No Associations Between Telomere Length and Age-Sensitive Indicators of Physical Function in Mid and Later Life

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Telomere length, which declines with age, has been hypothesized to act as an indicator of biological aging. If it fulfills this purpose, shorter telomere length should correlate with age-related loss of physical function, independent of age. In this cross-sectional Australian population study, the associations between peripheral blood leukocyte telomere length and age-sensitive indicators of physical function (lung function, blood pressure, and grip strength) were examined in two narrow age range cohorts aged 44–49 years (n = 351) and 64–70 years (n = 295). Telomere length was correlated with systolic blood pressure but only for women of the younger cohort and in the opposite direction to that expected (partial \( r = .181, p = .017 \)). This evidence does not provide support for the hypothesis that telomere length is related to age-associated changes in physical function.

Key Words: Biological aging—Biomarker—Physical function—Telomere length.

Received October 27, 2009; Accepted March 24, 2010

Decision Editor: Rafael de Cabo, PhD

INTRODUCTION

The use of chronological age to indicate an individual’s current state of functioning and health is a relatively imprecise measure due to the intra- and interindividual variability in the rates of age-related decline (1–3). Efforts continue to identify biomarkers of aging that may be used to provide more information regarding an individual’s biological health or functional capacity (biological age) than chronological age alone (4,5). Telomere length has been proposed as a biomarker of aging (6–8).

Telomeres are eukaryotic nucleoprotein structures located at the ends of chromosomes that act as protective caps contributing to genomic integrity and stability (9,10). Human telomeres shorten with increasing age (11,12) and in response to sporadic events, such as increased oxidative stress (13,14). Critically shortened telomeres may lead to the loss of the telomere cap structure and trigger telomere dysfunction, chromosomal abnormalities, and replicative senescence (10,15–17), which may contribute to organismal aging and age-related disease (18). Shortened telomere length in humans has been associated with (i) poorer performance on age-sensitive measures, such as cognitive performance (19); (ii) the presence of age-related conditions, such as insulin resistance (20), and age-related diseases, such as stroke and myocardial infarction (21); and (iii) a higher mortality rate in several population studies (22,23). These data suggest that telomere length is implicated in the biological and functional changes observed with aging.

If telomere length indicates biological age, it should correlate with “normal” aging processes such as the changes observed in physical function (5,6), including the decrease in muscle strength (24,25) and lung function and the increase in systolic blood pressure (5,26,27). Unbiased assessment of the relationship between telomere length and physical function parameters requires the study of representative population samples. The few population studies that have examined these relationships to date have failed to demonstrate significant relationships between the telomere length and the markers of physical function, lung function, and handgrip strength (28,29). Cardiovascular measures, such as pulse pressure, have been inversely associated with telomere length but the results are inconsistent (21,30–32).

These studies have used small samples consisting only of older individuals. The relationships between telomere length and age-sensitive measures may vary over the lifetime reflecting fluctuations in the rate of change of both (11,33), as well as the confounding effects of genetic, environmental, and stochastic factors. More investigations using community-based samples at different stages of the life span are required to detect possible age-specific effects. These may be
difficult to detect in studies employing age-heterogeneous samples and are more appropriately investigated using sequential narrow age range cohorts (34). In this cross-sectional Australian population study, we assess the relationships between telomere length and measures of physical function in two sequential narrow age range cohorts of middle-aged and older adults. We test the hypothesis that telomere length is inversely correlated with performance on age-sensitive measures of physical function.

**Materials and Methods**

**Participants**

The sample was drawn from a larger community-based longitudinal Australian study, the Personality and Total Health (PATH) Through Life Project, which examines the health and well-being of participants who were aged 20–24, 40–44 (40+), and 60–64 (60+) years at the first wave of data collection. Approval for this study was obtained from the Human Research Ethics Committee of the Australian National University, and informed consent was provided by all participants. The details of the recruitment of PATH Study participants are described elsewhere (35). Briefly, the sample was recruited by sampling from the compulsory electoral roll, and most interviews were carried out in the person’s home. Self-report data were collected by computer, and physical tests were administered by a trained interviewer. Cross-sectional results presented here are from Wave 2 data, collected 4 years later, and the participants are a subsample drawn from the PATH 40+ and 60+ Cohorts. The telomere substudy comprised participants who donated a blood sample for genetic analyses at Wave 2. Although a variety of measures were collected in the PATH Study, this article focuses on the relationship between physical function and telomere length.

**40+ Telomere Cohort**

At Wave 2, more than 25% of the PATH 40+ Cohort (n = 656) were invited to participate in a brain imaging substudy, which involved undergoing a brain scan and providing a blood sample. Of the 503 participants who expressed an interest in participating, 431 underwent a brain magnetic resonance imaging scan of which 372 donated a blood sample for DNA extraction. Leukocyte telomere length was successfully estimated in 351 individuals with a mean age of 46.8 years (SD = 1.4). Participants in the 40+ Telomere Cohort were slightly older and more likely to report that they were either employed part time or unemployed than the remainder of the PATH 40+ Cohort but did not differ in terms of sex and marital status, years of education, Symbol Digit Modalities Test (36) or depression scale scores (37).

**60+ Telomere Cohort**

The 60+ substudy was begun at Wave 1 when all PATH 60+ Cohort participants were invited to participate in a brain imaging substudy of which 479 participated. At Wave 2 data collection, 297 participants also provided a blood sample before the commencement of this study for DNA extraction. Leukocyte telomere length was successfully estimated in 297 individuals; two participants were identified as extreme outliers using z scores (>3.29 or <-3.29) (38) and were excluded from further analyses. The 60+ Telomere Cohort had a mean age of 66.7 years (SD = 1.4). Participants in the 60+ Telomere Cohort performed better on the Symbol Digit Modalities Test and were more likely to report that they were unemployed or worked part time than the rest of the 60+ PATH Cohort. However, these two groups did not differ in terms of age, sex, years of education, marital status, the Mini-Mental State Examination (39), or depression scale scores.

**Telomere Length**

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA 96 DNA Blood Kit (#51162; Qiagen, GmbH, Hilden, Germany). Control samples consisted of human saliva and cell line genomic DNA (Jurkat, 293s, HeLa; low- and high–molecular weight telomere genomic DNA from the TeloTAGGG Telomere Length Assay Kit; Roche, Basel, Switzerland). DNA from saliva samples was extracted using an Oragene DNA Self-Collection Kit (DNA Genotek, Ottawa, Ontario, Canada), and cell line DNA was extracted using a QIAamp DNA Blood Mini Kit (#51106; Qiagen).

Telomere length was estimated in all samples by quantitative real-time polymerase chain reaction (Q-PCR) as described by Cawthon (40), with modifications as described below. Briefly, two Q-PCRs were performed for each sample, a single-copy gene-specific Q-PCR (acidic ribosomal phosphoprotein, 36B4 or RPLP0) and a telomere-specific Q-PCR. From the Q-PCR results, a telomere/single-copy gene (T/S) ratio was calculated for each sample and normalized to the T/S ratio of a reference sample. This normalized T/S ratio was used as the estimate of relative telomere length.

Telomere length estimation for each of the age cohorts was performed separately. Each Q-PCR contained approximately 5–10 ng DNA dispensed into duplicate 384-well plates using a liquid handling robot (EpMotion 5070; Eppendorf, Hamburg, Germany). One plate was used for the telomere Q-PCR and the other was used for the 36B4 Q-PCR. Samples were assayed in quadruplicate, and the four replicates were placed together in corresponding positions on each of the duplicate plates. DNA was dried down and resuspended in the appropriate Q-PCR mix, which contained the primers of interest, 1x Master Mix (Platinum SYBR Green Master Mix, #11744-500; Invitrogen, Carlsbad, CA) and H2O to a total volume of 10 μL. The final concentration of each of the two primers in the telomere Q-PCR (Tel1b: 5’ CGG TTT GTG TGG TGG GTG GTG
The relative telomere length results were validated by comparing the relative telomere estimates (T/S ratio) across assays for the positive controls, which were assayed by comparing the relative telomere estimates (T/S ratio) using the comparative Ct method after confirmatory analyses. Ten to thirteen percent of the samples had outliers. For the 40+ Telomere Cohort, 6% of the samples were not improved to acceptable levels by removal of an aberrant outlier (ie, SD > 0.2) and were omitted from further analyses. For the 60+ Telomere Cohort, 3.7% of the samples were not improved but the assays were repeated successfully.

For the 40+ Telomere Cohort, the average coefficients of variation (CVs) for Ct scores were 0.43% for the single-copy gene Q-PCR and 0.76% for the telomere Q-PCR, based on a set of positive controls (human cell line and saliva DNA) assayed in quadruplicate across five plates. Similarly, for the 60+ Telomere Cohort, the average CVs for Ct scores were 0.53% and 0.62% for the single-copy gene and telomere Q-PCRs, respectively.

Relative telomere length was estimated from Ct scores (T/S ratio) using the comparative Ct method after confirming that the two types of Q-PCR yielded similar amplification efficiencies (42). The interassay variation was assessed by comparing the relative telomere estimates (T/S ratio) across assays for the positive controls, which were assayed on every experimental plate. Average interassay CVs for the relative telomere estimates were 8.82% and 3.44% for the 40+ and 60+ Telomere Cohorts, respectively.

The relative telomere length results were validated by comparison with an alternate method, the telomere restriction fragment method (TRF; TeloTAGGG Telomere Length Assay kit, #2 209 136; Roche), which provides a direct estimate of telomere length in kilobases. This was undertaken separately for each age cohort. In a preliminary analysis, the kilobase estimate was used to determine if there was a significant difference in length between the two age cohorts, with the older cohort expected to have longer mean telomere length. All the primary analyses utilized the relative telomere length results.

**Sociodemographic, Anthropometric, Lifestyle, Mental Health, and Cognitive Measures**

Height and weight were self-reported using questions from the Australian National Health Survey (43). Years of education and marital status were also recorded. Socioeconomic status was assessed by occupation, and participants were classified into three occupational categories: professional, white collar, and blue collar based on the Australian Standard Classification of Occupations (44). Participants were classified as current, past, or never-smokers (45). Alcohol consumption was self-reported using questions from the Alcohol Use Disorders Identification Test (46). Weekly consumption was determined from the quantity and frequency responses and took into account any episodes of binge drinking (47,48). Daily use of antioxidant vitamins was defined as the consumption of vitamins and/or multivitamins 6–7 days a week for 6 months or more. The frequency and levels of physical activity were assessed using measures from the UK Whitehall II study (49,50). A continuous measure of the total number of hours of physical activity per week was derived from these responses (51). Depressive symptoms in the last 4 weeks were assessed using the Goldberg Depression Scale (37). Performance on the Symbol Digit Modalities Test was measured in both cohorts, whereas the Mini-Mental State Examination was administered to the 60+ Cohort only. The presence of the lung conditions, asthma, chronic bronchitis, and emphysema and the current use of antihypertensive medication were self-reported.

**Indicators of Physical Function**

Lung function was measured using a Micro Spirometer (Micro Medical Ltd, Rochester, Kent, UK). Three forced expiratory volume measurements were recorded, and the highest value was used in analyses. Handgrip strength using the dominant hand was measured over two trials with a dynamometer (Smedley’s Dynamometer, Tokyo, Japan), and the mean was used. Two blood pressure measurements were taken while the participant was seated and the mean used for analyses. Participants were designated definite hypertensive if they self-reported treatment for hypertension, had a mean systolic blood pressure measurement greater than or equal to 160 mmHg, and/or a mean diastolic blood pressure greater than or equal to 95 mmHg.
Table 1. Characteristics of Study Participants by Age Cohort

<table>
<thead>
<tr>
<th>Trait</th>
<th>Category</th>
<th>40+ (n = 351)</th>
<th>60+ (n = 295)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere length (kb), median (IQR)</td>
<td>Men</td>
<td>5.65 (1.37)</td>
<td>4.74 (0.61)</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>155 (44.2)</td>
<td>158 (53.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>196 (55.8)</td>
<td>137 (46.4)</td>
</tr>
<tr>
<td>Age (y), M ± SD</td>
<td>Current</td>
<td>46.8 ± 1.4</td>
<td>66.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>14.00 (16.75)</td>
<td>171 (58.0)</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>14.00 (2.62)</td>
<td>137 (46.4)</td>
</tr>
<tr>
<td>Education (y), M ± SD</td>
<td>Current</td>
<td>4.00 (8.00)</td>
<td>4.00 (8.62)</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>4.00 (1.24)</td>
<td>4.00 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>2.25 ± 8.00</td>
<td>2.25 ± 8.00</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td>Current</td>
<td>45 (12.8)</td>
<td>17 (5.8)</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>117 (33.3)</td>
<td>107 (36.3)</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>189 (53.8)</td>
<td>171 (58.0)</td>
</tr>
<tr>
<td>Goldberg depression score, M ± SD</td>
<td>Current</td>
<td>2.34 ± 2.33</td>
<td>1.78 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>2.34 ± 2.33</td>
<td>1.78 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>14.00 (16.75)</td>
<td>14.00 (16.75)</td>
</tr>
<tr>
<td>Number alcoholic drinks/week, median (IQR)</td>
<td></td>
<td>10.50 (11.50)</td>
<td>14.00 (16.75)</td>
</tr>
</tbody>
</table>
| Telomere length. Participants of the 60+ Telomere Cohort had significantly shorter telomeres (median = 4.74 kb, IQR = 0.61) than the 40+ Telomere Cohort (median = 5.65 kb, IQR = 1.37; p < .0001, equal variances not assumed). However, within each of the narrow age range cohorts, telomere length was not significantly correlated with age (p > .05).

Statistical Analyses

Descriptive data are presented as means and standard deviations for continuous measures, medians and inter-quartile ranges (IQR) for continuous variables with distributions that deviated from normality and frequencies (%) for categorical data. All cases with data were included in analyses. Missing information was minimal for all variables except for physical activity where the data were imputed in the following manner: missing values were replaced with the median values for the entire PATH sample when split by age group or replaced with zero when the frequency of activity was never/hardly ever. The major outcome variable, relative telomere length, was positively skewed and was log_{10} transformed. Alcohol consumption, physical activity, and body mass index (BMI, kilograms per meters squared) measures were also log_{10} transformed. Analyses were undertaken to assess whether variables previously reported to be associated with telomere length, sex, antioxidant vitamin use, physical activity, alcohol consumption, tobacco smoking, and socioeconomic status were significant covariates of telomere length. There were no significant sex differences in telomere length (p > .05). Higher alcohol consumption was associated with shorter telomere length for the 60+ Telomere Cohort (r = -.155, p = .008). Physical activity was inversely correlated with telomere length for the entire 40+ Telomere Cohort only (r = -.114 p = .034). The appropriate analyses were therefore adjusted for alcohol consumption and physical activity. Because most of the variables were associated with age, inferential analyses were also adjusted for age. Due to the correlation between height and (i) grip strength and (ii) lung function, these variables were adjusted for height. Lung function analyses were also adjusted for tobacco smoking, and those who reported lung conditions were omitted from the analyses. BMI was positively correlated with blood pressure, and the relevant analyses were adjusted for BMI and for the current use of antihypertensive medication.

Simple bivariate and partial correlation coefficients were computed to describe the associations between continuous variables. Differences between groups were assessed using independent t tests or analysis of variance when the outcome variable was continuous and chi-square tests for associations between categorical variables. The majority of analyses were performed within each age cohort. Due to sex differences on most measures, the analyses were also undertaken separately for each sex. Statistical tests were undertaken using SPSS v17 (SPSS Inc., Chicago, IL) and a significance level of 0.05 was used.

Results

Characteristics of the two age cohorts are shown in Table 1. There were more women in the 40+ Telomere Cohort than in the 60+ Telomere Cohort (55.8% vs 46.4%). Fewer current smokers were present in the older cohort and older participants undertook more hours of physical activity. For the older cohort, a higher proportion took daily antioxidant vitamins and more individuals were classified as hypertensive compared with the younger cohort.

Cross-sectional age group differences in telomere length were assessed using the TRF kilobase measure (log_{10}) of telomere length. Participants of the 60+ Telomere Cohort had significantly shorter telomeres (median = 4.74 kb, IQR = 0.61) than the 40+ Telomere Cohort (median = 5.65 kb, IQR = 1.37; p < .0001, equal variances not assumed). However, within each of the narrow age range cohorts, telomere length was not significantly correlated with age (p > .05).

The descriptive statistics for the physical function measures are detailed in Table 2. In general, poorer performances on the lung function and grip strength tests were observed for the older cohort. Mean systolic blood pressure was also higher for the older cohort. As shown in Table 2, for the younger 40+ Telomere Cohort, telomere length was significantly positively correlated with systolic blood pressure for the entire cohort after adjustment for age, alcohol consumption, physical activity, BMI, and antihypertensive medication (partial r = .127, p = .022). Similarly, telomere length was positively correlated with systolic blood pressure for 40+ women only, before (r = .157, p = .029) and after adjustments for covariates (partial r = .181, p = .017). For the older 60+ Telomere Cohort, telomere length was not associated with any of the physical function measures (p > .05).

Discussion

In this Australian population-based study, telomere length was in general not associated with performance on indicators of physical function in two narrow age range cohorts of middle-aged and older adults. The current study found little support for the premise that telomere length is
Table 2. Correlations Between Telomere Length and Indicators of Physical Function by Age Cohort and Sex

<table>
<thead>
<tr>
<th>Indicator of Physical Function</th>
<th>Age Cohort</th>
<th>n</th>
<th>M ± SD or Median (IQR)</th>
<th>r</th>
<th>p</th>
<th>Partial r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV highest reading</td>
<td>40+</td>
<td>302</td>
<td>1.859 ± 0.362</td>
<td>−0.033</td>
<td>.566</td>
<td>−0.035</td>
<td>.551</td>
</tr>
<tr>
<td></td>
<td>40+ Men</td>
<td>137</td>
<td>2.122 ± 0.253</td>
<td>−0.147</td>
<td>.086</td>
<td>−0.144</td>
<td>.098</td>
</tr>
<tr>
<td></td>
<td>40+ Women</td>
<td>165</td>
<td>1.641 ± 0.285</td>
<td>0.088</td>
<td>.260</td>
<td>0.085</td>
<td>.286</td>
</tr>
<tr>
<td></td>
<td>60+</td>
<td>265</td>
<td>1.513 ± 0.342</td>
<td>−0.016</td>
<td>.844</td>
<td>−0.015</td>
<td>.857</td>
</tr>
<tr>
<td></td>
<td>60+ Men</td>
<td>147</td>
<td>1.689 ± 0.331</td>
<td>−0.014</td>
<td>.864</td>
<td>−0.015</td>
<td>.749</td>
</tr>
<tr>
<td></td>
<td>60+ Women</td>
<td>118</td>
<td>1.294 ± 0.200</td>
<td>0.115</td>
<td>.215</td>
<td>0.138</td>
<td>.144</td>
</tr>
<tr>
<td>Grip strength</td>
<td>40+</td>
<td>351</td>
<td>21.598 ± 6.081</td>
<td>−0.027</td>
<td>.609</td>
<td>−0.01</td>
<td>.989</td>
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<tr>
<td></td>
<td>40+ Men</td>
<td>155</td>
<td>26.705 ± 4.466</td>
<td>−0.015</td>
<td>.851</td>
<td>−0.002</td>
<td>.980</td>
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<tr>
<td></td>
<td>40+ Women</td>
<td>196</td>
<td>17.560 ± 3.675</td>
<td>0.042</td>
<td>.561</td>
<td>0.062</td>
<td>.393</td>
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<tr>
<td></td>
<td>60+</td>
<td>293</td>
<td>18.533 ± 5.153</td>
<td>−0.019</td>
<td>.743</td>
<td>0.008</td>
<td>.498</td>
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<tr>
<td></td>
<td>60+ Men</td>
<td>157</td>
<td>21.886 ± 4.202</td>
<td>0.059</td>
<td>.460</td>
<td>0.055</td>
<td>.501</td>
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<tr>
<td></td>
<td>60+ Women</td>
<td>136</td>
<td>14.664 ± 2.981</td>
<td>0.019</td>
<td>.826</td>
<td>0.012</td>
<td>.893</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>40+</td>
<td>350</td>
<td>80.48 (10.01)</td>
<td>0.055</td>
<td>.305</td>
<td>0.085</td>
<td>.125</td>
</tr>
<tr>
<td></td>
<td>40+ Men</td>
<td>155</td>
<td>84.46 (10.00)</td>
<td>0.054</td>
<td>.507</td>
<td>0.067</td>
<td>.419</td>
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<td>40+ Women</td>
<td>195</td>
<td>77.31 (8.84)</td>
<td>0.098</td>
<td>.171</td>
<td>0.124</td>
<td>.101</td>
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<tr>
<td></td>
<td>60+</td>
<td>293</td>
<td>81.06 (9.58)</td>
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<td>.368</td>
<td>0.085</td>
<td>.149</td>
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<tr>
<td></td>
<td>60+ Men</td>
<td>157</td>
<td>82.86 (9.83)</td>
<td>0.039</td>
<td>.627</td>
<td>0.048</td>
<td>.562</td>
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<tr>
<td></td>
<td>60+ Women</td>
<td>136</td>
<td>78.98 (8.86)</td>
<td>0.103</td>
<td>.231</td>
<td>0.122</td>
<td>.167</td>
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<tr>
<td>Systolic</td>
<td>40+</td>
<td>347</td>
<td>123.67 (15.84)</td>
<td>0.092</td>
<td>.087</td>
<td>0.127</td>
<td>.022*</td>
</tr>
<tr>
<td></td>
<td>40+ Men</td>
<td>152</td>
<td>130.22 (13.55)</td>
<td>0.015</td>
<td>.856</td>
<td>0.038</td>
<td>.649</td>
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<tr>
<td></td>
<td>40+ Women</td>
<td>194</td>
<td>118.29 (15.21)</td>
<td>0.157</td>
<td>.029*</td>
<td>0.181</td>
<td>.017*</td>
</tr>
<tr>
<td></td>
<td>60+</td>
<td>293</td>
<td>138.30 (18.22)</td>
<td>0.036</td>
<td>.536</td>
<td>0.060</td>
<td>.312</td>
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<tr>
<td></td>
<td>60+ Men</td>
<td>157</td>
<td>141.03 (18.32)</td>
<td>0.053</td>
<td>.510</td>
<td>0.050</td>
<td>.546</td>
</tr>
<tr>
<td></td>
<td>60+ Women</td>
<td>136</td>
<td>135.16 (17.65)</td>
<td>0.044</td>
<td>.604</td>
<td>0.068</td>
<td>.443</td>
</tr>
</tbody>
</table>

Notes: All partial correlations were adjusted for age, alcohol consumption (log10), and physical activity (log10). BMI = body mass index; FEV = forced expiratory volume; IQR = inter-quartile range; T/S = telomere length. Telomere length was shorter for the 60+ Telomere Cohort participants compared with the 40+ Telomere Cohort but was not correlated with age within each cohort. This is not totally unexpected due to the narrow range of ages found within each cohort and the high level of variation in telomere length found among individuals of the same age (53). There are several studies that have failed to find a significant relationship between telomere length and age, many of which have used limited age range samples (54).

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There are several studies that have failed to find a significant relationship between telomere length and age, many of which have used limited age range samples (54).

Our kilobase estimates of telomere length are comparable with previous estimates that are based on the relative telomere length (T/S ratio) for similar age samples (22,55,56). The difference in telomere length between the two age cohorts implies a loss of approximately 50 base pairs per year, which is in close agreement with previous cross-sectional studies (57–62).

Ideally, telomere length should be measured in tissues specific for the physical domain under investigation but for practical reasons measurements are usually restricted to cells collected in blood samples. Several reports suggest that leukocyte telomere length positively correlates with length in other tissues within an individual (7,63–65), although this appears not to be the case for all cell types (66). Because the present study and prior reports have found few if any associations between markers of physical function...
and telomere length in leukocytes, measurement of telomere length in other cell types from the domains under investigation may be more relevant and informative. For example, telomere length measured in lung tissue may be more appropriate to investigate the relationships between telomere length and lung function. This may explain our and others’ failure to find a relationship between leukocyte telomere length and measures of age-related physical performance.

Limitations of the present study include the relatively small sample sizes of each of the telomere cohorts, decreasing the power to find significant associations of small effect size. On the other hand, the present sample size would have been sufficient to detect a medium or large effect size ($r \geq .3$); with a sample size of 85 or more required to have more than 80% power (67). Telomere integrity may also be affected by mutations, damage, and modifications to telomere-associated proteins and telomere DNA. In the present study, these parameters were not examined. DNA extraction under conditions designed to minimize oxidative stress, which may inhibit the accurate estimation of telomere length using the Q-PCR method (68), was not undertaken. Nevertheless, the majority of published studies using the Q-PCR protocol have used standard DNA extraction procedures. Minimization of measurement error is another concern. The relatively high interassay CV for the 40+ Telomere Cohort may have attenuated any significant relationships. However, this CV result is consistent with the range of interassay CVs reported by other studies, which is usually less than 10% (69–71). Conversely, the relatively low CV for the 60+ Telomere Cohort in comparison to prior studies (40,69,71,72) and the absence of significant findings suggests that there are small or no associations between these measures and telomere length in this cohort of older adults. Alternate methods of telomere length estimation, such as the recently described absolute Q-PCR method (68), may provide a superior method for telomere length estimation. This method is reported to have greater accuracy and reproducibility than the current Q-PCR method used herein and has the advantage of providing an absolute estimate. In future studies, strategies should be undertaken to minimize telomere length measurement error. It should also be acknowledged that in general, measurement error is a pervasive problem in research. Other potential telomere length confounders not measured in this study include paternal age at the time of the participant’s birth (73) and life stress (74).

This study adds to the growing body of evidence about the relationship between leukocyte telomere length and age-sensitive physical function measures. Like previous studies, it suggests that leukocyte telomere length is not correlated with indicators of physical function in middle-aged and older adults. Therefore, other measures may prove to be superior and more useful indicators of physical aging, such as the levels of CDKN2A p16INK4a (75). Longitudinal studies are needed to overcome the limitations of cross-sectional studies, and larger samples are required to detect statistical associations of small effect size.

**Funding**

This study was supported by an Australian National Health and Medical Research Council (NHMRC) Program Grant 179805 and an R.M. Gibson Grant from the Australian Association of Gerontology. A.F.J. and H.C. are funded by NHMRC Fellowships.

**Acknowledgments**

We would like to acknowledge and thank the PATH participants and the PATH Project staff. We would also like to thank Professor Kaarin Anstey, Centre for Mental Health Research, Australian National University, and Dr. Ruth Parslow, Australian Centre for Post-Traumatic Mental Health, University of Melbourne for insightful discussions regarding this work.

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