Research Article

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# The Influence of Alcohol Consumption, Cigarette Smoking, and Physical Activity on Leukocyte Telomere Length

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## Abstract

**Background:** Telomeres protect from DNA degradation and maintain chromosomal stability. Short telomeres have been associated with an increased risk of cancer at several sites. However, there is limited knowledge about the lifestyle determinants of telomere length. We aimed to determine the effect of three factors, known to be important in cancer etiology, on relative leukocyte telomere length (rLTL): alcohol consumption, smoking, and physical activity.

**Methods:** This cross-sectional study included 477 healthy volunteers ages 20 to 50 years who completed a questionnaire and provided a fasting blood sample. Multiplex quantitative real-time PCR (qPCR) was used to measure rLTL. Regression coefficients were calculated using multiple linear regression while controlling for important covariates.

**Results:** There was no association between alcohol consumption and rLTL. Daily smokers and those in the middle and lower tertile of pack-years smoking had shorter rLTL than never daily smokers ($P = 0.02$). Data were suggestive of a linear trend with total physical activity ($P = 0.06$). Compared with the lowest quartile, the highest quartile of vigorous physical activity was associated with longer rLTL. A significant linear trend of increasing rLTL with increasing vigorous physical activity was observed ($P = 0.02$).

**Conclusions:** Cigarette smoking and vigorous physical activity have an impact on telomere length. Smoking was related to shorter telomere length while vigorous physical activity was related to longer telomeres.

**Impact:** The findings from this study suggest that lifestyle may play an important role in telomere dynamics and also suggest that engaging in healthy behaviors may mitigate the effect of harmful behaviors on telomere length. *Cancer Epidemiol Biomarkers Prev; 25(2); 374–80. ©2015 AACR.*



## Introduction

Telomeres are repeating "TTAGGG" DNA sequences located at the ends of linear chromosomes. They protect chromosomes from damage and, consequently, are important for maintaining chromosomal stability (1). As replicative machinery cannot completely copy the ends of linear chromosomes, 50 to 100 base pairs of telomeric DNA are lost at each cell division; this results in a gradual telomere attrition with increasing age (2). In recent years, research has demonstrated a relationship between short telomeres and aging-related diseases, including many forms of cancer. Evidence from two recent meta-analyses supports an inverse association between telomere length and overall risk of cancer, stratified analysis by subtype support an association with lung, bladder, esophageal, gastric, and renal cancers, while results for breast and colorectal cancer were inconsistent (3, 4).

Critically short telomeres ordinarily trigger either cell death or replicative senescence (5, 6). However, rarely cells may bypass cell death or senescence and continue to divide, acquiring mutations and genetic aberrations that may eventually result in malignant transformation (5, 6). Replicative senescence may indirectly promote cancer initiation by contributing to immunosenescence (7), an age-dependent decrease in immunologic functioning, as well as a lack of immunosurveillance (8), the body's ability to monitor and recognize aberrant cells, which are associated with the development of aging-related diseases, such as cancer (9, 10).

Due to the nature of their molecular structures, telomeres are particularly vulnerable to oxidative stress (11), and DNA damage that occurs as a result of oxidative stress may accelerate telomere shortening. It has been well documented that oxidative stress is associated with shorter telomeres (12–16). In addition, oxidative stress often accompanies inflammation and can contribute to leukocyte telomere shortening by promoting cell turnover and replicative senescence (17–19).

Various physiologic, environmental, and lifestyle factors can affect oxidative stress and inflammation, and presumably telomere length. Smoking (20, 21) and alcohol consumption (22, 23) cause increased oxidative damage and inflammation. Both chronic and acute alcohol consumption promote increased production of reactive oxygen species (ROS) and enhanced peroxidation of lipids, proteins, and DNA (23–25). Cigarette smoke contains many oxidants and free radicals (26) that can both directly and indirectly cause oxidative damage to DNA (27). Regular physical activity is associated with decreased levels of oxidative stress and inflammation (28, 29) as well as increased antioxidant and enzyme activity levels (28, 30) and has been shown to regulate telomere-stabilizing proteins (31).

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The objective of this cross-sectional study was to determine the association between the following factors and relative leukocyte telomere length (rLTL): alcohol consumption, cigarette smoking, and physical activity.

## Materials and Methods

### Design and source population

This analysis is nested within a cross-sectional study that recruited 678 healthy male and female volunteers ages 20 to 50 years. Volunteers were recruited from Ottawa, Ontario, Kingston, Ontario and Halifax, Nova Scotia. Complete study design details and subject eligibility and exclusion criteria have been described elsewhere (32). Briefly, participants were recruited between 2006 and 2008 by informed consent in approximately equal numbers by age and sex. Each volunteer was asked to complete a self-administered research questionnaire and provide a fasting blood sample for biochemical and genetic analysis. Ethics approval for the study was obtained from the Queen's University Health Science Research Ethics Board. Of the 678 recruited participants, 80 were excluded from this study because of an inadequate blood sample, 69 were excluded due to insufficient DNA, a further 25 were excluded because an accurate rLTL measurement could not be obtained, and an additional 28 subjects were excluded because of missing covariate information. The final sample size for this analysis consisted of 477 individuals with available DNA and complete questionnaire and biochemical data.

### Exposure measurement

Demographic and lifestyle information, including alcohol consumption, cigarette smoking, and physical activity, was assessed by questionnaire. Alcohol consumption was estimated using validated, global, quantity–frequency questions adapted from the 1996 Ontario Drug Monitor (ODM). The definition of one standard alcoholic beverage [1 alcoholic beverage = 12oz beer (1 bottle), 5oz wine (1 glass), or 1.5oz liquor] was provided in the questionnaire. Information was collected on the typical number of drinking occasions over the past month and the typical number of drinks consumed per drinking occasion. Weekly alcohol consumption was estimated using data on frequency and quantity of alcohol consumption, according to recommendations from the ODM Technical Guide (33). Alcohol consumption was categorized into four levels—abstainer, low, moderate, and high—according to published low-risk drinking guidelines (34). The abstainer category included both lifetime abstainers and former drinkers and was defined as 0 drinks/week. Low consumption was defined as an intake of more than 0 but fewer than 7 drinks/week, while moderate and high consumption had sex-specific category definitions. Females consuming more than 7 but fewer than 10 drinks/week and males consuming more than 7 but fewer than 15 drinks/week were classified as moderate. High consumption was defined as greater than 10 drinks/week for females and greater than 15 drinks/week for males.

Daily smoking status was categorized as daily smoker, former daily smoker, and never daily smoker. Participants who reported currently smoking at least one cigarette per day over the past month were classified as daily smokers. Those who were not currently smoking, but reported smoking at least one cigarette a day for at least six months at some point in their lives were classified as former daily smokers. Otherwise, participants were coded as never daily smokers. For this analysis, packs per day were calculated assuming a 20-cigarette pack. For daily smokers, smoking duration was calculated from the age smoking started to age at the time of questionnaire completion, and for former daily smokers, age started smoking to age stopped smoking. Cumulative smoke exposure was calculated as the product of the number of packs smoked per day and smoking duration. Pack-years were categorized into tertiles.

Physical activity was measured using the short-form International Physical Activity Questionnaire (IPAQ), which was developed and validated for use in adults ages 15 to 69 years (35). The IPAQ short-form instrument measures walking, as well as moderate-intensity and vigorous-intensity activities across four domains: leisure-time physical activity, domestic and gardening activities, work-related physical activity, and transport-related physical activity. Total physical activity metabolic equivalent of task (MET)-minute scores were calculated by summing duration, in minutes, and frequency, in days, of walking, moderate-intensity, and vigorous-intensity activities. The IPAQ scoring protocol (36) was used to guide data cleaning and categorization of total physical activity into high, moderate, and low categories. High activity was defined as vigorous-intensity activity on at least 3 days and accumulating at least 1,500 MET-minutes/week; or any combination of walking, moderate- or vigorous-intensity activities equivalent to at least 3,000 MET-minutes/week. Moderate activity was defined as at least 20 minutes of vigorous activity per day on 3 or more days per week; 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day; or any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 MET-minutes/week. Low activity was defined as either no activity reported or any amount of activity that did not meet the criteria for moderate or high. Vigorous physical activity was considered as a separate variable. Vigorous physical activity level was calculated according to the IPAQ continuous score protocol (36), by multiplying 8 METs by the number of minutes of vigorous physical activity per day and by the number of vigorous days per week; finally, this variable was categorized into quartiles.

### Measurement of relative leukocyte telomere length

Blood sampling, processing, and aliquoting procedures are described in detail elsewhere (32). Briefly, a whole-blood sample was obtained from each study participant at enrollment and stored at −80°C until DNA extraction. Genomic DNA was isolated from blood according to the manufacturer's instructions, using the 5Prime ArchivePure DNA Blood Kit. Extracted DNA was eluted in Tris-EDTA (TE) buffer. The concentration and purity of each sample was measured with a NanoDrop 2000 UV-Vis spectrophotometer and stored at −20°C until analysis.

rLTL was measured by monochrome multiplex qPCR described by Cawthon (37). This assay measures the relative ratio of telomere repeat copy number (T) to single gene copy number (S) (human betaglobin) in participant samples compared with a reference DNA sample, producing a relative T/S ratio measure. Twenty nanograms of template DNA diluted in 10 μL of molecular grade water (VWR) was added to 15 μL of master mix and prepared as reported by Cawthon (37) for a final reaction volume of 25 μL. Primer oligonucleotides were manufactured by Integrated DNA Technologies per sequence descriptions reported by Cawthon (37). Samples from all individuals were assayed in triplicate on 96-well plates using a BioRad CFX96 Real-Time PCR

Detection System set to the thermal cycling profile specified by Lan and colleagues (38). Five concentrations (3-fold serial dilution: 150, 50, 16.7, 5.5, and 1.9 ng) of a pooled DNA sample were included on each plate, in triplicate, providing data for the generation of standard curves. Standard curve efficiencies ranged from 90% to 110% and were used for relative quantification of rLTL by the Pfaffl method (39). rLTL for each sample was standardized to a reference sample prepared from pooled DNA from a 29-year-old female and 49-year-old male. Statistical tests of reliability were calculated from a one-way analysis of variance model using mean square error as an estimate of within-person standard deviation. The intraclass correlation coefficient for triplicate measures was 0.99, and the intra-assay coefficient of variation was 8.98%. The inter-assay coefficient of variation of 6.39% was calculated on 10 quality control samples run on three different plates. Samples that did not amplify or that generated a signal outside the dynamic range of the standard curve were excluded from analyses. A measure of rLTL could not be obtained for 4.7% of the samples with available DNA; however, these samples did not consistently belong to any specific age group, suggesting that the method did not systematically fail for samples with long or short telomeres.

### Statistical analysis

Multivariable linear regression was used to determine the relation between rLTL and measures of the following factors: alcohol consumption, cigarette smoking, and physical activity. Exposure metrics for smoking and physical activity included daily smoking status, tertiles of cumulative pack-years smoking, total physical activity, and quartiles of vigorous physical activity. Potential covariates included age, sex, ethnicity, body mass index (BMI), and lipid ratio (cholesterol to HDL ratio). A parsimonious model predicting rLTL from among these covariates was selected using backward elimination with a liberal $P$ value of 0.2 and alcohol, cigarette smoking, and physical activity forced in the model. The final parsimonious model included age, sex, and lipid ratio and was used to adjust the analyses of the lifestyle exposures of interest. Participants were recruited at study centers based in three different municipalities; thus, a random-effects parameter was included in the model to account for cluster sampling. A $P$ value for trend across ordered categorical variables was calculated by assigning integer values to categories and assessing the parameter as a continuous variable (40). All statistical tests were two-tailed and conducted in SAS 9.3 (SAS Institute).

## Results

A summary of rLTL distribution and the covariates considered is presented in Table 1. rLTL was approximately normally distributed with a right skew, mean of 0.85, and standard deviation of 0.30. Subjects were ages 20 to 50; the distribution of age was approximately univariate. Age was inversely associated with rLTL; every 10-year increase in age was associated with an rLTL decrease of 0.060 relative T/S ratio units ($P < 0.01$). The study population was 57% female, predominantly Caucasian, and 44% of the study population were overweight or obese. Age-adjusted effects are presented for the covariates considered (sex, ethnicity, BMI, and lipid ratio); none of these covariates were predictive of rLTL in this analysis. Age, sex, and lipid ratio met the inclusion criteria for the parsimonious model and the analysis of the lifestyle exposures of interest controls for this set of covariates.

**Table 1.** Characteristics of the study population and regression coefficients from a multiple linear regression adjusted for age

| Characteristic | *N* (%) | Coefficient ± SE | *P*[a] |
|---|---|---|---|
| Age, y | | | <0.0001 |
| 20–29 | 192 (40) | Ref. | |
| 30–39 | 138 (30) | −0.061 ± 0.032 | |
| 40–50 | 147 (31) | −0.13 ± 0.032[b] | |
| Age (per 10 years) | — | −0.060 ± 0.015 | <0.0001 |
| Sex | | | 0.16 |
| Male | 205 (43) | Ref. | |
| Female | 272 (57) | −0.038 ± 0.027 | |
| Ethnicity | | | 0.35 |
| Caucasian | 409 (86) | Ref. | |
| Asian | 33 (7) | 0.046 ± 0.053 | |
| Other | 35 (7) | −0.054 ± 0.051 | |
| Body mass index (kg/m$^2$) | | | 0.53 |
| Normal | 266 (56) | Ref. | |
| Overweight | 144 (30) | −0.022 ± 0.030 | |
| Obese | 65 (14) | −0.042 ± 0.040 | |
| $P_{trend}$ | | 0.3 | |
| Lipid ratio (mmol/L)[c] | | | 0.19 |
| Q1 | 122 (26) | Ref. | |
| Q2 | 116 (24) | 0.004 ± 0.037 | |
| Q3 | 120 (25) | −0.062 ± 0.037 | |
| Q4 | 119 (25) | 0.012 ± 0.039 | |
| $P_{trend}$ | | 0.82 | |

Abbreviations: SE, standard error; Ref., referent.
[a]Calculated by *F*-statistic from random-effects ANCOVA adjusted for age that included a random-effects parameter representing study center, except for age (per 10 years), which was calculated from simple linear regression.
[b]$P < 0.01$.
[c]Lipid ratio is the cholesterol HDL ratio.

Table 2 contains the results of the multivariable linear regression with age-adjusted and fully adjusted effects presented. This description of results focuses on the fully adjusted models. In multivariable linear regression, alcohol consumption was not related to rLTL ($P = 0.57$). Compared with never daily smoking, current daily smoking was associated with shorter rLTL ($P = 0.02$). Pack-years of smoking was a significant predictor of rLTL ($P < 0.01$) and those with cumulative pack-years of smoking in the lower and middle tertile had shorter rLTL than never smokers. The coefficient estimates for current daily smoking indicate that, on average, rLTL was 0.096 relative T/S ratio units shorter for current daily smokers than never daily smokers. Being in the lower and middle tertiles of pack-years smoking was associated with, on average, 0.089 and 0.14 relative T/S ratio unit shorter rLTL compared with never smokers.

While total physical activity was not a significant predictor in the model, there was evidence of a positive dose–response relationship with rLTL across categories of total physical activity ($P_{trend} = 0.06$; Table 2). Several studies have reported longer telomeres in endurance-trained athletes; thus, we considered vigorous physical activity separately from total physical activity. Vigorous physical activity in the fourth, relative to the first, quartile was associated with longer telomere length ($P < 0.01$). rLTL was 0.12 relative T/S ratio units longer for those in the fourth quartile of vigorous physical activity compared with the first and there was a significant linear trend of increasing rLTL with greater vigorous physical activity ($P = 0.01$).

## Discussion

An association of rLTL with alcohol consumption was not observed in this population of healthy 20 to 50 year old men

**Table 2.** Results from a multivariable linear regression presenting adjusted regression coefficients relating lifestyle exposure variables to rLTL

| | | Age adjusted[a] | | Fully adjusted[b] | |
|---|---|---|---|---|---|
| **Lifestyle exposure** | ***N* (%)** | **Coefficient ± SE** | ***P*[c]** | **Coefficient ± SE** | ***P*[c]** |
| Alcohol consumption | | | 0.66 | | 0.57 |
| Abstainer | 45 (9) | −0.006 ± 0.046 | | −0.014 ± 0.046 | |
| Low | 325 (68) | Ref. | | Ref. | |
| Moderate | 68 (14) | −0.046 ± 0.038 | | −0.055 ± 0.039 | |
| High | 39 (8) | −0.025 ± 0.049 | | −0.024 ± 0.050 | |
| $P_{trend}$ | | 0.39 | | 0.41 | |
| Smoking status | | | 0.01 | | 0.02 |
| Never daily smoker | 325 (68) | Ref. | | Ref. | |
| Former daily smoker | 90 (19) | −0.078 ± 0.035[d] | | −0.065 ± 0.035 | |
| Current daily smoker | 62 (13) | −0.094 ± 0.040[d] | | −0.096 ± 0.041[d] | |
| Pack-years of smoking | | | 0.002 | | 0.003 |
| Never daily smoker | 326 (68) | Ref. | | Ref. | |
| Lower tertile (>0–6.25) | 66 (14) | −0.10 ± 0.038[e] | | −0.089 ± 0.039[d] | |
| Middle tertile (>6.25–16) | 43 (9) | −0.14 ± 0.046[e] | | −0.14 ± 0.048[e] | |
| Higher tertile (>16) | 42 (9) | 0.009 ± 0.048 | | 0.007 ± 0.050 | |
| $P_{trend}$ | | 0.08 | | 0.10 | |
| Total physical activity | | | 0.12 | | 0.27 |
| Low | 42 (9) | Ref. | | Ref. | |
| Moderate | 225 (47) | 0.050 ± 0.048 | | 0.038 ± 0.049 | |
| High | 210 (44) | 0.089 ± 0.048 | | 0.071 ± 0.050 | |
| $P_{trend}$ | | 0.04 | | 0.06 | |
| Vigorous physical activity (MET-min) | | | 0.003 | | 0.005 |
| Q1 (0) | 147 (31) | Ref. | | Ref. | |
| Q2 (>0–<840) | 91 (19) | 0.054 ± 0.038 | | 0.040 ± 0.038 | |
| Q3 (840–<1920) | 134 (28) | 0.013 ± 0.034 | | 0.000068 ± 0.038 | |
| Q4 (≥1920) | 103 (22) | 0.13 ± 0.037[e] | | 0.12 ± 0.038[e] | |
| $P_{trend}$ | | 0.006 | | 0.01 | |

Abbreviations: SE, standard error; Ref., referent.

[a]The age-adjusted model controlled for age and included a random-effects parameter representing study center.

[b]The fully adjusted models controlled for age, sex, and lipid ratio, while also adjusting for the other lifestyle exposures of interest. For example, the model with alcohol consumption adjusts for age, sex, lipid ratio, smoking status, and total physical activity. These models also included a random-effects parameter representing study center.

[c]Overall *P* value computed by *F*-statistic.

[d]$P < 0.05$ in comparison to referent computed by *t* statistic.

[e]$P < 0.01$ in comparison to referent computed by *t* statistic.

and women. However, rLTL was inversely associated with current daily smoking and pack-years smoked. The results are suggestive of a linear trend of longer telomere length with increasing total physical activity, although this was not statistically significant. Vigorous physical activity was positively associated with rLTL.

Oxidative stress and inflammation are associated with shorter telomeres and likely mediate the association between lifestyle exposures and rLTL. Oxidative stress causes damage to DNA, and under a state of high oxidative stress, accumulation of single-strand breaks is the major cause of telomere shortening (15, 41). Telomeres are particularly sensitive to base oxidation by ROS because of the large number of guanine (G) nucleotides in their repeating TTAGGG sequences (41, 42). Environmental and lifestyle factors can act to either promote or mitigate oxidative stress. ROS are generated as a byproduct of alcohol metabolism (24) and cigarette smoke (26, 27); in addition, both alcohol and cigarette smoke can interfere with antioxidative defense (21, 23). Regular physical activity augments the physiological antioxidant response (28, 29). Additionally, physical activity promotes energy expenditure and helps maintain energy balance; thus, being physically active may reduce obesity (43) and the associated levels of oxidative stress and inflammation (44). The extent of ROS damage to telomere DNA depends on a balance between factors that promote oxidative stress and those that augment antioxidative defense.

While moderate alcohol consumption, such as that observed in our study, does not appear to affect telomere length, evidence from other studies suggests that heavy drinking is associated with shorter telomeres. Our findings differed from the results of two other studies with a primary focus on the relationship between alcohol and rLTL. Contrary to our observations, both reported an association between alcohol intake and rLTL: one in a cross-sectional analysis in a group of males with alcohol abuse (45) and the other in longitudinal analysis in a group of older businessmen (46). The range of alcohol consumption levels reported by these studies was well above that seen in our population of healthy volunteers. The reported association of alcohol consumption with rLTL was observed only with alcohol intakes of above 28 drinks/week.

Despite a relatively large sample size ($n = 477$), this study had limited statistical power to investigate the relationship with alcohol consumption due to a low proportion of subjects being classified as having moderate or high consumption. *A priori* the comparison of high ($n = 39$) versus low alcohol consumption ($n = 325$) would have 80% power (at 0.05 significance) to detect a difference of one half of a standard deviation in rLTL. In addition, the fully adjusted model controlled for five other variables, which may further reduce statistical power. However, the sample size of 477 is sufficient to model this number of parameters, and the standard error associated with regression coefficients was similar in age-adjusted and fully adjusted models.

It is also possible that the time of exposure measurement, at healthy midlife, by Strandberg and colleagues (46), represents a more biologically relevant exposure measure for the relationship

between alcohol consumption and rLTL. Alcohol use at this age is more stable than alcohol consumption in young adulthood and is likely more representative of a lifetime cumulative exposure. Cumulative lifetime intake likely has an effect on rLTL, but cumulative exposure is hard to measure and the best cross-sectional representation of this may be a measure of alcohol use at midlife. The measure used in this study may not capture the biologically relevant exposure for all participants, which may explain why no relationship was observed.

Our observation of a relationship of rLTL with current smoking and pack-years of smoking is consistent with the existing literature. Seven other studies have demonstrated an inverse association between smoking and rLTL (47–52). Additionally, one study reported a 3-fold increased yearly telomere attrition rate in smokers compared with non-smokers (47).

Our results corroborate reports of a positive linear trend of increasing rLTL with total physical activity and vigorous physical activity (53, 54). However, some studies have also reported a U-shaped relationship between physical activity and telomere length and found that moderate physical activity was associated with longer telomere length than both inactivity and vigorous physical activity (55, 56). Others have reported longer telomere length in endurance-trained athletes relative to both moderately active non-athletes (57) and sedentary peers (58). In addition to promoting increased antioxidant enzyme activity, greater physical activity may indirectly protect rLTL by moderating the effect of psychological stress (59). Notably, both short- and long-term exercise have been shown to elevate telomerase activity (30, 31, 60).

This study explored the relationship between prevalent and modifiable lifestyle exposures and a biomarker of early effect, which is suspected to play a role in carcinogenesis. Although epidemiologic and mechanistic evidence provide some support for the hypotheses tested, the possibility for spurious results exists and statistically significant findings in this relatively novel area of research merit replication. We used a prior probability of 0.1 in the calculation of the false-positive report probability (FPRP) for the risk factors examined to reflect the moderate support for these relationships in the literature (61, 62). The FPRP was less than 17% for the statistically significant findings observed.

Determinants of telomere length are poorly understood and much of the existing literature is inconsistent. This study contributes to a relatively new area of research with limited existing knowledge. Issues of temporality, typical of most cross-sectional analysis, do not apply here unless we invoke the unlikely possibility that individuals with shorter telomeres are prone to consume more alcohol, smoke more, or exercise less throughout life. The age range of 20 to 50 years is a further strength of this study as telomere length exhibits instability and steeper loss in the very young or very old, while midlife is marked by a gradual loss in telomere length (63). This allowed us to model telomere length during a period of life where telomere loss is gradual, may be better represented by a linear model, and where age may have a smaller influence. Additionally, this study examined the impact of a small number of lifestyle factors on telomere length. Epidemiologic and mechanistic evidence provides support for the hypotheses tested, and therefore the prior probability that these relationships exist would be high (61). This reduces the probability that the significant associations observed in this study are false positives.

Despite these important strengths, our study has several limitations. As this was a cross-sectional analysis, lifestyle factors and telomere length were measured at a single point in time. A one-time measure of smoking, alcohol consumption and physical activity may not capture a cumulative lifetime exposure, which may be the more appropriate exposure window for this question and may also contribute to exposure misclassification. Thus, the inherent imprecision in a single measurement of both lifestyle factors and telomere length may non-differentially influence the observed estimates of association. However, good short-term reliability in measurement of telomere length over a period of months has been demonstrated (64), and longitudinal panel studies have also demonstrated some degree of stability in lifestyle factors measured in adult populations (65–71).

The monochrome multiplex qPCR method for measuring telomere length is commonly used in epidemiologic studies and is scalable for high throughput analysis (72, 73). However, it measures mean leukocyte telomere length in relative T/S ratio units and does not yield absolute values or percentage of short telomeres. Methods that quantify mean telomere length have limited sensitivity for detection of differences in telomere length between cells in a sample cell population, and the ability to detect small changes in telomere length is reduced. Variation may also arise because leukocytes consist of a mixture of cell subsets with different individual telomere lengths; however, there is a strong correlation in telomere length between cell subsets within an individual and inter-individual differences in telomere length are much greater than intra-individual differences between cell subsets (74). Additionally, measurement variation is greater for the qPCR method of measuring rLTL than for the Southern blot method (75); however, while the Southern blot method may be more precise, it is often limited in use as it requires large amounts of DNA and cannot be performed on DNA with even modest degradation (75).

The potential for unmeasured or residual confounding exists as we have insufficient information about other factors that may be associated with healthy aging and may also be related to telomere length. Finally, our study population was 20 to 50 years old and primarily Caucasian; thus, generalizability to other populations may be limited.

Currently, there is a lack of widely applicable reference ranges for telomere length, and it is also unclear the extent of which telomere length is modifiable. Whether changes in behavioral and environmental factors can improve telomere length in a clinically meaningful way, which results in a reduced risk of disease, is currently being investigated. A comparison of regression coefficients obtained in this study for the lifestyle exposures to that observed for age provides some interpretation of the clinical significance of these lifestyle factors in relation to telomere length. The coefficient for vigorous physical activity suggests a 20-year decrease in biologic age for those participating in 60 minutes of vigorous physical activity 4 times a week and the coefficient for current smoking suggests a 16-year increase in biologic age for current smokers compared with never daily smokers.

While telomeres normally shorten over a period of decades, it has been hypothesized that sustained shorter-term increases in telomere length may alter the long-term trajectory of telomere attrition in such a way that decreases risk of age-related diseases (76). This is based on observations of increased lifespan in animal models with upregulated telomerase activity (77) and in human studies improvements in health that coincided with a modest increase in telomerase following behavioral interventions (60, 78). When telomere length was dichotomized at the median,

those with longer telomere length lived an average of 5 years longer than those with shorter telomeres (9). This may be due to a delay in senescence and associated pathologies among those with longer telomeres.

In conclusion, in this study rLTL was associated with vigorous physical activity and smoking, but was not associated with alcohol consumption. These results support the hypothesis that lifestyle factors may influence telomere dynamics and leukocyte telomere shortening. Longitudinal studies that measure telomere length at multiple time points and adjust for important environmental and genetic covariates are needed to confirm these results. To assess the effect of these lifestyle exposures directly on telomere attrition, a prospective study that measures telomere length at multiple time points is required.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** L. Latifovic, W.D. King, T.E. Massey
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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** L. Latifovic, S.D. Peacock, T.E. Massey, W.D. King
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** L. Latifovic, T.E. Massey, W.D. King
**Writing, review, and/or revision of the manuscript:** L. Latifovic, S.D. Peacock, T.E. Massey, W.D. King
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** W.D. King
**Study supervision:** T.E. Massey, W.D. King

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