LTL, age and race were controlled in analyses of partial correlations. All analyses were performed using SPSS version 20.

## Results

### Telomere Length and Demographic Characteristics

Mean LTL for the total sample was  $0.951 \pm 0.147$  T/S and ranged from 0.73 to 1.34, with a median of 0.940). Log transformation was sufficient to normalize the skewed distribution of LTL in this sample and was used for all analyses. The mean age of the sample was  $61 \pm 1.8$  (SD) years (Table 1). There were no significant racial group differences in LTL, education, income, marital status, or age.

As expected, LTL was inversely correlated with age ( $r = -.355$ ,  $p = .026$ ). While LTL was unrelated to education or marital status, it was correlated with income ( $\rho = .318$ ,  $p = .049$ ) such that the higher the woman’s income, the longer her LTL. After controlling for age and race, income was no longer statistically significant (partial  $r = .275$ ,  $p = 0.100$ ) and other demographic variables remained nonsignificant when age and race were controlled.

### Telomere Length and Health Characteristics

As seen in Table 1, over half of the sample perceived their health as very good or excellent. Perceived general health status was unrelated to LTL or age in this sample. Perceived health did not differ by race, yet African American women had significantly higher body mass index ( $p = 0.001$ ), systolic ( $p = 0.014$ ), and diastolic blood pressures ( $p = 0.035$ ). Waist/hip ratio and self-reported days of regular physical activity were not significantly different by race and there were no significant racial group differences in number of children or age-related factors such as age at menarche or menopause.

Anthropometric measures (BMI, waist circumference, waist/hip ratio and current weight) were not significantly related to LTL. After controlling for age and race, current weight was the only anthropometric variable that approached a statistically significant relationship to LTL (partial  $r = .298$ ,  $p = .073$ ). Age at menarche and age at menopause were not associated with LTL. LTL did not differ between women who were mothers ( $n=21$ ) or not ( $n = 18$ ), but mothers had significantly higher BMI ( $t = 2.03$ ,  $p = .050$ ) than women without children, and the relationship between number of children and LTL approached significance ( $\rho = .273$ ,  $p = .093$ ) in this small sample. After controlling for age and race, the number of children was significantly related to LTL (partial  $r = .341$ ,  $p = .039$ ) such that women with no children had shorter LTL.

The African American women had significantly higher blood pressures compared to Whites (Table 1). While systolic pressure was unrelated to LTL in bivariate and partial correlations, there was an inverse correlation between diastolic pressure and LTL ( $r = -.254$ ,  $p = .119$ ) that improved after controlling for age and race (partial  $r = -.307$ ,  $p = .064$ ). While the higher the diastolic pressure, the shorter LTL, the relationship was not statistically significant in this small sample.

### Telomere Length and Psychosocial Characteristics

The PSS-10 and CES-D scores are reported in Table 1. These two psychosocial variables were highly correlated ( $r = .85$ ,  $p < .001$ ) but neither were related to LTL or to perceived general health status. There was a significant difference in PSS-10 and CES-D scores by race; African American women perceiving higher stress ( $t = 2.5$ ,  $p = .02$ ) and depression symptoms (unequal variance  $t = 2.5$ ,  $p = .02$ ) than Whites. The two groups did not differ on self-reported history of sexual harassment or abuse and there was no difference in LTL between women with and without a history of abuse or sexual harassment. After controlling for age and race, only the bivariate relationship between perceived stress and LTL became stronger and statistically significant (partial  $r = -.335$ ,  $p = .043$ ).

### Discussion

There were no statistically significant demographic differences by race in this small sample of postmenopausal women. The majority of the sample perceived their general health as 'very good' or 'excellent' but African American women had higher perceived stress (PSS-10) scores and depression symptom (CES-D) scores compared to White women. African American women also had significantly higher BMI and blood pressure, but none of

the women in this sample had diastolic blood pressures above 90 mmHg. We hypothesized that general health status would be associated with LTL, yet it was unrelated to LTL and unrelated to other factors, including BMI, blood pressure, perceived stress and depression symptoms.

LTL did not differ by race in this small sample, however the African American women trended toward longer LTL in comparison to the White women, which supports findings from other larger studies (Hunt et al., 2008; Zhu et al., 2011). Given the current literature and risk profiles for African American women, we expected to find shorter LTL in our sample of African Americans compared to White women. The significance of longer LTL for African American women in this cohort of postmenopausal women is unclear and conflicts with studies that link adiposity and hypertension with shorter LTL (Al-Attas et al., 2010; Bhupatiraju et al., 2012; Honig, Kang, Schupf, Lee, & Mayeux, 2012; Laimer et al., 2015; Revesz, Milaneschi, Verhoeven, Lin, & Penninx, 2015). Some researchers reported that African Americans trend towards longer LTL and that LTL declines at a faster rate, which may contribute to health disparities (Rewak et al., 2014; Zhu et al., 2011). This study was not designed to measure the rate of LTL shortening; the sample was also relatively young and there were no longitudinal measures of LTL. The lack of a significant difference in LTL by race may be a statistical power issue with our small sample, however there are many factors that can mediate LTL shortening, including lifestyle and environmental exposures. Evaluation of these factors was beyond the scope of this small study but remains important, especially as African American women continue to suffer disproportionately from poor health outcomes and inequality in health care services (Agency for Healthcare Research and Quality, 2011).

As expected, age was correlated with telomere length in this sample of women despite a narrow age range and supports prior research (Cherif, Tarry, Ozanne, & Hales, 2003; Oeseburg et al., 2010). In addition, findings from this study add to women's health research by finding no relationship between LTL and measures of reproductive age such as age at menarche or age at menopause.

The number of children in this sample ranged from 0 to 4, and was related to LTL. Surprisingly, having a child (yes/no) was not associated with LTL, but the number of children appeared protective for maintaining LTL. This finding was strengthened when controlling for age and race. The positive relationship between number of children and LTL and the protective influence of having more children on LTL needs further research. There may be a protective effect from female hormones during pregnancy, or a higher level of social support in larger families. At least one study found longer LTL associated with having more children, and the authors suggested that levels of estradiol experienced during pregnancy might counter the adverse physiological effects anticipated to occur from multiple pregnancies (Barha et al., 2016). Recent research also proposes that social support may have a protective effect on LTL such that higher levels of social and spousal support are associated with longer LTL (Barger & Cribbet, 2016; Barha et al., 2016). Although spousal support was not evaluated in this study, others found that spousal support, in contrast to support from other sources, was associated with longer telomere length (Barger & Cribbet, 2016).



Only after controlling for age and race did perceived stress become a significant correlate of LTL. Perceived stress and depression symptoms were highly correlated measures in this sample, and both are concepts strongly linked with race, socioeconomic status, and health outcomes (Geronimus et al., 2010; Giurgescu et al., 2015; Jackson, Rowley, & Curry Owens, 2012). Yet it was perceived stress rather than depression that was related to shorter LTL in our sample. One potential explanation is that depression symptoms measured with the CES-D only indicate frequency of depression symptoms within the past week, while the PSS-10 is a more global measure of perceived stress. This variable may represent an underlying, intangible factor influencing severity and prevalence of certain health conditions for women.

Chronic perceived stress and trauma have been associated with LTL in women (Geronimus et al., 2010; Humphreys et al., 2012). However, few women in this sample reported traumatic events such as a history of sexual abuse or harassment, making any significant group differences unlikely due to the small sample. Other chronic stressors can include childcare, and other researchers have reported that mothers of chronically ill children have shorter LTL relative to the length of time performing caregiving duties (Epel et al., 2004). Due to the many links between perceived stress and adverse health outcomes, perceived stress is now accepted as a health determinant (Cohen et al., 2012; Epel et al., 2004), supporting its association with shorter LTL found in this study.

Income and diastolic pressure as well as BMI and depression were correlated with LTL, but these relationships were no longer significant after controlling for age and race. It is important to note that no participant in our sample had a diastolic pressure of 90 or greater, therefore it is not surprising that this variable was not significant and thus neither supports or conflicts with other studies. Correlates such as waist-hip ratio, smoking, and poverty have been associated with shorter LTL among other samples of women (Geronimus et al., 2010), and these variables need to be explored in future studies with larger more diverse samples of women.

Aging is inevitable, but a greater degree of understanding about the aging process and long-term physiological effects of hypertension, low income, high body weight, perceived stress, and women's roles and parenting responsibilities could improve health promotion. The postmenopausal transition is a key life phase during which women experience risk for certain health conditions such as heart disease and cancer. Although this narrow-aged cohort of women progressed from pre-menopause to post-menopause in relatively good health, understanding the demographic, health, and social factors that contributed to accelerated shortening of LTL is necessary for health promotion and longevity.

## Strengths & Limitations

This study explored the effects of salient demographic, health, and psychosocial variables on LTL in a relatively small sample of midlife women. The study has a number of strengths that include a specific focus on postmenopausal women of similar age from the same geographic area and standardized measures obtained by research staff. However the small sample size and descriptive design limit our findings and replication is required in larger more diverse



samples. Correlational measures are limited by their inability to determine causation although findings in this small study support other reports (Barger & Cribbet, 2016). The results from this sample would be difficult to generalize to all postmenopausal women since the sample was limited to women in the San Francisco Bay area and environmental stressors may vary geographically. The influence of geographical location or climate on LTL in humans is unknown. The cross-sectional nature of the study limits the ability to assess long-term effects of health and psychosocial characteristics on current LTL in this study. In addition, this current sample participated in the original study and their responses may be biased by their prior participation in this ancillary study. Given our small convenience sample, relationships noted in this study require replication in different samples from more diverse populations that can better represent midlife women in a broader context of lifestyles and socioeconomics within the United States.

## Recommendations for Future Research

Our sample was limited to midlife women of African American and White descent only. Sampling from a larger population of women from more diverse racial groups, including Asians and Latinas is highly recommended. Future studies should be designed to examine the influence of childbearing and chronic perceived stress on LTL over time. Demographic and psychosocial variables should be included in future studies to assess their potential moderating or modifying effects on telomere shortening to improve understanding of aging and associated health risks.

## Conclusions

Aging is a complex biological process influenced by a variety of interrelated factors. Researchers have yet to uncover how timing of certain stressors and the environment may further influence aging and the risk for adverse health outcomes. We found several demographic, health, and psychosocial correlates of LTL in this sample of postmenopausal women. Many of the correlation coefficients were diminished when controlling for age and race, yet perceived stress and number of children emerged as a significant correlates after controlling for age and race. While older age was the strongest correlate of shorter telomere length, having fewer children and higher perceived stress also influenced the telomere length in this sample. As our society continues to age and healthcare inequalities persist, the importance of understanding how and why certain factors influence our vulnerability to disease and poor health remains an important aspect of women's health across the lifespan.

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**Callouts**

1. Chronic stress, age, weight, race, and socioeconomic status have been associated with accelerated shortening of leukocyte telomere length.
2. Shorter leukocyte telomere length was related to chronological age but unrelated to age at menarche or menopause.
3. After controlling for age and race, higher perceived stress and having fewer children were associated with shorter leukocyte telomere length in this sample of women.

**Table 1**

## Demographic and Health Characteristics by Race

Participant Characteristics	African American n = 14	White n = 25	Total sample N = 39
	Mean ± SD	Mean ± SD	Mean ± SD
Leukocyte telomere length	.971 ± .140	.940 ± .152	.951 ± .147
Age (yrs)	60.9 ± 1.49	61.6 ± 1.98	61.3 ± 1.83
Age at menarche (yrs)	13.1 ± 1.41	12.5 ± 1.62	12.8 ± 1.55
Age at first child (yrs)	27.1 ± 7.97	28.3 ± 5.90	27.7 ± 6.76
Number of children	1.21 ± 1.05	.95 ± 1.31	1.05 ± 1.21
Age at menopause (yrs)	50.7 ± 6.23	52.4 ± 3.67	51.8 ± 4.77
Body mass index	33 ± 5.50 **	27 ± 4.39	29 ± 5.51
Waist-hip ratio	.86 ± .06	.84 ± .05	.85 ± .06
Systolic blood pressure	137 ± 15.9 *	117 ± 29.2	125 ± 26.5
Diastolic blood pressure	74 ± 7.6 *	67 ± 12.4	69 ± 11.3
Perceived Stress Scale	18.6 ± 6.17 *	14.1 ± 5.12	15.7 ± 5.87
Center for Epidemiologic Studies-Depression	17.3 ± 10.92 *	9.1 ± 6.98	12.1 ± 9.34
	n (%)	n (%)	n (%)
Perceived general health status			
<i>excellent or very good</i>	5 (35.7)	14 (56.0)	19 (48.7)
Routine exercise (Yes)	11 (84.6)	22 (88.0)	33 (86.8)
History of abuse (Yes)	6 (42.9)	5 (20.8)	11 (28.2)
Sexual harassment (Yes)	4 (30.8)	3 (12.0)	7 (17.9)
Education - college graduate	10 (71.5)	24 (96.0)	34 (87.2)
Marital Status			
Married/partnered	7 (50.0)	16 (64.0)	23 (59.0)
Single/Divorced	7 (50.0)	9 (36.0)	16 (41.0)
Income < \$ 30,999	5 (35.7)	1 (4.0)	6 (15.4)
> \$ 81,000	6 (42.9)	16 (64.0)	22 (56.4)

\* p value .05,

\*\* p value .001

**Table 2**

Correlates of Leukocyte Telomere Length (log transformed)

Control variables	Bivariate Correlations with Leukocyte Telomere Length	Partial Correlations with Leukocyte Telomere Length (controlling for age & race)
Age	-.355	
Race (White=1; Black=2)	-.110	
Income	.174	.275
Number of children	.273	<b>.341</b>
Diastolic blood pressure	-.254	-.307
Current weight	.215	.298
Perceived Stress Scale	-.125	<b>-.335</b>

Note. Bolded values indicate statistical significance for bivariate and partial correlations ( $p < .05$ ).

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