

Comparison of Telomere Length in Black and White Teachers From South Africa: The Sympathetic Activity and Ambulatory Blood Pressure in Africans Study

ROLAND VON KÄNEL, MD, NICO T. MALAN, DSc, MARK HAMER, PhD, AND LEONÉ MALAN, RN, PhD

Objective: Telomere length is a marker of biological aging that has been linked to cardiovascular disease risk. The black South African population is witnessing a tremendous increase in the prevalence of cardiovascular disease, part of which might be explained through urbanization. We compared telomere length between black South Africans and white South Africans and examined which biological and psychosocial variables played a role in ethnic difference in telomere length. **Methods:** We measured leukocyte telomere length in 161 black South African teachers and 180 white South African teachers aged 23 to 66 years without a history of atherosclerotic vascular disease. Age, sex, years having lived in the area, human immunodeficiency virus (HIV) infection, hypertension, body mass index, dyslipidemia, hemoglobin A1c, C-reactive protein, smoking, physical activity, alcohol abuse, depressive symptoms, psychological distress, and work stress were considered as covariates. **Results:** Black participants had shorter (median, interquartile range) relative telomere length (0.79, 0.70–0.95) than did white participants (1.06, 0.87–1.21; $p < .001$), and this difference changed very little after adjusting for covariates. In fully adjusted models, age ($p < .001$), male sex ($p = .011$), and HIV positive status ($p = .023$) were associated with shorter telomere length. Ethnicity did not significantly interact with any covariates in determining telomere length, including psychosocial characteristics. **Conclusions:** Black South Africans showed markedly shorter telomeres than did white South African counterparts. Age, male sex, and HIV status were associated with shorter telomere length. No interactions between ethnicity and biomedical or psychosocial factors were found. Ethnic difference in telomere length might primarily be explained by genetic factors. **Key words:** cardiovascular disease, ethnicity, genetics, psychological stress, telomere.

BMI = body mass index; **BP** = blood pressure; **CRP** = C-reactive protein; **CVD** = cardiovascular disease; **DNA** = deoxyribonucleic acid; **γ -GT** = γ -glutamyltransferase; **HbA1c** = glycosylated hemoglobin A1c; **HDL-C** = high-density lipoprotein cholesterol; **HIV** = human immunodeficiency virus; **Q-PCR** = quantitative real-time polymerase chain reaction; **SABPA** = Sympathetic Activity and Ambulatory Blood Pressure in Africans; **SES** = socioeconomic status; **T-C** = total cholesterol.

INTRODUCTION

Telomeres are deoxyribonucleic acid (DNA)–protein complexes at the ends of eukaryotic chromosomes providing protection against genome instability—promoting events with potentially catastrophic health outcomes (1). During somatic cell division, the ribonucleoprotein enzyme telomerase compensates for terminal telomeric DNA losses by lengthening the ends of telomeres through RNA-templated addition of telomeric sequences (2). Repair processes do not fully balance telomere attrition, so telomeres grow shorter with repeated cell divisions.

Mean telomere length of replicating somatic cells is an indicator of biological age and is modified by many factors including genetic, sociodemographic, cardiometabolic, life-style, and psychosocial ones (2,3). Specifically, shortened telomere length has been linked to male sex (4), low socioeconomic status (SES) (5), and poorer health habits like smoking (5), low physical activity (6), and high alcohol consumption (7). In terms of psychosocial measures, shorter telomeres have been

linked to depressive symptoms (8), psychological distress (9), and work-related stress (10). Oxidative stress and low-grade inflammation may contribute to telomere attrition in multiple diseases including atherosclerotic vascular diseases (11) like coronary heart disease (12) and ischemic stroke (13). Accordingly, the metabolic cardiovascular risk factors hypertension (14), obesity (15), dyslipidemia (16), and elevated blood glucose (16) have been linked to shortened telomeres.

There has been a dramatic increase in the prevalence of cardiovascular disease (CVD) in South Africans, much of it being a consequence of their transition from a traditional African to a modernized “Western” life-style (17). Stroke and coronary heart disease rank among the most prevalent CVD in South Africa (18), where the usual risk factors of hypertension, obesity, smoking, alcohol abuse, physical inactivity, and inappropriate diet are all relevant (19). Moreover, urbanization may inflict psychosocial stress in South Africans with unfavorable consequences for their cardiovascular health (20). Telomere shortening through an aggregation of life-style, cardiometabolic risk factors, human immunodeficiency virus (HIV) infection (21), and psychosocial stress could be one mechanism to explain the excessive CVD risk in South Africans. Regarding psychosocial characteristics being associated with telomere length (8–10) and ethnicity, we previously showed that relative to white South Africans, black South Africans have higher levels of psychological distress, work stress, and depressive symptoms, as well as greater rates of moderate depression (22,23).

Telomere length has not previously been investigated in the black population of South Africa. Moreover, studies on telomere length in African Americans are sparse, whereby the paradigm that links telomere length with CVD risk is based mainly on data from non-African populations (24). In population-based studies, African Americans showed longer (5,24–27) and shorter (28) age- and sex-adjusted telomere length compared with white Americans. However, African Americans tend to show ancestry primarily from Western Africa with a genetic makeup that is quite different from black South Africans (29).

From the Department of Neurology (R.v.K.), Inselspital, Bern University Hospital, and Department of Clinical Research (R.v.K.), University of Bern, Switzerland; Department of Psychosomatic Medicine (R.v.K.), Clinic Barmelweid, Barmelweid, Switzerland; Hypertension in Africa Research Team (HART) (N.T.M., M.H., L.M.), Faculty of Health Sciences, North-West University, Potchefstroom, North-West, South Africa; and Department of Epidemiology and Public Health (M.H.), University College London, London, UK.

Address correspondence and reprint requests to Roland von Känel, MD, Department of Psychosomatic Medicine, Clinic Barmelweid, CH-5017 Barmelweid, Switzerland. E-mail: roland.vonkaenel@barmelweid.ch

Received for publication January 6, 2014; revision received July 16, 2014.

DOI: 10.1097/PSY.0000000000000123

TELOMERE LENGTH IN SOUTH AFRICANS

Therefore, the primary aim of this study was to compare leukocyte telomere length between black South African teachers and white South African teachers with an equal sex distribution and socioeconomic background and free of clinically overt atherosclerotic vascular disease. To the extent that there would be an ethnic difference in telomere length, the secondary aim of our study was to explore whether sociodemographic factors, HIV infection, and cardiometabolic, life-style, and psychosocial characteristics (2–11,14–17,21) would explain an ethnic difference in telomere length.

MATERIALS AND METHODS

Study Participants

The participants of this study were recruited as part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study conducted between February 2008 and May 2009 (SABPA I). Data presented here were collected between February 2011 and May 2012 as part of the follow-up investigation (SABPA II) when leukocyte telomere length was measured. The study protocol applies with the Declaration of Helsinki (30) and was approved by the ethics review board of the North-West University, Potchefstroom Campus (0003607S6). All participants provided written informed consent before participation. The SABPA I study had a target population comparative design and recruited 409 black and white South African teachers aged 25 to 65 years, working in the Dr Kenneth Kaunda Education district in the North West Province, South Africa. This selection assured a homogenous sample from a similar socioeconomic class. Exclusion criteria for SABPA I included pregnancy, lactation, and vaccination or blood donation within 3 months before participation (22). There were 359 teachers participating in SABPA II study (attrition rate, 12.2%), whereby a similar proportion of black and white South Africans ($p = .44$) and of men and women ($p = .42$) were lost to follow-up.

For the purpose of the present study, we excluded three participants with a history of atherosclerotic vascular disease (myocardial infarction, $n = 2$; stroke, $n = 1$) and 15 with missing telomere data, yielding a final sample of 341 teachers (161 blacks, 180 whites) for the statistical analysis. We applied the Expectation-Maximization algorithm to replace a few missing data points ($n = 23$) in 16 participants: high-density lipoprotein (HDL) cholesterol ($n = 1$), hemoglobin A1c (HbA1c; $n = 5$), C-reactive protein (CRP; $n = 1$), total energy expenditure ($n = 4$), γ -glutamyltransferase (γ -GT; $n = 2$), depressive symptoms ($n = 3$), psychological distress ($n = 2$), and work stress ($n = 5$).

Study Design

On the morning of the first testing day (Monday through Thursday), between 7:00 AM and 8:00 AM, all participants were equipped with a 24-hour ambulatory blood pressure (BP) measurement device at their respective schools. At 3:00 PM, teachers were admitted to the multibedroom Metabolic Unit Research Facility of the North-West University. They were welcomed, received precounseling for HIV, and completed psychosocial questionnaires in a comfortable relaxing environment, well ventilated with mild temperatures. Demographic data and medical and smoking history were noted. All participants received a standardized dinner at 6:30 PM and had their last beverages (tea/coffee) and snacks at 8:30 PM, followed by recreational activities. They had no access to alcohol and refrained from caffeine, smoking, and exercising 12 hours before data sampling the next morning. All participants went to bed at around 10:00 PM and were woken at 6:30 AM on the second testing day. After the completion of anthropometric measurements, a registered nurse obtained fasting blood samples. Afterward, a physical activity monitor was fitted, and participants had breakfast and received post-counseling for HIV, feedback on health status, and an honorarium as token of appreciation (R100.00/US\$6). Participant confidentiality was maintained under all circumstances.

Assessment of Covariates

Sociodemographic Factors

We noted participants' age, sex, and the years they had been living in the Potchefstroom area as a proxy marker of urbanization-related stress.

Cardiometabolic Factors

We used the validated Cardiometers apparatus (CE0120; Meditech, Budapest, Hungary) with appropriate cuff sizes attached on the nondominant arm and instructions to comply with successful inflation rates to measure 24-hour ambulatory BP with 30-minute intervals during the day (8:00 AM–10:00 PM) and every hour during the night (31). The mean successful inflation rate was 88.6%. Participants continued with their normal daily activities and recorded any abnormalities on their ambulatory diary cards. In accordance with recently published guidelines, we defined "ambulatory hypertension" as an average 24-hour ambulatory BP of either at least 130 mm Hg systolic and/or at least 80 mm Hg diastolic (32).

With participants only in their underwear, we measured height and weight to the nearest 0.1 cm and 0.1 kg with calibrated instruments (Precision Health Scale; A&D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK) to calculate the body mass index (BMI; in kilograms per meter squared). Intraobserver and interobserver variability was less than 10%. Serum total cholesterol (T-C) and HDL-C levels were measured with the Konelab 20i (Thermo Fisher Scientific, Vantaa, Finland). We calculated the T-C/HDL-C ratio as an indicator of dyslipidemia. HbA1c was determined by a turbidometric inhibition immunoassay method from EDTA plasma (Integra 400; Roche, Basel, Switzerland). Ultra high-sensitivity CRP was measured in serum with a turbidimetric method (Unicel DXC 800; Beckman and Coulter, Germany).

Human Immunodeficiency Virus

HIV infection was determined with a rapid antibody test in plasma (First response kit; PMC Medical, Daman, India) and confirmed with the Pareekshak test (BHAT Bio-Tech, Bangalore, India).

Health Behaviors

Participants who indicated that they currently smoked at least one cigarette per day were categorized as current smokers and all others as noncurrent smokers. Serum levels of γ -GT activity were measured with an enzymatic colorimetric assay (Cobas Integra 400 plus) and used as a marker of alcohol abuse with cutoffs of at least 55 U/L for men and at least 38 U/L for women (33). For the assessment of physical activity, participants wore the Actiheart physical activity monitor (CamNtech Ltd, Upper Pendrill Court, Papworth Everard, Cambridgeshire, CB233UY, UK) apparatus for 7 days continuously. Total energy expenditure in kilocalories per day was computed, taking resting metabolic rate into account.

Psychosocial Measures

We used the nine-item Patient Health Questionnaire to measure the frequency of depressive symptoms during the prior 2 weeks (34). Each item is rated on a 4-point scale (0 = "not at all," 1 = "several days," 2 = "more than half the days," 3 = "nearly every day") giving a possible global score of 0 to 27. We additionally tested for a threshold effect of depressive symptom severity applying a cutoff of at least 10 defining "moderate depression"; this cutoff was previously shown to have a sensitivity of 88% and a specificity of 88% for major depression assessed by a structured interview (34).

With the 28-item General Health Questionnaire, we quantified perceived psychological distress in general related to symptoms of depression, anxiety, somatic complaints, and social withdrawal over the past few weeks (35). Each item is rated on a 4-point scale (1 = "not at all," 2 = "no more than usual," 3 = "rather more than usual," 4 = "much more than usual"); typical items are "Felt that you are ill?" and "Been getting scared or panicky for no good reason?" We applied the binary scoring method with the two least symptomatic answers scoring "0" and the two most symptomatic answers scoring "1." The global score provides a severity measure of total psychological distress (35).

The 20-item Teacher Stress Inventory was applied to measure work stress (36). Participants are requested to answer the question: "As a teacher, how great a source of stress are these factors to you?" Participants are then presented a list of potential work-related stressors (e.g., "difficult class," "pressure from principal and education officials"). Each item is rated on a 5-point scale ranging from 1 ("no stress") to 5 ("extreme stress"), yielding a global score of 20 to 100.

Measurement of Leukocyte Telomere Length

Leukocyte genomic DNA was extracted using a NucleoSpin 96 Blood Core kit (Machery Nagel, Düren, Germany) according to the manufacturer's instructions

and stored at -20°C . All samples were diluted to a concentration of 10 ng/ μl before the analyses. To prepare the reference DNA samples, all isolated DNAs were mixed together in equal proportions representing the average of all analyzed patients. We assessed telomere length by multiplex quantitative real-time polymerase chain reaction (Q-PCR) as previously described (37). Five concentrations of a reference DNA sample spanning a 75-fold range of DNA concentrations were prepared by serial dilution and analyzed in triplicate; these reactions provided the data for the generation of the standard curves used for relative quantification. All experimental DNA samples were assayed in triplicate with an average coefficient of variation of 16.4%. The intrabatch and interbatch control had a coefficient of variation of 4.3% and 4.8%, respectively. During this procedure, including blood preparation, DNA isolation, dilutions and Q-PCR, all precautions were taken to ensure repeatability and accuracy. This approach was chosen based on practical advantages of Q-PCR compared with Southern blot, and the good correlation was reported for the Q-PCR method compared with Southern Blot (37).

All PCRs were performed with CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) in a 25- μl volume in the biochemistry laboratory, North-West University (accredited by the Health Professional Council of South Africa). PCR reactions comprised 20 μl master mix (including 5 μl of 5 \times HOT FIREPol EvaGreen qPCR Mix Plus (no ROX; Solis BioDyne, Tartu, Estonia) and 5 μl of each experimental DNA sample. For multiplex Q-PCR, the telomere primer pair telg: AACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT and telc: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA (final concentrations 900 nM each) was combined with primers for the single copy gene human β -globin (hbg); hbg_u: CGGCGGCGGGCGGGCGGGCTGGGCG GCTTATCCACGTTACCTTG and hbg_d: GCCCGGCCCGCCGCGCCCGTC CCGCCGAGGAGAAGTCTGCCGTT (final concentrations 500 nM each), to give PCR products of 79 and 106 base pairs, respectively. All primers were manufactured by Integrated DNA Technologies (Coralville, IA).

The thermal cycling profile was as follows: Stage 1, 15 minutes at 95°C ; Stage 2, 2 cycles of 15 seconds at 94°C and 15 seconds at 49°C ; and Stage 3, 32 cycles of 15 seconds at 94°C , 10 seconds at 62°C , 15 seconds at 73°C with signal acquisition, 10 seconds at 84°C , and 15 seconds at 87°C with signal acquisition. The 73°C reads provided the Ct values for the amplification of the

telomere template (in early cycles where the hbg signal is still at baseline); the 87°C reads the Ct values for the amplification of the hbg template (at this temperature, there is no signal from the telomere PCR product because it is fully melted). After thermal cycling and raw data collection were complete, Ct values generated by CFX Manager Software Version 1.6 (Bio-Rad) were exported to REST software (Relative Expression Software Tool), version REST-384. PCR efficiency for each primer set was calculated by serial dilutions of reference DNA using the REST software tool. The relative telomere length per cell was also calculated using the REST software as the ratio (T/S) between relative content of telomere PCR product (T) and hbg PCR product (S). The T/S ratio was used the measure of leukocyte telomere length in all analysis presented here and was calculated for each individual participant relative to the average telomere length of the total analyzed group (344 participants).

Statistical Analysis

Data were analyzed using SPSS version 21.0 statistical software for Windows (SPSS Inc, Chicago, IL). The level of significance was set at $p < .05$ (two tailed). To obtain more normally distributed values, we log₁₀ transformed values for telomere length, age, years having stayed in the area, systolic BP, diastolic BP, T-C, HDL-C, BMI, HbA1c, CRP, γ -GT, total energy expenditure, depressive symptoms, and symptoms of psychological distress and work stress before the analyses. We applied independent t test and Pearson χ^2 test for group comparisons on continuous and categorical data, respectively. Pearson correlation and partial correlation analyses (controlling for age and sex) were used to estimate the bivariate association between telomere length and covariates.

We used univariate analysis of covariance to compute the relationship between ethnicity and telomere length. We only covaried for those variables that were significantly associated with telomere length in the bivariate correlation analysis, after controlling for age and sex as standard confounders of telomere length. Covariates were selected a priori based on previously described and/or hypothesized relationships with telomere length (2–11,14–17,21): a) sociodemographic factors (age, sex, number of years having lived in the area) and HIV infection status, b) metabolic factors (hypertension, BMI, T-C/HDL-C ratio, HbA1c, CRP),

TABLE 1. Participant Characteristics.

Variables	Black South Africans ($n = 161$)	White South Africans ($n = 180$)	p
Age, y	47.0 (42.0–54.0)	50.0 (44.0–58.0)	.269
Female sex, n (%)	76 (47.2)	92 (51.1)	.471
Having lived in the area, y	38.0 (21.5–48.0)	23.0 (13.0–32.0)	<.001
HIV infection, n (%)	21 (13.0)	0 (0)	<.001
Systolic blood pressure, mm Hg	133.0 (123.5–145.0)	122.0 (116–130)	<.001
Diastolic blood pressure, mm Hg	83.0 (75.5–91.0)	75.0 (69.0–80.0)	<.001
Ambulatory hypertension, n (%)	109 (67.7)	63 (35.0)	<.001
Body mass index, kg/m^2	30.1 (25.7–34.8)	27.9 (24.4–30.7)	.005
Total cholesterol, mmol/l	4.41 (3.81–5.27)	4.24 (3.68–4.88)	.008
HDL cholesterol, mmol/l	0.96 (0.79–1.13)	0.97 (0.80–1.23)	.144
Total cholesterol/HDL cholesterol	4.68 (3.83–5.90)	4.31 (3.23–5.32)	.001
Hemoglobin A1c, %	5.84 (5.59–6.13)	5.48 (5.29–5.64)	<.001
C-reactive protein, mg/L	4.63 (1.83–8.51)	1.19 (0.58–2.36)	<.001
Current smoker, n (%)	23 (14.3)	25 (13.9)	.466
γ -Glutamyltransferase, U/L	38.4 (22.9–69.8)	18.2 (11.9–31.6)	<.001
Alcohol abuse, n (%)	61 (37.9%)	16 (8.9%)	<.001
Total energy expenditure, kcal/d	2896 (2416–3639)	3045 (2480–3892)	.284
Depressive symptoms (score)	7.88 (5.00–11.0)	5.00 (3.00–8.00)	<.001
Moderate depression, n (%)	58 (36.0)	34 (18.9)	<.001
Distress symptoms (score)	5.00 (1.00–10.0)	2.00 (0.00–7.00)	<.001
Work stress (score)	71.0 (58.0–82.0)	63.0 (49.0–73.0)	<.001

HIV = human immunodeficiency virus; HDL = high-density lipoprotein.

Data are given as number of observations (n) and percentage of total (%) or median with interquartile range (25th–75th percentile).

TELOMERE LENGTH IN SOUTH AFRICANS

c) life-style factors (current smoking, total energy expenditure, alcohol abuse), and d) psychosocial factors (depressive symptoms, moderate depression, psychological distress, work stress).

We further undertook a moderator analysis for all covariates that were significantly different between black and white participants and thus might differently affect telomere length depending on ethnicity. For this purpose, we probed for significant two-way interactions between ethnicity and each such covariate controlling for age and sex, and in the case of psychosocial characteristics, additionally for other significant covariates. Effect sizes are expressed as partial η^2 .

RESULTS

Study Participants

Table 1 summarizes the characteristics of the 341 study participants per ethnic group. Compared with white participants, black participants had been living longer in the Potchefstroom area. All of the HIV-positive individuals were black. As compared with their white counterparts, black participants had a worse metabolic profile showing more ambulatory hypertension; higher levels of BMI, HbA1c, and CRP; and higher T-C/HDL-C ratio. Regarding life-style factors, there was evidence for significantly more alcohol abuse in black participants than in white participants. Psychosocial stress in the form of depressive symptoms, distress, and perceived work stress was significantly higher in black participants relative to white participants.

Associations Between Telomere Length and Covariates Sociodemographic Factors

Telomeres (median, interquartile range) were shorter in men compared with women (0.89, 0.74–1.04 versus 0.94, 0.76–1.17; $p = .030$) and in relation to greater age ($r = -0.16$, $p = .003$). All of the subsequent bivariate correlation analyses were adjusted for age and sex as standard confounders of telomere length. There emerged significant correlations between shorter telomeres and longer residency in the area ($r = -0.16$, $p = .004$) and HIV infection ($r = -0.23$, $p < .001$).

Cardiometabolic Factors

Shorter telomeres were associated with higher systolic ($r = -0.12$, $p = .029$) and diastolic ($r = -0.13$, $p = .016$) BP as well as with hypertension status ($r = -0.13$, $p = .014$). Shorter telomeres were also found in those with higher BMI ($r = -0.13$, $p = .018$) and CRP ($r = -0.26$, $p < .001$) levels. The relationships between telomere length and HbA1c ($r = -0.10$, $p = .074$) and the T-C/HDL-C ratio was not significant ($r = -0.04$, $p = .46$).

Life-Style Factors

Shorter telomeres correlated with higher γ -GT levels ($r = -0.34$, $p < .001$) and also with alcohol abuse ($r = -0.22$, $p < .001$). In contrast, telomere length did not relate to smoking status ($r = 0.04$, $p = .48$) and total energy expenditure ($r = 0.02$, $p = .69$).

Psychosocial Factors

All of the relationships of telomere length with measures of psychosocial stress pointed in the expected inverse direction, but failed to reach statistical significance: depressive symptoms ($r = -0.11$, $p = .051$), moderate depression ($r = -0.06$, $p = .30$), symptoms of distress ($r = -0.08$, $p = .13$), and perceived work stress ($r = -0.07$, $p = .21$).

Association Between Ethnicity and Telomere Length

The median (interquartile range) of the relative telomere length (T/S ratio) was 0.91 (0.75–1.09) in the total sample, whereby black South Africans had shorter telomeres than white South Africans (0.79, 0.70–0.95 versus 1.06, 0.87–1.21; $p < .001$). Figure 1 shows the average relative telomere length in original units for black participants in comparison with white participants with adjustment for those covariates, which were significantly associated with telomere length in the above correlation analysis (i.e., age, sex, years having lived in the area, HIV infection, hypertension, BMI, CRP, and alcohol abuse). Across five models with subsequent adjustment for sets of covariates, ethnicity was significant for all group comparisons ($p < .001$), with black participants consistently showing shorter average relative telomere length than their white counterparts. Moreover, regardless of any covariate adjustments, the absolute difference in telomere length changed very little between black and white participants.

Table 2 shows the final model for the relation of ethnicity with the log-transformed telomere length with adjustment for those covariates, which were significantly related to telomere length in the above correlation analyses (i.e., age, sex, years having lived in the area, HIV infection, hypertension, BMI, CRP, and alcohol abuse). In addition to shorter telomeres in black

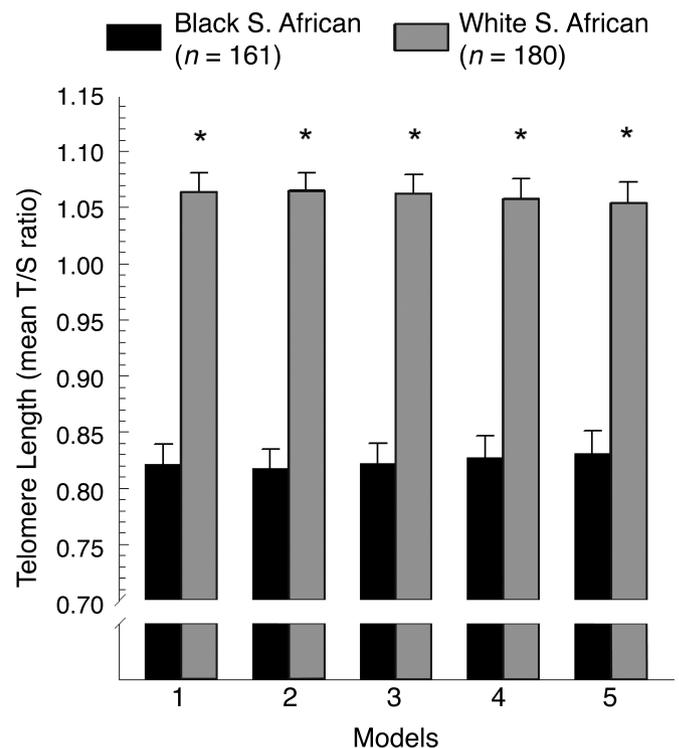


Figure 1. Leukocyte telomere length per ethnic group. Error bars show the mean \pm SEM of leukocyte telomere length (T/S ratio) in black South Africans and white South Africans with covariate adjustment in five models: (1) no adjustment; (2) adjusted for age and sex; (3) adjusted for covariates in model 2 plus years having lived in the area and human immunodeficiency virus infection status; (4) adjusted for covariates in model 3 plus ambulatory hypertension, body mass index, and C-reactive protein; and (5) adjusted for covariates in model 4 plus alcohol abuse. The comparison between black participants and white participants on telomere length was significant at * $p < .001$ in all models. SEM = standard error of the mean; T/S = ratio between relative content of telomere polymerase chain reaction product and human β -globin polymerase chain reaction product.

TABLE 2. Univariate Analysis of Covariance for Leukocyte Telomere Length

Entered Variables	Unstandardized B Coefficient	Standard Error	<i>p</i>	Partial η^2
Intercept	0.582	0.146	<.001	0.046
Black South African ethnicity	-0.098	0.014	<.001	0.131
Age	-0.280	0.065	<.001	0.053
Female sex	0.030	0.012	.011	0.020
Years having lived in the area	0.006	0.020	.77	0.000
Human immunodeficiency virus infection	-0.054	0.024	.023	0.016
Ambulatory hypertension	0.016	0.013	.20	0.005
Body mass index	-0.085	0.068	.21	0.005
C-reactive protein	-0.006	0.012	.59	0.001
Alcohol abuse	-0.016	0.014	.25	0.004

All continuously scaled data were entered as \log_{10} -transformed values. Effect sizes are expressed as partial η^2 .

participants than white participants (corresponding to model 5 in Fig. 1), significantly shorter telomeres were also found in those with advanced age, in men compared with women, and in HIV-positive versus HIV-negative participants.

Moderator Analysis

Most of the covariates of telomere length, including psychosocial characteristics, were significantly different between black and white participants (Table 1) and thus might differ in their association with telomere length depending on ethnicity. However, adjusting for age and sex, ethnicity did not significantly interact with any covariate to determine telomere length: years having lived in the area ($p = .63$), hypertension ($p = 1.00$), BMI ($p = .40$), T-C/HDL-C ratio ($p = .46$), HbA1c ($p = .17$), CRP ($p = .072$), and alcohol abuse ($p = .59$).

In terms of psychosocial characteristics, there were also no significant interactions of ethnicity with depressive symptoms ($p = .48$), moderate depression ($p = .23$), psychological distress ($p = .21$), and work stress ($p = .12$) in determining telomere length, controlling for age and sex. Moreover, these results did not change with additional adjustment for years having lived in the area, HIV infection, hypertension, BMI, CRP, and alcohol abuse (all p values $\geq .11$ for interactions between ethnicity and psychosocial characteristics).

DISCUSSION

The main finding from this study is that black South African teachers had significantly shorter telomeres compared with their white counterparts, even after controlling for a range of socio-demographic, HIV-infection-related, cardiometabolic, life-style, and psychosocial factors which have previously been identified as important correlates of telomere length; this suggests that the observation of shortened telomeres in black South Africans versus white South Africans is a robust one. Among the significant correlates of telomere length, ethnicity showed the greatest effect size for an association with telomere length in absolute terms, explaining 13.1% of the variance in the fully adjusted

model; to compare, the well-known correlates age and sex explained substantially less of the variance in telomere length.

We found that sociodemographic, HIV-infection-related, cardiometabolic, life-style, and psychosocial factors which significantly differentiated black and white participants did not emerge as significant moderators of the relation between ethnicity and telomere length after adjustment for all other covariates. This might indicate that genetic factors more than those investigated in our study contributed to shorter telomeres in black South Africans than white South Africans (2,3). Previous studies reported heritability estimates ranging from 34% to 82% (3); for instance, there might be gene polymorphisms that contributed toward telomere length (38), but we did not explore this. However, it should be emphasized that demographic and health-related variables are not irrelevant for telomere length maintenance, but rather of equal importance in black and white South Africans.

We confirmed several previously reported independent associations of telomere length with age, sex, and HIV infection. Women had longer telomeres than did men, whereas older and HIV-positive individuals had shorter telomeres than did younger participants and those HIV negative (4,21). Although showing the expected inverse relationship, we did not find a significant association between psychosocial factors and telomere length, namely, depression, psychological distress, and work stress; this is contrary to some previous studies (8–10), but similar to others (39,40). Psychosocial risk factors of CVD cover different domains of social adversities, personality traits, and negative affect (41). Further studies are needed to answer the question whether cellular aging as reflected by telomere length is a pathway that critically links psychosocial stress to CVD risk and the molecular mechanisms underlying these relationships in South Africans. The underlying mechanism might be complex and, as was revealed for various physical stressors disrupting telomere homeostasis (42), will likely differ in their effects on telomere attrition. For instance, a recent study found shorter telomeres but higher telomerase activity in high-hostile versus low-hostile men, whereby the heightened telomerase activity was interpreted as a compensatory response in men with high levels of cynical hostility (43).

To our knowledge, this is the first study to examine telomere length in black South Africans compared with white South Africans. Interestingly, except from one study also showing shorter telomeres in African Americans compared with whites and also Hispanics (28), the available literature suggests that African Americans have longer (age- and sex-adjusted) telomeres than do white (5,24–27). Similarly, African American adolescents aged 14 to 18 years also had longer telomeres than did their white counterparts (44). Altogether, these population-based studies cover the whole age distribution from adolescence to old age, suggesting that telomeres are consistently longer in African Americans compared with whites up to the age of 80 years (24).

We mention two explanations that might apply to the discrepancy with our study showing that black South Africans had markedly shorter telomeres than white South Africans. First, South Africans have quite a different genetic makeup compared with African Americans. It has been revealed that most African

TELOMERE LENGTH IN SOUTH AFRICANS

Americans have mixed ancestry from different regions of western Africa, whereas black South Africans show the most private alleles within Africa (29). Second, although the aforementioned studies, including ours, were all cross sectional, one of the few longitudinal studies on telomere length found that African Americans had longer telomeres than did whites, but that telomere shortening occurred relatively faster in African Americans over a follow-up of 6 to 10 years (45). Moreover, metabolic factors (46), but also depressive symptoms (8), result in more rapid telomere length attrition, and our black South African participants were clearly worse off in these variables than their white South African counterparts. Our cross-sectional analysis could only capture a snapshot of telomere length across the life span. Theoretically, our black participants could have begun life with longer telomeres (similar to African Americans) than their white counterparts, but experienced exaggerated telomere length attrition due to their progressively increasing CVD risk factors. This idea concurs with the notion that urbanization is a key stress factor in black South Africans in terms of both physical and psychological health sequelae (20), and indeed, an increased cardiometabolic risk was demonstrated in South Africans attempting to cope with an urban life-style compared with their rural counterparts (47). In addition, this hypothesis is consistent with recent evidence indicating a differential role of telomere shortening at various stages of atherosclerosis, with preferential involvement in advanced vessel pathology and acute vascular syndromes (48–50). However, we acknowledge that our study lacked a comparison group of rural South Africans, so the assumed trajectory in telomere attrition due to the sequel of urbanization must remain speculative.

As shortened telomeres have been implied in CVD risk (51), telomere biology might partly explain the rapidly increasing risk of incident atherothrombotic disease and CVD risk factors in the African population (12–20). The increased CVD risk in South Africans as related to urbanization might be a function of time an individual has been living in an urban environment. We found that the longer participants had been living in the Potchefstroom area, the shorter were their telomeres. However, this relationship disappeared after adjustment for covariates, and it also did not differ by ethnicity. It should be emphasized that “years participants had been living in the area” is a very crude proxy measure of urbanization stress, as this variable does not take into account how participants dealt with the stress. Therefore, more sophisticated measures of urbanization should be used to probe for an association with telomere length in future studies. Additional psychosocial variables, such as cultural differences, might also be important to investigate in this context (52).

The comprehensive assessment of several important correlates of telomere length and the comparable socioeconomic background of participants to equate major health disparities due to differences in the SES were strengths of our study. However, the study also had its limitations. Our results may not transfer to more rural populations in South Africa, those with a lower SES, elderly South African individuals, and those with clinically apparent atherothrombotic disease. Also, we did not assess nutrition status and telomerase activity, both of which might account for differences in telomere length between ethnicities (19,38,43).

Because circulating white cells reproduce rapidly and thus differ in telomere kinetics from, for instance, heart or nerve cells, leukocyte telomeres only can act as a marker, not an indicator of telomere length in other tissues (53).

Taken together, we found strong evidence for shorter telomeres in black South Africans compared with their white counterparts independent of important correlates of telomere length. Genetic factors rather than sociodemographic, HIV-infection-related, cardiometabolic, life-style, and psychosocial ones might have accounted for this ethnic difference. The extent to which relatively shorter telomere length in black South Africans contributes to their increased risk of CVD needs to be explored further.

The authors are grateful to Péter Szabolcs, Chrissie Lessing, and Tina Scholtz for their technical assistance and support and to Dr. Oksana Levants for performing PCR analysis.

Source of Funding and Conflicts of Interest: The study was partly funded by The Metabolic Syndrome Institute, France; the Medical Research Council; National Research Foundation; ROCHE Diagnostics; North-West University; and North-West Department of Education, South Africa. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the National Research Foundation does not accept any liability in regard thereto. The authors declare no conflicts of interest.

REFERENCES

1. Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett* 2005;579:859–62.
2. Blackburn EH. Telomere states and cell fates. *Nature* 2000;408:53–6.
3. Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, Albrecht E, Amin N, Beekman M, de Geus EJ, Henders A, Nelson CP, Steves CJ, Wright MJ, de Craen AJ, Isaacs A, Matthews M, Moayyeri A, Montgomery GW, Oostra BA, Vink JM, Spector TD, Slagboom PE, Martin NG, Samani NJ, van Duijn CM, Boomsma DI. Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur J Hum Genet* 2013;21:1163–8.
4. Mayer S, Brüderlein S, Perner S, Waibel I, Holdenried A, Ciloglu N, Hasel C, Mattfeldt T, Nielsen KV, Möller P. Sex-specific telomere length profiles and age-dependent erosion dynamics of individual chromosome arms in humans. *Cytogenet Genome Res* 2006;112:194–201.
5. Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, Epel ES. Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999–2002. *Soc Sci Med* 2013;85:1–8.
6. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, Kimura M, Lu X, Spector TD, Aviv A. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med* 2008;168:154–8.
7. Pavanello S, Hoxha M, Dioni L, Bertazzi PA, Sneghi R, Nalesso A, Ferrara SD, Montisci M, Baccarelli A. Shortened telomeres in individuals with abuse in alcohol consumption. *Int J Cancer* 2011;129:983–92.
8. Phillips AC, Robertson T, Carroll D, Der G, Shiels PG, McGlynn L, Benzeval M. Do symptoms of depression predict telomere length? Evidence from the west of Scotland Twenty-07 Study. *Psychosom Med* 2013;75:288–96.
9. Huzen J, van der Harst P, de Boer RA, Lesman-Leege I, Voors AA, van Gilst WH, Samani NJ, Jaarsma T, van Veldhuisen DJ. Telomere length and psychological well-being in patients with chronic heart failure. *Age Ageing* 2010;39:223–7.
10. Ahola K, Sirén I, Kivimäki M, Ripatti S, Aromaa A, Lönnqvist J, Hovatta I. Work-related exhaustion and telomere length: a population-based study. *PLoS One* 2012;7:e40186.
11. Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. Telomere length assessment: biomarker of chronic oxidative stress? *Free Radic Biol Med* 2008;44:235–46.

12. Brouillette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ; West of Scotland Coronary Prevention Study Group. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet* 2007;369:107–14.
13. Ding H, Chen C, Shaffer JR, Liu L, Xu Y, Wang X, Hui R, Wang DW. Telomere length and risk of stroke in Chinese. *Stroke* 2012;43:658–63.
14. Lung FW, Ku CS, Kao WT. Telomere length may be associated with hypertension. *J Hum Hypertens* 2008;22:230–2.
15. Lee M, Martin H, Firpo MA, Demerath EW. Inverse association between adiposity and telomere length: The Fels Longitudinal Study. *Am J Hum Biol* 2011;23:100–6.
16. Huzen J, Wong LS, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, Cawthon RM, Benus GF, van der Horst IC, Navis G, Bakker SJ, Gansevoort RT, de Jong PE, Hillege HL, van Gilst WH, de Boer RA, van der Harst P. Telomere length loss due to smoking and metabolic traits. *J Intern Med* 2014;275:155–63.
17. Sliwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, Stewart S. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. *Lancet* 2008;371:915–22.
18. Bradshaw D, Groenewald P, Laubscher R, Nannan N, Nojilana B, Norman R, Pieterse D, Schneider M. Initial Burden of Disease Estimates for South Africa, 2000. Cape Town: South African Medical Research Council; 2003.
19. Alberts M, Urdal P, Steyn K, Stensvold I, Tverdal A, Nel JH, Steyn NP. Prevalence of cardiovascular diseases and associated risk factors in a rural black population of South Africa. *Eur J Cardiovasc Prev Rehabil* 2005;12:347–54.
20. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation* 2005;112:3562–8.
21. Pathai S, Lawn SD, Gilbert CE, McGuinness D, McGlynn L, Weiss HA, Port J, Christ T, Barclay K, Wood R, Bekker LG, Shiels PG. Accelerated biological ageing in HIV-infected individuals in South Africa: a case-control study. *AIDS* 2013;27:2375–84.
22. von Känel R, Hamer M, Malan NT, Scheepers K, Meiring M, Malan L. Procoagulant reactivity to laboratory acute mental stress in Africans and Caucasians, and its relation to depressive symptoms: The SABPA Study. *Thromb Haemostasis* 2013;110:977–86.
23. Boshoff SM, Potgieter JC, Ellis SM, Malan L. Validation of the Teacher Stress Inventory (TSI) in a South African context: The SABPA Study. Master-Thesis. Potchefstroom, South Africa: North-West University, Potchefstroom Campus, 2011:1–58.
24. Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, Eckfeldt JH, Berenson GS, Aviv A. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. *Aging Cell* 2008;7:451–8.
25. Elbers CC, Garcia ME, Kimura M, Cummings SR, Nalls MA, Newman AB, Park V, Sanders JL, Tranah GJ, Tishkoff SA, Harris TB, Aviv A. Comparison Between Southern Blots and qPCR Analysis of Leukocyte Telomere Length in the Health ABC Study. *J Gerontol A Biol Sci Med Sci* 2014;69:527–31.
26. Hofmann JN, Baccarelli A, Schwartz K, Davis FG, Ruterbusch JJ, Hoxha M, McCarthy BJ, Savage SA, Wacholder S, Rothman N, Graubard BI, Colt JS, Chow WH, Purdue MP. Risk of renal cell carcinoma in relation to blood telomere length in a population-based case-control study. *Br J Cancer* 2011;105:1772–5.
27. Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, Tracy RP, Hardikar S, Aviv A. Leukocyte telomere length and mortality in the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci* 2011;66:421–9.
28. Diez Roux AV, Ranjit N, Jenny NS, Shea S, Cushman M, Fitzpatrick A, Seeman T. Race/ethnicity and telomere length in the Multi-Ethnic Study of Atherosclerosis. *Aging Cell* 2009;8:251–7.
29. Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, Hirbo JB, Awomoyi AA, Bodo JM, Doumbo O, Ibrahim M, Juma AT, Kotze MJ, Lema G, Moore JH, Mortensen H, Nyambo TB, Omar SA, Powell K, Pretorius GS, Smith MW, Thera MA, Wambebe C, Weber JL, Williams SM. The genetic structure and history of Africans and African Americans. *Science* 2009;324:1035–44.
30. World Medical Association. Declaration of Helsinki. Ethical principles of medical research involving human subjects. *J Indian Med Assoc* 2009;107:403–5.
31. Barna I, Keazei A, Dunai A. Evaluation of Meditech ABPM-04 ambulatory blood pressure measuring device according to the British Hypertension Society protocol. *Blood Press Monit* 1998;3:363–8.
32. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F; Task Force Members. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013;31:1281–357.
33. Hastedt M, Büchner M, Rothe M, Gapert R, Herre S, Krumbiegel F, Tsokos M, Kienast T, Heinz A, Hartwig S. Detecting alcohol abuse: traditional blood alcohol markers compared to ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) measurement in hair. *Forensic Sci Med Pathol* 2013;9:471–7.
34. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001;16:606–13.
35. Goldberg DP, Hillier VF. A scaled version of the General Health Questionnaire. *Psychol Med* 1979;9:139–45.
36. Boyle GJ, Borg MG, Falzon JM, Baglioni AJ. A structural model of the dimensions of teacher stress. *Br J Educ Psychol* 1995;65:29–67.
37. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002;30:e47.
38. Rehkopf DH, Dow WH, Rosero-Bixby L, Lin J, Epel ES, Blackburn EH. Longer leukocyte telomere length in Costa Rica's Nicoya Peninsula: a population-based study. *Exp Gerontol* 2013;48:1266–73.
39. Ladwig KH, Brockhaus AC, Baumert J, Lukaschek K, Emeny RT, Kruse J, Codd V, Häfner S, Albrecht E, Illig T, Samani NJ, Wichmann HE, Gieger C, Peters A. Posttraumatic stress disorder and not depression is associated with shorter leukocyte telomere length: findings from 3,000 participants in the population-based KORA F4 study. *PLoS One* 2013;8:e64762.
40. Fujishiro K, Diez-Roux AV, Landsbergis PA, Jenny NS, Seeman T. Current employment status, occupational category, occupational hazard exposure and job stress in relation to telomere length: the Multiethnic Study of Atherosclerosis (MESA). *Occup Environ Med* 2013;70:552–60.
41. von Känel R. Psychosocial stress and cardiovascular risk: current opinion. *Swiss Med Wkly* 2012;142:w13502.
42. Romano GH, Harari Y, Yehuda T, Podhorzer A, Rubinstein L, Shamir R, Gottlieb A, Silberberg Y, Pe'er D, Ruppin E, Sharan R, Kupiec M. Environmental stresses disrupt telomere length homeostasis. *PLoS Genet* 2013;9:e1003721.
43. Brydon L, Lin J, Butcher L, Hamer M, Erusalimsky JD, Blackburn EH, Steptoe A. Hostility and cellular aging in men from the Whitehall II cohort. *Biol Psychiatry* 2012;71:767–73.
44. Zhu H, Wang X, Gutin B, Davis CL, Keeton D, Thomas J, Stallmann-Jorgensen I, Mookken G, Bundy V, Snieder H, van der Harst P, Dong Y. Leukocyte telomere length in healthy Caucasian and African-American adolescents: relationships with race, sex, adiposity, adipokines, and physical activity. *J Pediatr* 2011;158:215–20.
45. Aviv A, Chen W, Gardner JP, Kimura M, Brimacombe M, Cao X, Srinivasan SR, Berenson GS. Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. *Am J Epidemiol* 2009;169:323–9.
46. Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation* 2005;111:2171–7.
47. Malan L, Malan NT, Wissing MP, Seedat YK. Coping with urbanization: a cardiometabolic risk? The THUSA study. *Biol Psychol* 2008;79:323–8.
48. Willeit P, Willeit J, Brandstätter A, Ehrenbach S, Mayr A, Gasperi A, Weger S, Oberhollenzer F, Reindl M, Kronenberg F, Kiechl S. Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. *Arterioscler Thromb Vasc Biol* 2010;30:1649–56.
49. De Meyer T, Rietzschel ER, De Buyzere ML, Langlois MR, De Bacquer D, Segers P, Van Damme P, De Backer GG, Van Oostveldt P, Van Criekinge W, Gillebert TC, Bekaert S; Asklepios Study Investigators. Systemic telomere length and preclinical atherosclerosis: the Asklepios Study. *Eur Heart J* 2009;30:3074–81.
50. Vasan RS, Demissie S, Kimura M, Cupples LA, White C, Gardner JP, Cao X, Levy D, Benjamin EJ, Aviv A. Association of leukocyte telomere length with echocardiographic left ventricular mass: the Framingham Heart Study. *Circulation* 2009;120:1195–202.
51. Serrano AL, Andrés V. Telomeres and cardiovascular disease: does size matter? *Circ Res* 2004;94:575–84.
52. Chae DH, Nuru-Jeter AM, Adler NE, Brody GH, Lin J, Blackburn EH, Epel ES. Discrimination, racial bias, and telomere length in African-American men. *Am J Prev Med* 2014;46:103–11.
53. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev* 2000;119:89–99.