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RESEARCH PAPER

Telomere length and its relationships with lifestyle and behavioural factors: variations by sex and race/ethnicity

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Abstract

Background: Adherence to healthy lifestyles/behaviours promotes healthy ageing. However, little is known about whether age, sex and/or race/ethnicity moderate associations of lifestyle/behavioural factors with relative telomere length (RTL), a potential biomarker of ageing.
Methods: We included 749 midlife to older non-Hispanic White ($n = 254$), Black ($n = 248$) and Hispanic ($n = 247$) US participants [mean (standard deviation) age = 69.3 (7.2) years; women: 50.5%]. We extracted genomic DNA from peripheral leucocytes. RTL was assayed using real-time quantitative polymerase chain reaction. Multivariable regression was used to examine associations between lifestyle/behavioural exposures (i.e. physical activity, alcohol consumption, smoking and depression) with RTL.
Results: Increasing chronological age was associated with shorter RTL ($P < 0.01$). Higher physical activity was associated with longer RTL (P-trend = 0.03); daily versus never/rare alcohol consumption and 30+ versus <5 smoking pack-year were associated with shorter RTLs (P-trend = 0.02). Associations varied significantly by sex and race/ethnicity. The association between physical activity and longer RTL appeared strongest among non-Hispanic Whites (P-interaction = 0.01). Compared to men, women had stronger associations between heavy smoking and shorter RTLs (P-interaction = 0.03). Light/moderate alcohol consumption (monthly/weekly) was associated with longer RTL among non-Hispanic Whites, while daily consumption was related to shorter RTLs among Blacks and Hispanics (P-interactions < 0.01). Associations of daily alcohol and heavy smoking with shorter RTLs were particularly apparent among Black women.

Conclusion: We observed novel variations by sex and race/ethnicity in associations between lifestyle/behavioural factors and RTL. Further work is needed to replicate these findings and to address potential public health implications for modifying strategies by sex or across racial/ethnic groups to optimise lifestyles/behaviours for healthy ageing.

Keywords: telomere length, biomarker, older people, health disparities, race/ethnicity

Key Points

- Higher physical activity was related to longer telomeres, while higher alcohol and smoking use were related to shorter telomeres.
- Race/ethnicity and sex influence the strength and direction of associations of lifestyle/behavioural factors and telomere length.
- Association of higher physical activity and longer telomeres was stronger among non-Hispanic Whites compared to minority groups.
- Associations of daily alcohol consumption and shorter telomeres were strongest among Black and Hispanic women.
- The association of heavy smoking and shorter telomeres was strongest among Black women.

Introduction

Telomeres, repetitive DNA sequences (TTAGGG) at the end of chromosomes, are essential for genome stability during cell division [1]. Telomere length (TL) is considered a potential biological ageing marker due to its established role in cellular ageing and apoptosis. Inflammation, oxidative stress and/or DNA damage mechanisms may accelerate age-associated telomere shortening. Modifiable lifestyle/behavioural factors may differentially influence such sources of telomere shortening and, thus, affect health and lifespan [2].

Physical activity may have a protective influence on TL by reducing inflammation and oxidative stress [3, 4]. However, associations between physical activity and TL have been mixed, with reports of positive [5–9], inverted 'U' [10, 11] or null associations [12, 13]. Furthermore, few studies evaluated associations between types of physical activity (including low-to-intermediate intensity exercise) and TL [6, 14]. The relation of alcohol to health outcomes appears U−/J-shaped. While moderate alcohol consumption has been associated with lower risk of cardiovascular disease (CVD) and overall mortality, adverse outcomes are observed with heavy alcohol consumption [15, 16]. TL is an emerging quantitative biomarker of health in ageing, and shorter TL is linked to increased mortality [17, 18]. Thus, it can be hypothesised that light/moderate alcohol consumption may be associated with longer TL, while heavy alcohol consumption may be related to shorter TL. However, studies on relations of alcohol to TL have been inconclusive [19–21]. Smoking may be related to cellular damage and, thus, telomere shortening [22]. Some, [23–25] but not all [26, 27] studies have found that higher smoking is associated with shorter telomeres. Finally, evidence suggests that poor behavioural health, including depression, is associated with telomere shortening [28, 29].

Little is known about whether associations of lifestyle/behavioural factors with TL differ by sex and/or race/ethnicity. Evidence suggests that Blacks and Hispanics compared to non-Hispanic Whites experience accelerated age-associated telomere shortening [30]. Furthermore, minority versus non-Hispanic Whites may have higher prevalence of unhealthy lifestyle/behavioural factors (e.g. physical inactivity, higher depression burden) that may influence TL [31, 32]. Potential variations in risk factor-biological ageing associations may provide an important explanatory link in understanding racial/ethnic disparities [33].

We hypothesised that unhealthy lifestyle/behavioural factors (i.e. physical inactivity, heavy alcohol and smoking use and depression) would be associated with shorter telomeres, and that light/moderate alcohol consumption would be associated with longer telomeres compared to either no or heavy consumption, among ∼800 midlife and older adults. Further, we addressed potential variations in associations by age, sex and/or race/ethnicity.

Methods

Study participants

Participants were from VITAL-DEP (VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention, NCT01696435) [34], a late-life depression prevention ancillary study to the VITAL (NCT01169259) trial [35, 36]. VITAL consists of 25,871 men and women, aged 50+ and 55+ years, respectively, in a 2 × 2 factorial randomised trial of cancer and CVD prevention using vitamin D3 and/or marine omega-3 supplementation; thus, all participants were free of heart disease or cancer at baseline. Additional VITAL-DEP eligibility criteria are provided elsewhere (Appendix 1). VITAL included 70% non-Hispanic white participants and 30% were members of racial/ethnic minority groups (i.e. Black, Hispanic, Asian and other, multiple or unspecified), as described elsewhere [35, 36].

Sample selection

In VITAL, Black and Hispanic adults were younger at baseline, reflecting earlier onset of CVD morbidity and

mortality in these groups [37–39]. Thus, we used age-stratified sampling to select 800 VITAL-DEP participants with comparable age distributions, by 10-year age groups between 50 and 100, across racial/ethnic groups. To increase power to address racial/ethnic differences, participants were randomly selected within age strata so that one-third of the sample was non-Hispanic White, one-third was Black, and one-third was Hispanic; sex was balanced across groups. Participants with ≥1 microgram of genomic DNA were included. We excluded participants with missing relative telomere length (RTL) data ($n = 8$) or self-reported information on lifestyle/behavioural factors ($n = 43$) (Appendix 1). Excluded ($n = 51$) versus included ($n = 749$) participants were similar with regard to key characteristics: e.g. mean RTL (0.4 in both groups) and mean body mass index (BMI) (28 kg/m^2 in both groups).

Assessment of physical activity

Physical activity was ascertained using a validated questionnaire method [40]. Participants reported average hours/week engaged in specific recreational activities and daily number of climbed stairs. Each activity was assigned a metabolic equivalent task (MET) value [41]. Total *amount* of physical activity was calculated by summing MET-hours/week from all recreational activities and climbing stairs. Based on its distribution, MET-hours/week was categorised into: 0–4.99, 5–29.99, and 30+ MET-hours/week. *Frequency* of physical activity was categorised into <1, 1 to <3.5, 3.5 to <7 and 7+ hours/week. *Types* of physical activity included low-to-intermediate (walking, weightlifting, yoga/stretching/toning, bicycling) and vigorous intensity (running, jogging, lap swimming, tennis, intense aerobics) activities. Regarding validity of self-reported physical activity in our sample, we observed an inverse correlation between BMI and physical activity (Spearman rho (ρ) = −0.28, $P < 0.001$).

Assessment of alcohol and smoking consumption

Alcohol consumption and cigarette smoking were ascertained via questionnaires. Alcohol consumption was categorised by frequency: rare/never, monthly, weekly and daily. Cumulative smoking exposure was summarised as pack-years and categorised into three groups: 0–4.99, 5–29.99 and 30+ pack-years; smoking was also categorised as never, past, or current.

Assessment and measures of depression

In VITAL-DEP, depression status was characterised by presence of current symptoms, diagnosis and/or treatment of depression [34]. Current depressive symptoms were ascertained via annual questionnaires using the Patient Health Questionnaire-8 (PHQ-8), which has clinical validity for identifying depression [42,43] and is well-validated among diverse samples of older adults [44, 45]. Participants also reported on past history of depression.

Ascertainment of covariates

Demographic characteristics included age (years), sex (men/women), race/ethnicity (non-Hispanic White, Black, Hispanic), education (<high school, high school diploma, attended/graduated from college, post-graduate) and income (<$15,000, $15,000–49,999, $50,000–89,999, $90,000–120,000, >$120,000). Lifestyle and health factors included BMI (18.5–24.99, 25–29.99, ≥30 kg/m^2) and self-reported history of hypertension, diabetes, current use of cholesterol-lowering medications and multivitamin use.

RTL assay

Details on RTL assay methods and quality control (QC) procedures are provided elsewhere [46]. Briefly, genomic DNA was extracted from peripheral blood leukocytes and RTL was measured using quantitative real-time polymerase chain reaction. Average RTL was calculated as the exponentiated T/S ratio, defined as a Telomere repeat copy number to Single gene (36B4) copy number (T/S) corrected for a reference sample [47]. QC samples were interspersed across plates along with participants' samples and were run in triplicates to assess inter- and intra-assay reliability; the average coefficient of variation (CV) for the exponentiated T/S ratio was 6.6%.

Statistical analysis

Participants' characteristics were compared across racial/ethnic groups. Further, we evaluated the relationships of age, sex and race/ethnicity with RTL. We used multivariable linear regression analyses to estimate associations of physical activity, smoking, alcohol consumption and depression measures with RTL. Total physical activity was included as either a continuous (standardised z-score) or categorical variable. We computed mean-centred PHQ-8 score, categorised history of depression as a binary variable (yes/no), and used an interaction term to estimate combined effects of current depressive symptoms (PHQ-8) and history of depression; main effects were interpreted when the indicator term for the interaction was zero. Trend tests were used to assess linear relationships between lifestyle/behavioural exposures and RTL, and we used the linear step-up method of Benjamini and Hochberg for multiple hypothesis testing [48]. Separately, we ran multiplicative interaction tests to assess effect modification by age, sex and race/ethnicity of the primary associations, and also conducted stratified analyses.

For all analyses, education and income were coded as binary variables (<post-graduate versus post-graduate; <$50,000 versus ≥$50,000); BMI < 18.5 kg/m^2 was considered missing. Regression models were initially adjusted for age and sex (model 1), and additionally adjusted for race/ethnicity, education, income, BMI, physical activity, alcohol, smoking, and depression measures, including main and interaction effects of current depressive symptoms and past-history of depression (model 2). A final model further adjusted for health-related variables as described above (model 3). Mean differences in RTLs, with 95% confidence

intervals (CI), were calculated. Because smoking may reduce exercise capacity and affect participation in physical activity, [49] we explored in a sensitivity analysis whether associations between physical activity and RTL were stronger among participants with never-smoking or past-smoking rather than current-smoking status.

All analyses were performed using SAS version 9.4 (SAS, Cary, NC) and R 3.2.2. Two-tailed $P < 0.05$ was considered statistically significant. False discovery rate (FDR) is the expected proportion of Type-I errors (false positives) in null hypothesis testing when performing multiple comparisons; FDR < 0.05 was the significance cut-off used for multiple-hypothesis testing. The study was approved by the Institutional Review Board at Partners HealthCare-Brigham and Women's Hospital.

Results

Table 1 shows participant characteristics by racial/ethnic group. Black participants had lower physical activity levels than other groups. Minorities were less likely to consume daily alcohol or to have a heavy smoking history, compared to non-Hispanic Whites. There were no differences by race/ethnicity in current depressive symptoms (measured as PHQ-8 score). Compared to non-Hispanic Whites, minority adults had higher BMI and medical comorbidity, and lower income and post-graduate education levels. RTL was inversely correlated with age ($\rho = -0.12$, $P < 0.01$). After multivariable adjustment, age was significantly associated with shorter RTL ($\beta = -0.02$, $P < 0.01$), but there were no significant associations of sex or race/ethnicity with RTL. Approximately 25% of participants reported 30+ MET-hours/week (men reported higher physical activity than women [median (IQR): 18.4 (6.1–38.7) versus 12.7 (3.0–27.9)]).

Table 2 shows results for associations between physical activity and RTL. There was a significant positive association between total amount of physical activity and RTL (P-trend = 0.03); compared to those with low physical activity (<5 MET-hours/week), those with high physical activity (30+ MET-hours/week) had significantly longer telomeres. When we examined physical activity-RTL associations using frequency of physical activity, regardless of activity type, there was a non-significant trend of higher physical activity frequency with longer RTLs (P-trend = 0.07). Vigorous, but not low-to-intermediate, intensity physical activity appeared to have a positive association with RTL (P-trend = 0.05). The physical activity-RTL association varied by race/ethnicity: estimates in favour of a positive association between higher total amount of physical activity and longer RTL were observed among non-Hispanic Whites but not Hispanics (P-interaction = 0.005) (Figure 1). There were no interactions of physical activity with age or sex.

Associations of alcohol consumption, smoking and depression with RTL are presented in Table 3. Overall, higher alcohol consumption and smoking levels were related to shorter telomeres (P-trends = 0.02). Alcohol-RTL associations varied by race/ethnicity (Figure 1). Although light/moderate alcohol consumption (monthly/weekly) was positively related to RTL among non-Hispanic Whites, weekly or daily alcohol consumption was associated with shorter RTLs among Blacks and Hispanics; daily alcohol-shorter RTL associations were especially apparent among Black and Hispanic women (Figure 2).

Heavy smoking (30+ pack-years), compared to no/minimal smoking (<5 pack-years), was related to shorter RTLs (adjusted mean difference (95% CI) = −0.04 (−0.08, −0.01)). Associations varied by sex and race/ethnicity (Figure 1). Estimates for the association of heavy versus no/minimal smoking with RTLs were stronger among women than men (P-interaction = 0.03) and among non-Hispanic White and Black compared to Hispanic adults (P-interaction = 0.03) (Figure 1). When stratifying by sex and race/ethnicity (Figure 2), this association was particularly strong among Black women (adjusted mean difference (95% CI) = −0.16 (−0.27, −0.05)).

There were no associations of current depressive symptoms or past depression history with RTLs. Finally, in a sensitivity analysis, we observed positive associations between amount of physical activity and RTL among those with never or past smoking, but not current smoking (data not shown).

Discussion

In a large, racially/ethnically diverse sample of midlife and older adults, we observed significant associations of lifestyle/behavioural factors with RTL, and variations in several associations by sex and/or race/ethnicity. Specifically, we observed that higher physical activity was associated with longer RTL; this association was strongest among non-Hispanic Whites. Light/moderate alcohol consumption (monthly/weekly) was related to longer RTL among non-Hispanic Whites, whereas daily alcohol consumption was related to shorter RTL among Blacks and Hispanics, especially women. Heavy smoking was related to shorter RTL, and estimates were stronger among women, especially Black women.

Our results are consistent with prior literature demonstrating positive associations between physical activity and TL [5–9]. Our study provides more granular data on relations of total amount, frequency and type (intensity level) of physical activity to RTL, as well as potential variations by race/ethnicity. Results suggest that the potential benefits of exercise with respect to biological ageing may not be uniform across racial/ethnic groups. Furthermore, an exploratory analysis suggested positive associations between physical activity and RTL among those who never smoked or quit smoking, but not among those with current smoking; this may have clinical implications regarding smoking cessation as a proactive approach to optimise benefits of physical activity on healthy ageing.

As with PA, our results for smoking are consistent with hypotheses and prior findings. In a meta-analysis of 30

Table 1. Baseline characteristics of study sample by race/ethnicity groups*

Characteristic	Full sample[a] ($n = 749$)	Non-Hispanic White ($n = 254$)	Black ($n = 248$)	Hispanic ($n = 247$)
Age in years, mean (SD)	69.3 (7.2)	69.9 (7.0)	68.6 (7.6)	69.5 (6.8)
Women, %	49.0	53.2	52.0	41.7
Post-graduate education, %	42.1	53.0	38.7	34.4
Income ≥50,000 per year, %	62.8	74.8	54.7	58.5
BMI in kg/m^2, mean (SD)	28.2 (5.4)	26.7 (4.4)	29.6 (6.2)	28.3 (5.3)
Physical activity, MET-hours/week, median (IQR)[b]	15.7 (4.4–31.8)	17.9 (6.5–31.5)	10.9 (3.1–27.7)	18.1 (4.0–40.6)
Frequency of physical activity[c], hours/week, %				
<1	25.0	18.5	30.7	25.9
1 to <3.5	22.8	25.6	25.8	17.0
3.5 to <7	22.0	28.4	16.5	21.1
7+	30.2	27.6	27.0	36.0
Low-intermediate intensity physical activity[d] in hours/week, %				
< 0.75	24.6	17.7	29.4	26.7
0.75 to <2	17.5	19.3	22.2	10.9
2 to <5	20.7	23.2	16.5	22.3
5+	37.3	39.8	31.9	40.1
Vigorous intensity physical activity[e] in hours/week, %				
<0.5	62.4	62.6	63.7	60.7
0.5 to <1.5	14.4	13.8	15.3	14.2
1.5 to <3	13.9	15.0	14.5	12.2
3+	9.4	8.7	6.5	13.0
Cigarette smoking, in smoking pack-years,[f] %				
Never/minimal cigarette smokers (0.00–4.99)	68.5	69.3	63.7	72.5
Moderate cigarette smokers (5.00–29.99)	23.8	22.4	28.2	20.7
Heavy cigarette smokers (30+)	7.7	8.3	8.1	6.9
Alcohol consumption, %				
Rare/never	33.1	22.8	43.6	33.2
Monthly	8.3	5.1	10.5	9.3
Weekly	37.8	43.7	34.7	34.4
Daily	21.0	28.4	11.3	23.1
Multivitamin use, %	43.0	45.7	40.8	42.5
Hypertension, %	55.3	45.8	70.2	50.0
Diabetes, %	18.8	12.6	23.8	20.2
High cholesterol, %	38.4	39.7	35.8	39.8
PHQ-8 score, median (IQR)	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.0 (0.0–1.0)
Past, but not current, history of diagnosed depression, %	8.3	8.7	8.5	7.7
Relative telomere length (T/S ratio), mean (SD)	0.42 (0.13)	0.41 (0.12)	0.44 (0.14)	0.42 (0.13)

Abbreviation: SD, standard deviation; IQR, interquartile range; T/S: exponentiated telomere/single gene ratio *Figures for percentages may not add to 100.0 due to rounding. [a]For normally distributed continuous variables, this column contains mean (standard deviation) for non-missing responses. For non-normally distributed continuous variables, this column contains median (interquartile range) and percentages for categorical variables. [b]Total physical activity was based on MET-hours per week in the leisure time recreational activities: walking, jogging, running, bicycling, intense aerobic exercise, lower intensity exercise: yoga/stretching/toning, tennis, lap swimming, weight lifting, other exercise and reported number of flights of stairs climbed daily. [c]Frequency of physical activity includes the amount of all recreational activities per week (hours/week). [d]Low to intermediate intensity physical activity includes walking, weightlifting, yoga, stretching, toning, bicycling. [e]Vigorous intensity physical activity includes running, jogging, lap swimming, tennis, intense aerobics. [f]Cigarette smoking was measured as smoking pack-years [(average cigarettes per day/20)*total years smoked]. Never smokers were considered as having zero smoking pack-years.

studies, Astuti et al. [25] observed shorter telomeres among those with current versus no smoking (standardised mean difference (95% CI) = −0.11 (−0.16, −0.07)); the analyses also suggested a dose–response association between smoking pack-years and shorter RTL. Our finding of variation by sex in the smoking-RTL association is also consistent with prior data: in a large cross-sectional study of 5,624 older adults, the association between current smoking and shorter telomeres was stronger among women than men [50]. Our novel report of further variation by race/ethnicity in inverse smoking-telomere associations, especially among Black women, suggests biological paths by which smoking may contribute to health disparities affecting women and minorities.

Reported alcohol-RTL associations have been less consistent than those observed for physical activity and smoking. We observed a significant trend of higher alcohol consumption and shorter RTL, but racial/ethnic variation was noted: light/moderate alcohol consumption was associated with longer RTL among non-Hispanic Whites, but daily alcohol consumption appeared inversely related to RTL among Blacks and Hispanics. Regarding biological mechanisms, moderate alcohol consumption exerts beneficial health effects through reduction of inflammation—which could protect telomeres against shortening [51–53]. Although biological mechanisms underlying daily alcohol-shorter RTL associations have not been well established, some

Table 2. Association of physical activity measures with relative telomere length (exponentiated telomere/single gene ratio)

Exposures	Model 1[a]	Model 2[b]	Model 3[c]	*P*-trend[d]
Continuous, total physical activity, Per SD MET-hours/week	0.01 (−0.00, 0.02)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.03*
Categories, total physical activity, MET-hours/week				
<5, *n* = *208*	Ref	Ref	Ref	
5 to 29.99, *n* = *343*	−0.01 (−0.03, 0.01)	−0.00 (−0.02, 0.02)	−0.00 (−0.02, 0.02)	
30 or over, *n* = *198*	0.01 (−0.01, 0.04)	0.03 (0.00, 0.06)	0.03 (0.00, 0.06)	0.03*
Categories, frequency of physical activity[a], hours/week				
<1, *n* = *187*	Ref	Ref	Ref	
1 to 3.49, *n* = *171*	−0.01 (−0.04, 0.01)	−0.01 (−0.03, 0.02)	−0.01 (−0.04, 0.02)	
3.50 to 6.99, *n* = *165*	−0.01 (−0.04, 0.01)	−0.00 (−0.03, 0.02)	−0.00 (−0.03, 0.02)	
7 or over, *n* = *226*	0.01 (−0.02, 0.03)	0.02 (−0.01, 0.05)	0.02 (−0.00, 0.05)	0.07
Low to intermediate intensity physical activity, hours/week				
<0.75, *n* = *184*	Ref	Ref	Ref	
0.75 to 1.99, *n* = *131*	−0.00 (−0.03, 0.02)	0.00 (−0.03, 0.03)	−0.00 (−0.03, 0.03)	
2 to 4.99, *n* = *155*	−0.00 (−0.03, 0.02)	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.03)	
5 or over, *n* = *279*	0.01 (−0.02, 0.03)	0.02 (−0.01, 0.04)	0.02 (−0.01, 0.04)	0.13
Vigorous intensity physical activity, hours/week				
<0.5, *n* = *467*	Ref	Ref	Ref	
0.50 to 1.49, *n* = *108*	−0.01 (−0.03, 0.02)	−0.00 (−0.03, 0.03)	0.00 (−0.03, 0.03)	
1.50 to 4.99, *n* = *104*	−0.00 (−0.03, 0.03)	0.01 (−0.02, 0.03)	0.01 (−0.02, 0.03)	
5 or over, *n* = *70*	0.03 (−0.00, 0.06)	0.03 (0.00, 0.07)	0.04 (0.01, 0.07)	0.05

Note: Relationship of continuous total physical activity and RTL varied by race/ethnicity: *P*-interaction for Hispanic ethnicity = 0.005. *Estimates remained significant after adjusting for multiple testing FDR < 0.05 for main exposures. All definitions for measures of physical activity are provided in the footnotes of Table 1 (no. b–f). [a]Model 1 was adjusted for age (years) and sex. [b]Model 2 was adjusted for demographic and lifestyle/behavioral factors. [c]Model 3 was adjusted for demographic, lifestyle/behavioural and health-related factors. [d]*P*-trend across exposure categories was calculated based on Model 3.

Table 3. Associations of alcohol, smoking consumption and depression variables with relative telomere length (exponentiated telomere/single gene ratio)

Exposures	Model 1[c]	Model 2[d]	Model 3[e]	P Trend[f]
Alcohol consumption[a]				
Rare/never, *n* = *248*	Ref	Ref	Ref	
Monthly, *n* = *62*	−0.00 (−0.04, 0.03)	0.00 (−0.03, 0.04)	0.00 (−0.03, 0.04)	
Weekly, *n* = *282*	−0.02 (−0.05, −0.00)	−0.02 (−0.04, 0.00)	−0.02 (−0.04, 0.00)	
Daily, *n* = *157*	−0.03 (−0.06, −0.01)	−0.03 (−0.06, −0.00)	−0.03 (−0.06, −0.00)	0.02*
Cigarette smoking pack-years[b]				
0 to <5, *n* = *513*	Ref	Ref	Ref	
5 to <30, *n* = *178*	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)	−0.01 (−0.04, 0.01)	
30+, *n* = *58*	−0.04 (−0.07,-0.00)	−0.04 (−0.08, −0.01)	−0.04 (−0.08, −0.01)	0.02*
PHQ-8 Score	0.00 (−0.00, 0.01)	0.00 (−0.00, 0.01)	0.00 (−0.00, 0.01)	0.50
Past but not current, history of diagnosed depression				
No, *n* = *687*	Ref	Ref	Ref	
Yes, *n* = *62*	−0.01 (−0.04, 0.02)	−0.00 (−0.04, 0.03)	−0.00 (−0.04, 0.03)	0.81

*Estimates remained significant after adjusting for multiple testing FDR < 0.05 for main exposures. [a]Relationship of alcohol consumption and RTL varied by race/ethnicity. *P*-interactions for race/ethnicity were as follows: *P*-interaction for Black race and monthly and weekly alcohol consumption = 0.001 and 0.003; *P*-interaction for Hispanic race and weekly and daily alcohol consumption = 0.005 and 0.02. [b]Relationship of cigarette smoking pack-years and RTL varied by sex and Hispanic race/ethnicity: *P*-interaction for sex (for both smoking pack-years category: 0.03; *P*-interaction for Hispanic ethnicity for heavy smoking use (30+ smoking pack-years) = 0.03. [c]Model 1 was adjusted for age (years) and sex. [d]Model 2 was adjusted for demographic and lifestyle/behavioural factors. [e]Model 3 was adjusted for demographic, lifestyle/behavioural and health-related factors. [f]P Trend across exposure categories was calculated based on Model 3.

evidence suggests that daily alcohol consumption may induce cascades of cellular dysfunction that potentiate oxidative stress, which could shorten telomeres [54, 55]. Further, Blacks and Hispanics may experience more rapid age-related telomere shortening than non-Hispanic whites [30]; thus, potential negative consequences for TL of alcohol consumption may be greater among minorities compared to non-Hispanic Whites. However, we cannot exclude the possibility of unmeasured confounding: light/-moderate alcohol consumption may be correlated with social/behavioural patterns (e.g. social engagement that may promote longer TL) [56] and such correlations could be stronger among non-Hispanic whites than minorities. Finally, studies identified that genomic determinants of TL may vary by race/ethnicity [57]; thus, genetic variations may explain racial/ethnic differences in alcohol-RTL associations.

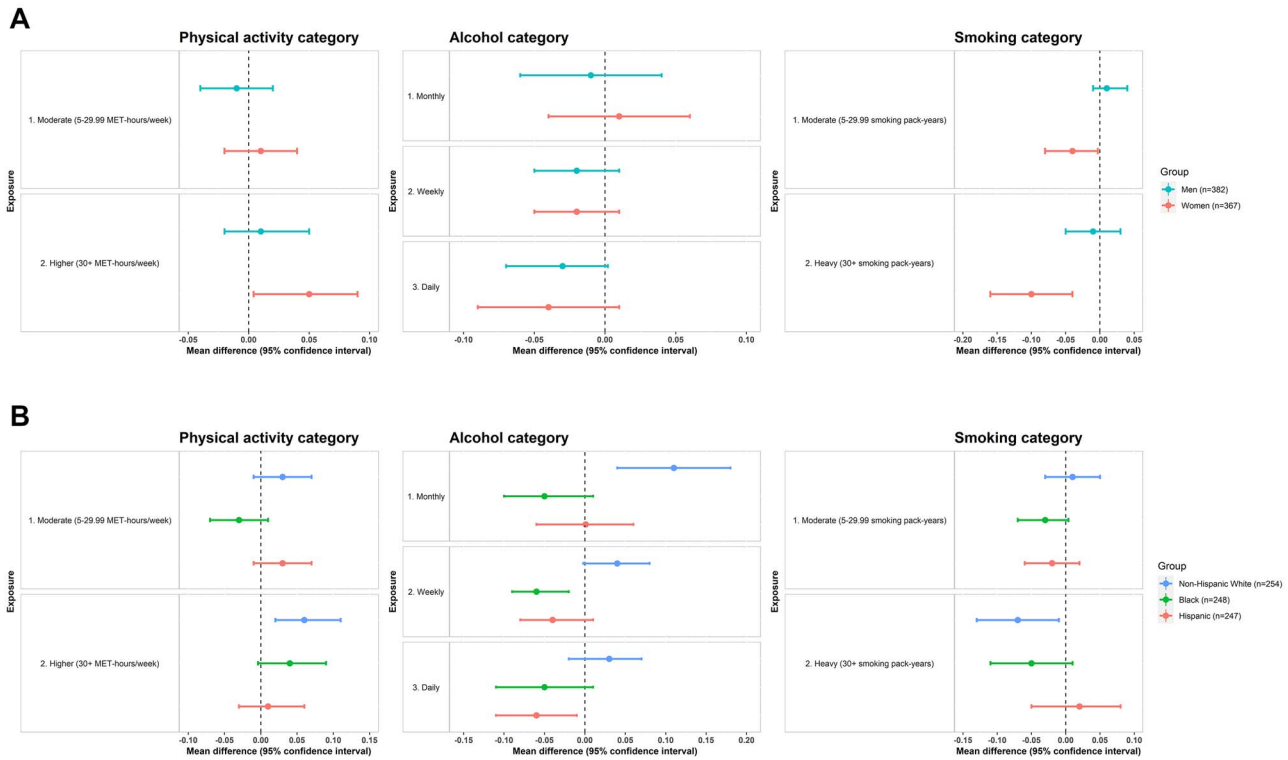

Figure 1. **A.** Associations of lifestyle and behavioural factors with relative telomere length (exponentiated telomere/single gene ratio), stratified by sex Figure 1B**.** Associations of lifestyle/behavioural factors with relative telomere length (exponentiated telomere/single gene ratio), stratified by race/ethnicity. Note: There were no statistically significant results for the associations of current depressive symptoms or past, but not current, history of diagnosed depression with RTL. Thus, stratified results for these variables are not illustrated in Figure 1A and B. For physical activity categories: Low physical activity (< 5 MET-hours/week) was the reference category. For alcohol consumption categories: Rare/never was the reference category. For Smoking categories: Never/minimal smoking (0–4.99 smoking pack-years) was the reference category. In Figure 1A and B, multivariable regression models were used to compute mean differences and CIs among moderate and higher versus lowest (reference) categories of selected lifestyle/behaviours exposures (i.e. physical activity, alcohol consumption and cigarette smoking). Models were adjusted for demographic, lifestyle/behavioural and health-related factors.

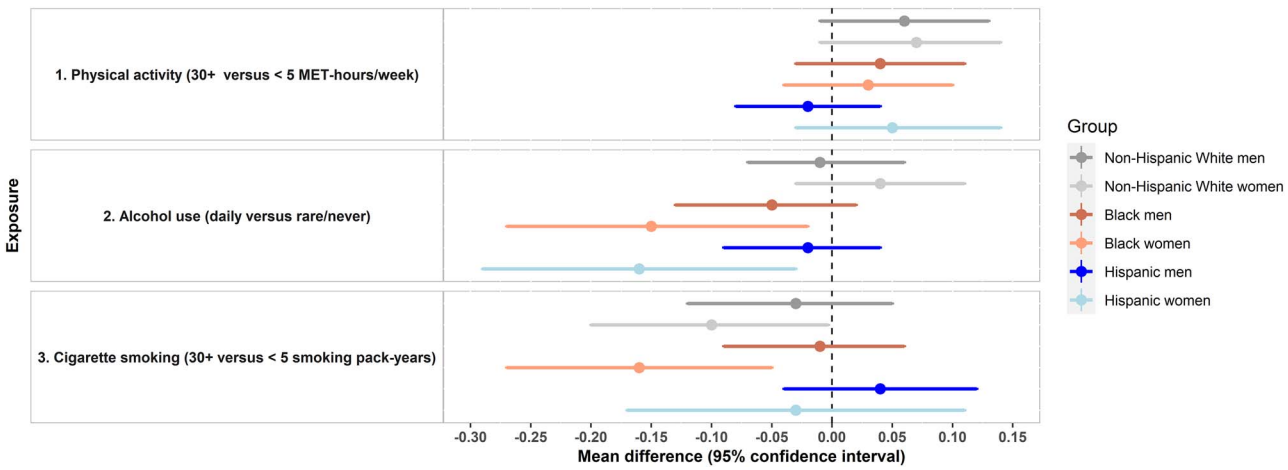

Figure 2. Associations of lifestyle/behavioural factors with relative telomere length (exponentiated telomere/single gene ratio): summary of variations by sex and racial/ethnic groups. Multivariable regression models were used to compute mean difference and Cis of RTL among highest versus lowest categories of selected lifestyle/behavioural exposures (i.e. physical activity, alcohol consumption, cigarette smoking). Models were adjusted for demographic, lifestyle/behavioural and health-related factors.

Therefore, our study findings highlight the need for future work to investigate the biological pathways that may underlie potential racial/ethnic variation in alcohol consumption-biological ageing associations.

Finally, prior evidence suggests that clinical-level psychiatric symptoms or disorders may be related to biological ageing [29, 46, 58]. Prior findings regarding depression and TL have been compelling, but not uniform, in the existing literature [59, 60]. We observed suggestive but non-significant associations between past depression and shorter RTLs, but no association between current depressive symptoms and RTLs. As VITAL-DEP was a depression prevention study, the sample did not include participants with prevalent depression or clinically significant depressive symptoms. Thus, the distribution of depressive symptoms was restricted to the mild range—in contrast with prior studies that included patient/clinical samples with broader ranges of depression severity [28, 29].

Strengths of this include its diverse, well-characterised sample and examination of sex and racial/ethnic differences in lifestyle/behaviour-telomere associations. We also acknowledge limitations. First, the study was cross-sectional; longitudinal studies are necessary to examine associations between lifestyle/behavioural factors and prospective change in RTL. Second, misclassification of self-reported exposures is a potential concern: random/non-differential misclassification could bias estimates towards the null, and differential item functioning (DIF) in self-reported measures (e.g. DIF by race/ethnicity) could bias estimates in either direction. However, our use of established, well-validated approaches to ascertain lifestyle/behavioural factors mitigates potential misclassification. Third, we did not measure social variables (e.g. social engagement) which may be correlated with alcohol consumption and might have influenced observed alcohol-RTL associations and variations by race/ethnicity; results should be interpreted with caution; additionally, as we did not collect information on binge drinking, it may underestimate daily alcohol-shorter RTL associations. Fourth, the sample size was relatively small to address additional variation in primary associations by sex and race/ethnicity; caution is needed regarding potential for chance findings.

In summary, we found significant associations between physical activity, alcohol consumption and cigarette smoking with TL, and novel sex and racial/ethnic variations in these associations. If confirmed, these results may have implications regarding approaches to optimise modifiable behaviours to reduce health disparities in ageing.

Supplementary Data: Supplementary data mentioned in the text are available to subscribers in *Age and Ageing* online.

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