Original Article

**Shorter Ends, Faster End? Leukocyte Telomere Length and Mortality Among Older Taiwanese**

Dana A. Glei,1 Noreen Goldman,2 Maxine Weinstein,1 and Rosa Ana Risques3

1Center for Population and Health, Georgetown University, Washington, District of Columbia. 2Office of Population Research, Princeton University, Princeton, New Jersey. 3Department of Pathology, University of Washington, Seattle, Washington.

Address correspondence to Dana A. Glei PhD, Center for Population and Health, Georgetown University, 5985 San Aleso Ct., Santa Rosa, CA 95409-3912. Email: dag77@georgetown.edu

**Abstract**

Recent studies have found mixed results regarding the association between leukocyte telomere length (LTL)—thought to be a marker of cellular aging—and all-cause mortality. Some studies have reported a significant inverse relationship, but others have not, perhaps in part owing to insufficient power. We examine the relationship using data from a nationally representative sample of older Taiwanese (54+ in 2000), which is larger (n = 942) than most previous studies, and which includes comprehensive information on potential confounders including white blood cell distribution and inflammatory markers. Results from a Cox hazards model demonstrate a small, but significant, association between LTL and mortality that is independent of age, sex, and lifestyle factors. White blood cell distribution, especially the proportion of neutrophils, is an important predictor of LTL; however, the association between LTL and mortality changes little controlling for white blood cell distribution. In contrast, the association between LTL and mortality weakens considerably (by 48%) after adjustment for inflammatory markers and homocysteine. Our results suggest that the relationship between short telomeres and mortality is tied to inflammation and homocysteine. Longitudinal studies are needed to explore bidirectional influences resulting from the fact that inflammation leads to shorter leukocyte telomeres, which in turn results in senescence, which exacerbates inflammation.

**Key Words:** Mortality—Telomeres—Biological aging—Taiwan—Inflammation.

**Decision Editor:** Rafael de Cabo, PhD

---

Telomeres—the repetitive deoxyribonucleic acid sequences at the end of chromosomes that protect them from fusion and degradation (1)—shorten with cell replication and oxidative damage. Eventually, telomeric deoxyribonucleic acid reaches a critical minimum length and fails to cap the end of the chromosomes, triggering the cell to stop dividing (ie, replicative senescence) (2). Cell senescence is believed to contribute to aging (3), which has led to the hypothesis that telomere shortening has a causal effect on mortality. Alternatively, shorter telomeres might be simply an indicator of other processes that affect aging and mortality. Scientists generally agree that shorter leukocyte telomere length (LTL) is associated with older age, male sex, Caucasian race, and possibly atherosclerosis (4,5). However, there is controversy regarding the association of LTL with other diseases of aging and with mortality, and the crucial question of whether shortening of the telomeres is a cause or a symptom of aging remains unanswered.

We have identified 13 studies that investigated the relationship between LTL and all-cause mortality in population-based samples: five found a significant inverse relationship (longer LTL was associated with lower mortality) (6–10); the other eight did not (11–18). Among three other studies based on twin samples, only one found a significant inverse association (19–21). Given
the substantial variation in LTL between individuals of similar age and health status, a large sample is required to draw meaningful conclusions (22). Only 5 of these 16 studies comprised at least 1,000 respondents with 250 or more deaths (7,9,10,14); three of these five reported a significant inverse association between LTL and mortality.

The extent of adjustment for potential confounders also varied considerably across the 16 studies. Many factors can influence both LTL and mortality (Figure 1), confounding the association between LTL and mortality and contributing to misleading results. The most consistent predictors of LTL are age, sex, and race (5). Other factors that have been shown, albeit inconsistently, to influence LTL include lifestyle factors such as smoking, physical activity, alcohol consumption, psychological stress, and socioeconomic status, as well as biological factors (eg, obesity, hypertension, cholesterol) and inflammatory markers (eg, interleukin-6 [IL-6] and C-reactive protein [CRP]) (5). The mechanisms that link these factors with LTL are complex and not completely understood. As shown in Figure 1, telomeres shorten because of cell replication and oxidative stress, two biological processes that are direct consequences of chronic inflammation (23). Thus, factors that lead to chronic inflammation, such as obesity and smoking, could indirectly cause telomere shortening. Importantly, however, telomere shortening can also cause inflammation via the induction of senescence. Cells with critically short telomeres enter senescence, a state of permanent replication arrest in which cells secrete numerous proinflammatory cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (3). Thus, the relationship between inflammation and LTL is likely to be bidirectional: telomere shortening leads to inflammation via the senescence-associated secretory phenotype, and chronic inflammation contributes to further telomere shortening via oxidative damage and faster turnover of leukocytes and hematopoietic stem cells. Lifestyle and biological health risk factors could contribute to this deleterious cycle at different levels (eg, producing chronic inflammation or oxidative stress) that are directly or indirectly linked to telomere shortening. However, these factors may also affect aging and mortality via alternative pathways independent of telomere shortening. For example, lifestyle factors can lead to chronic systemic low-grade inflammation and, in turn, oxidative stress, both of which can independently of their effects on telomeres—produce metabolic syndrome, deoxyribonucleic acid damage, and endothelial dysfunction that contribute to cardiovascular disease and other diseases of aging (24–26).

Thus, adjusting for confounders is fundamental to understanding the true association between LTL and mortality. Only 5 of the prior 16 studies adjusted for smoking (7,9,16,18,21); even fewer controlled for physical activity, obesity, alcohol use, socioeconomic status, inflammation, glucose, or high-density lipoprotein cholesterol. Because inflammation may be a consequence of telomere shortening, controlling for inflammation may lead to an underestimate of the overall effect of LTL on mortality. On the other hand, failure to control for inflammation may result in an overestimate of the effect of LTL because of confounding.

The distribution of leukocyte (white blood cell [WBC]) subtypes may affect LTL because LTL measurement is essentially a weighted average across a mix of different cell types. Although highly correlated, different WBC subsets have different telomere lengths (27–29). The distribution of WBC subsets varies with age and other factors such as the presence of acute infection or underlying disease. Thus, differences in the distribution of WBC subtypes could confound the results. For example, someone who is ill is likely to have a very high proportion of neutrophils. Because neutrophils tend to have longer

Figure 1. Conceptual model for the study of leukocyte telomere length and mortality. The confounders included in this study are indicated in bold italics. Chronic inflammation could be a confounder but also a mediator, since shorter telomeres lead to chronic inflammation through the induction of the SASP. HSC = hematopoietic stem cells; IGF-1 = insulin-like growth factor-1; SASP = senescence-associated secretory phenotype; SES = socioeconomic status. *In this study, we include the following inflammatory markers: leukocyte count and distribution, interleukin-6, C-reactive protein, soluble intercellular adhesion molecule 1, and soluble E-selectin.
telomere lengths than lymphocytes (28), such a person may have longer average LTL simply because of the WBC distribution. Yet, despite longer LTL, s/he may have a higher risk of mortality owing to underlying illness that increases the risk of death even while it inflates LTL (via its effect on the WBC distribution). None of the prior population-based studies adjusted for the distribution of WBC subtypes as a potential confounder.

In this study, we use data from a nationally representative sample of older Taiwanese to investigate the association between LTL and all-cause mortality. This dataset offers a large sample with a long follow-up period—nearly 1,000 respondents, more than 300 of whom died over 11 years. We control for the distribution of WBC subtypes (ie, lymphocytes, monocytes, neutrophils, eosinophils, basophils) as well as other potential confounders including demographic characteristics, lifestyle and biological health risk factors, and inflammatory markers. We have made a comprehensive effort to identify the possible biological associations between these factors, telomere length, and mortality, as represented in Figure 1. This integrative approach coupled with rigorous modeling has allowed us to determine the main predictors of LTL and to analyze and interpret their confounding influences on the association between LTL and mortality.

Methods

Data
The 2000 Social Environment and Biomarkers of Aging Study comprises a nationally representative sample of persons 54 and older in Taiwan, selected randomly using a multistage sampling design with oversampling of older persons (71+) and urban residents (30). In 2000, in-home interviews were completed with 1,497 respondents, 1,023 of whom also completed a physical examination. Exam participants did not differ significantly from nonparticipants in ways likely to introduce serious bias (31). Details regarding response rates are provided elsewhere (30). The study protocol was approved by human subjects committees in Taiwan and at Georgetown University and Princeton University.

On a scheduled day several weeks after a household interview, participants collected a 12-hour overnight urine sample (7 pm to 7 am), fasted overnight, and visited a nearby hospital the following morning where medical personnel drew a blood specimen and took blood pressure and anthropometric measurements. Compliance was extremely high: 96% fasted overnight and provided a urine specimen deemed suitable for analysis.

Survival status was ascertained by linkage to the Death Certificate file maintained by the Taiwan Department of Health and to the Household Registration file maintained by the Ministry of the Interior. After excluding 47 respondents who were missing LTL and another 34 individuals with missing data for a covariate, the analysis sample comprised 942 respondents (322 of whom died by December 31, 2011).

Measures

Average LTL
Leukocyte deoxyribonucleic acid was extracted by Union Clinical Laboratories (in Taiwan) using trimethyl ammonium bromide salts (dodecyl trimethyl ammonium bromide and cetyl-trimethyl-ammonium bromide). LTL, represented by the telomere-to-single copy gene (T/S) ratio, was measured at the University of Washington, using quantitative polymerase chain reaction with a few modifications from the method originally developed by Cawthon (32,33) (see Supplementary Material for details).

Potential confounders
We adjusted for a range of potential confounders that prior studies suggest affect LTL and are likely to affect mortality. These confounders (shown in bold italics in Figure 1) can be divided into four categories: demographic characteristics, lifestyle factors, inflammatory markers, and other biological parameters. Demographic characteristics included age and sex. Lifestyle factors comprised smoking history, physical activity, alcohol consumption, and educational attainment. Inflammatory markers included WBC count and distribution, IL-6, CRP, soluble intercellular adhesion molecule 1 (sICAM-1), and soluble E-selectin. We adjusted for the distribution of WBC subtypes using the percentages of monocytes, neutrophils, basophils, and eosinophils; % lymphocytes served as the reference group. IL-6 had an extremely skewed distribution; log-transformed values improved model fit. The remaining biological parameters included obesity, pulse pressure, high-density lipoprotein cholesterol, glucose metabolism, homocysteine, and insulin-like growth factor 1 (IGF-1). Obesity was measured by body mass index and a quadratic term was included to allow for the well-established nonlinear association with mortality (34). Pulse pressure was measured as the difference between systolic and diastolic blood pressure, and glucose metabolism as the level of glycosylated hemoglobin. Homocysteine is a nonprotein α-amino acid found in blood that is an indicator of cardiovascular disease (35). IGF-1 promotes proliferation, cell growth, and survival. Low levels of IGF-1 are associated with short telomeres and with age-related diseases (36,37).

Analytical Strategy
In the initial stage of analysis, we examined the predictors of LTL with four nested linear regression models. First, we adjusted for sex, age, and, in order to compensate for the sampling design, urban residence. Next, we added the distribution of WBC subtypes. Third, we added education and smoking history, which we consider to be exogenous predictors (ie, unlikely to be a consequence of LTL). Finally, we added the remaining behavioral and biological measures, which may be endogenous (eg, both a cause or consequence of LTL owing to the fact that short telomeres lead to inflammation and illness that, in turn, affect behavior and biomarker levels). Although lifestyle factors are likely to influence LTL via their effects on inflammation and other biological processes (as shown in Figure 1), it is impossible to establish the causal direction in a cross-sectional study. For example, sick people are less likely to exercise and may abstain from alcohol consumption because of their illness. These effects can inflate the apparent association between behavior and mortality. Thus, we add potentially endogenous confounders to the model last because the inclusion of endogenous variables would bias the coefficients (including the one for LTL).

In the second stage, we examined survival as a function of LTL. First, we calculated survival curves by long versus short LTL, split at the median. Next, we estimated a Cox hazards model with age as the “clock” to predict age-specific death rates adjusted for potential confounders. This modeling approach allows the underlying baseline hazard over age to assume whatever functional form best fits the data (ie, the log hazard is not constrained to be linear over age). Because age is likely to be a stronger predictor of mortality than duration of study, this model is superior to the conventional approach, which includes a control for age and uses duration of
study as the time metric. Initial tests (data not shown) showed no significant evidence that the age slope of mortality varied by sex. However, because there was evidence of nonproportional hazards for education (ie, the association with mortality was attenuated at older ages), we modeled education as a linear function of age. The models were fit in five stages: the first four models follow the same strategy described above for models predicting LTL; a fifth model demonstrates that changes in the coefficient for LTL are attributable primarily to selected covariates. That is, because we observed an attenuation in the coefficient for LTL in model 4, we estimated model 5 to demonstrate the minimal set of confounders that account for the attenuation.

The analyses were performed using Stata 12.1 (38). In all models, we used the robust estimator of variance to correct standard errors for clustering by primary sampling unit. In order to compare effect size across predictors, we standardized (mean = 0 and SD = 1) each of the continuous measures prior to fitting the models.

Results

Descriptive statistics are shown in Supplementary Table S1. About 30% of the sample died by the end of 2011 (average follow-up = 10.7 years).

As expected, LTL declines with age (correlation coefficient $r = -0.20$, $p < .001$, two sided, Supplementary Figure S1a). The distribution of WBC subtypes also varies by age: % neutrophils increases with age ($r = 0.19$, $p < .001$, two sided, Supplementary Figure S1b), whereas % lymphocytes declines with age ($r = -0.20$, $p < .001$, two sided, Supplementary Figure S1c). Granulocytes (ie, neutrophils, basophils, and eosinophils) have longer telomeres than lymphocytes, particularly at older ages (28). Therefore, the shift to a higher proportion of neutrophils at older ages could attenuate the age-related decline in LTL.

Table 1 shows the results from models predicting LTL. As expected, LTL declines with age ($\beta = -0.19$, 95% confidence interval [CI]: −0.28, −0.11) and is greater for women than men ($\beta = 0.20$, 95% CI: 0.03, 0.37) (Model 1). With adjustment for WBC distribution (Model 2), the age-related decline becomes slightly stronger ($\beta = -0.21$, 95% CI: −0.30, −0.12). Higher % neutrophils is associated with longer LTL, whereas higher % eosinophils is associated with shorter LTL. After adjustment for all covariates (Model 4), the significant predictors of LTL are age, neutrophils, and levels of IL-6.

The survival curves for the top versus bottom half of the LTL distribution are presented in Supplementary Figure S2. Individuals with longer LTL show a survival advantage relative to those with shorter LTL ($p = .004$).

Table 2 shows hazard ratios (HRs) from models predicting age-specific death rates. In the model that adjusts only for age, sex, and urban residence (Model 1), we find an inverse association between LTL and mortality (HR = 0.89 per SD, 95% CI: 0.81, 0.98). With adjustment for WBC distribution (Model 2), the HR for LTL barely changes. Inclusion of education and smoking history has no discernable influence on the coefficient for LTL (Model 3). Yet, with the addition of the remaining covariates (Model 4), the HR for LTL weakens (HR = 0.93, 95% CI: 0.87, 1.00), representing a 48% reduction in the effect size compared with Model 2 (ie, the coefficient—which is the log of the HR—changes from −0.133 in Model 2 to −0.069 in Model 4). This attenuation is almost entirely attributable to the addition of inflammatory markers (particularly IL-6 and sICAM-1) and homocysteine: A comparison of Model 5 with Model 2 reveals that the addition of only four variables (WBC count, IL-6, sICAM-1, and homocysteine) yields the same HR for LTL as in Model 4, which includes all the covariates. Furthermore, by comparing Model 4 with Model 5, we can deduce that adding the remaining variables has no effect on the HR for LTL, even though some of those covariates (eg, body mass index, glycosylated hemoglobin) significantly predict mortality.

Discussion

Our goal was to evaluate the strength of association between telomere length and mortality in a large cohort with long follow-up after controlling for potential confounders including WBC distribution and inflammatory markers. We found that shorter telomeres were associated with mortality independent of age, sex, education, and smoking history, although the magnitude of the effect was small (ie, 12% reduction in the mortality hazard per SD of LTL). The fact that we are able to detect such a small effect demonstrates that the study has sufficient statistical power. While WBC distribution, especially the proportion of neutrophils, was associated with LTL, the association between LTL and mortality changed little with adjustment for WBC distribution. Similarly, the relationship between shorter telomeres and mortality was independent of other factors including exercise, alcohol consumption, body mass index, high-density lipoprotein, glycosylated hemoglobin, pulse pressure, and IGF-1. However, the association weakened considerably (by 48%) after controlling for inflammatory markers (particularly IL-6 and sICAM-1) and homocysteine.

The potential of LTL as a biomarker of aging has been the focus of heated debate (4,5). Discrepancies between studies are typically attributed to differences in techniques for measuring telomere length and lack of statistical power. Another fundamental reason for discrepancy is the issue of confounding. LTL is the result of multiple environmental and biological factors that affect the individual (23). Two prominent biological factors that influence average LTL are the distribution of WBC subsets and the levels of inflammatory markers. While the association of LTL with inflammatory markers has been previously explored (39), the role of WBC distribution has been ignored until recently (40).

The WBC distribution is a potential confounder of the associations between LTL and aging outcomes because different WBC types have different telomere lengths and their distribution varies with age, disease, and other factors. Granulocytes, which comprise mostly neutrophils, have longer telomeres than lymphocytes and a slower rate of telomere shortening (28). Moreover, their percentage increases with age, as observed here, most likely due to a decrease of lymphopoiesis in the elderly (41). We find that higher % neutrophils—and to some extent lower % eosinophils—are associated with longer telomeres and, in the presence of controls for these factors, the association between age and LTL increases slightly. Nevertheless, controlling for WBC distribution has little effect on the association between LTL and mortality, indicating that the association of shorter telomeres with mortality is independent of the aging-related changes in WBC distribution. This result is consistent with a recent study of telomere length in centenarians and their offspring (40).

In contrast, the relationship between LTL and mortality was attenuated after adjustment for inflammatory markers (eg, IL-6 and sICAM-1) and homocysteine. Only 2 of the 16 population-based studies that analyzed the association between LTL and
### Table 1. Coefficients (95% CI) From Linear Regression Models Predicting Leukocyte Telomere Length†

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age†</td>
<td>−0.19***</td>
<td>−0.21***</td>
<td>−0.21***</td>
<td>−0.20***</td>
</tr>
<tr>
<td></td>
<td>(−0.282, −0.105)</td>
<td>(−0.296, −0.116)</td>
<td>(−0.299, −0.123)</td>
<td>(−0.287, −0.111)</td>
</tr>
<tr>
<td>Female</td>
<td>0.20*</td>
<td>0.20*</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(0.034, 0.370)</td>
<td>(0.024, 0.375)</td>
<td>(0.083, 0.386)</td>
<td>(−0.073, 0.354)</td>
</tr>
<tr>
<td>% Lymphocytes (omitted)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Monocytes§</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(−0.033, 0.069)</td>
<td>(−0.035, 0.067)</td>
<td>(−0.028, 0.080)</td>
<td></td>
</tr>
<tr>
<td>% Neutrophils§</td>
<td>0.09**</td>
<td>0.09**</td>
<td>0.12**</td>
<td>0.12**</td>
</tr>
<tr>
<td></td>
<td>(0.029, 0.156)</td>
<td>(0.029, 0.151)</td>
<td>(0.051, 0.182)</td>
<td></td>
</tr>
<tr>
<td>% Eosinophils§</td>
<td>−0.07*</td>
<td>−0.07*</td>
<td>−0.06</td>
<td>−0.06</td>
</tr>
<tr>
<td></td>
<td>(−0.148, −0.000)</td>
<td>(−0.146, −0.002)</td>
<td>(−0.132, 0.010)</td>
<td></td>
</tr>
<tr>
<td>% Basophils§</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>(−0.041, 0.105)</td>
<td>(−0.041, 0.106)</td>
<td>(−0.041, 0.112)</td>
<td></td>
</tr>
</tbody>
</table>

Never smoked (omitted)†

Current smoker

No exercise (omitted)

Exercises ≤2 times per wk

Exercises 3–5 times per wk

Exercises ≥6 times per wk

Never drinks (omitted)

Drinks sometimes

Drinks frequently

Leukocyte count§

log(IL-6)§

CRP§

tsCAM-1-1 §

sE-selectin§

BMI§

BMI squared§

HbA1c§

HDL§

Pulse pressure§

Homocysteine§

IGF-1§

Constant

−0.06

−0.07

−0.04

0.02

---

Notes: BMI = body mass index; CI = confidence interval; CRP = C-reactive protein; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein cholesterol; IGF-1 = insulin-like growth factor-1; IL-6 = interleukin-6; LTL = leukocyte telomere length; sE-selectin = soluble E-selectin; sICAM-1 = soluble intercellular adhesion molecule 1.

†All models adjust for urban residence; Models 3 and 4 also control for education (coefficients not shown).

One of the leukocyte categories must be omitted because they sum to 100%.

§This variable was standardized (M = 0, SD = 1) prior to fitting the model, as was LTL. So, this is a standardized coefficient (To create the quadratic term for BMI, we squared the standardized values for BMI).

*p < .05; **p < .01; ***p < .001, two sided.
Table 2. Hazard Ratios (95% CI) From Cox Models Predicting Age-Specific Mortality Through December 31, 2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte telomere length†</td>
<td>0.89* (0.807, 0.982)</td>
<td>0.88** (0.795, 0.965)</td>
<td>0.88** (0.801, 0.967)</td>
<td>0.93 (0.869, 1.003)</td>
<td>0.93 (0.858, 1.012)</td>
</tr>
<tr>
<td>Female</td>
<td>0.75* (0.583, 0.956)</td>
<td>0.86 (0.674, 1.103)</td>
<td>0.92 (0.692, 1.218)</td>
<td>0.77 (0.587, 1.011)</td>
<td></td>
</tr>
<tr>
<td>Urban resident</td>
<td>0.83 (0.641, 1.067)</td>
<td>0.81 (0.648, 1.013)</td>
<td>0.85 (0.679, 1.059)</td>
<td>0.82 (0.666, 1.019)</td>
<td></td>
</tr>
<tr>
<td>% Lymphocytes (omitted)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Monocytes†</td>
<td>1.17** (1.061, 1.281)</td>
<td>1.16** (1.052, 1.282)</td>
<td>1.17** (1.067, 1.292)</td>
<td>1.13** (1.038, 1.239)</td>
<td></td>
</tr>
<tr>
<td>% Neutrophils†</td>
<td>1.36*** (1.172, 1.582)</td>
<td>1.33*** (1.136, 1.555)</td>
<td>1.23** (1.055, 1.431)</td>
<td>1.23** (1.072, 1.401)</td>
<td></td>
</tr>
<tr>
<td>% Eosinophils†</td>
<td>1.18** (1.044, 1.335)</td>
<td>1.17* (1.032, 1.319)</td>
<td>1.16*** (1.063, 1.258)</td>
<td>1.14** (1.040, 1.259)</td>
<td></td>
</tr>
<tr>
<td>% Basophils†</td>
<td>1.14** (1.045, 1.238)</td>
<td>1.13* (1.027, 1.234)</td>
<td>1.13* (1.026, 1.235)</td>
<td>1.14** (1.041, 1.238)</td>
<td></td>
</tr>
<tr>
<td>Years of completed education†</td>
<td>0.66** (0.490, 0.885)</td>
<td></td>
<td>0.72 (0.497, 1.050)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education × (age − 54)†</td>
<td>1.01* (1.001, 1.024)</td>
<td></td>
<td>1.01 (0.997, 1.027)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked (omitted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>1.08 (0.743, 1.559)</td>
<td>1.00 (0.707, 1.421)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.46** (1.154, 1.842)</td>
<td></td>
<td>1.19 (0.894, 1.591)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No exercise (omitted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercises ≤2 times per wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercises 3-5 times per wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercises ≥6 times per wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never drinks (omitted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinks sometimes</td>
<td>0.80 (0.574, 1.117)</td>
<td>0.82 (0.525, 1.270)</td>
<td>0.98 (0.633, 1.513)</td>
<td>0.95 (0.746, 1.210)</td>
<td></td>
</tr>
<tr>
<td>Drinks frequently</td>
<td>0.45*** (0.306, 0.668)</td>
<td></td>
<td>0.82 (0.525, 1.270)</td>
<td>0.98 (0.633, 1.513)</td>
<td>0.95 (0.746, 1.210)</td>
</tr>
<tr>
<td>Leukocyte count†</td>
<td>1.12 (0.981, 1.268)</td>
<td>1.11 (0.980, 1.257)</td>
<td>1.32*** (1.186, 1.493)</td>
<td>1.32*** (1.189, 1.463)</td>
<td></td>
</tr>
<tr>
<td>log(IL-6)†</td>
<td>1.33*** (1.186, 1.493)</td>
<td></td>
<td>1.32*** (1.186, 1.493)</td>
<td>1.32*** (1.189, 1.463)</td>
<td></td>
</tr>
<tr>
<td>CRP†</td>
<td>0.99 (0.888, 1.099)</td>
<td></td>
<td>0.99 (0.888, 1.099)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sICAM-1†</td>
<td>1.32** (1.200, 1.457)</td>
<td></td>
<td>1.32** (1.200, 1.457)</td>
<td>1.35*** (1.238, 1.468)</td>
<td></td>
</tr>
<tr>
<td>sE-selectin†</td>
<td>0.96 (0.867, 1.075)</td>
<td>0.96 (0.864, 0.976)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>1.12 (0.998, 1.252)</td>
</tr>
<tr>
<td>BMI†</td>
<td>0.96 (0.867, 1.075)</td>
<td>0.96 (0.864, 0.976)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>1.12 (0.998, 1.252)</td>
</tr>
<tr>
<td>BMI squared‡</td>
<td>0.96 (0.867, 1.075)</td>
<td>0.96 (0.864, 0.976)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>1.12 (0.998, 1.252)</td>
</tr>
<tr>
<td>HbA1c†</td>
<td>1.22*** (1.083, 1.364)</td>
<td></td>
<td>1.22*** (1.083, 1.364)</td>
<td>1.22*** (1.083, 1.364)</td>
<td></td>
</tr>
<tr>
<td>HDL†</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>1.12 (0.998, 1.252)</td>
</tr>
<tr>
<td>Pulse pressure†</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>0.96 (0.836, 1.096)</td>
<td>1.12 (0.998, 1.252)</td>
</tr>
</tbody>
</table>

(Continued)
mortality included inflammatory markers as covariates; none of them included homocysteine. Fitzpatrick and colleagues (9) controlled for IL-6 and CRP; Weischer and colleagues (7) controlled for CRP only. Both studies found a significant association between LTL and mortality even after adjusting for inflammation. The study of LTL in centenarians and their offspring also reported a relationship between LTL and survival after adjustment for inflammatory and immune markers (IL-6, CRP, molar ratio of IGF-1 to IGF-binding protein 3, and cytomegalovirus serostatus), in addition to % lymphocytes (40). Attenuation of the association of LTL with mortality in our study but not in others may stem from our inclusion of several biomarkers (homocysteine, sICAM-1, and WBC count) that were not included in previous research. Our study highlights the importance of controlling for a comprehensive set of confounders, especially markers of inflammation, and shows limited utility of LTL as a biomarker of aging, given the small effect of shorter telomeres on mortality and its further attenuation with inflammation.

There are two possible explanations for the role of inflammation and homocysteine in attenuating the relationship of LTL to mortality. On one hand, inflammation and homocysteine may be confounders that influence both LTL and mortality (ie, the apparent effect of LTL on mortality may be partly spurious). On the other hand, they may mediate the link between LTL and mortality. The prevalent view in the literature supports the first scenario because chronic inflammation, which is a well-known feature of aging and a predictor of early mortality (42), causes telomere shortening via an increase in leukocyte turnover and the production of oxidative stress, which directly accelerates telomere attrition (23). The alternative view, however, is also plausible because critically short telomeres lead to replicative senescence, which induces chronic inflammation through the senescence-associated secretory phenotype (43). In the context of the immune system, senescent T cells, which accumulate in the elderly, have been well characterized. These cells have short telomeres, produce proinflammatory cytokines, and may contribute to many age-related pathologies (44).

This problem of bidirectionality has largely been ignored in the literature. Cross-sectional studies, such as this one, cannot distinguish between the two potential effects. Thus, longitudinal studies should be conducted to test whether inflammation predicts subsequent changes in LTL over time and whether LTL predicts subsequent changes in inflammation. However, if both prove true, then it will be difficult to separate the two pathways.

The main strengths of this study are the large, nationally representative sample with long follow-up; the comprehensive set of covariates; the rigorous measurement of LTL by quantitative polymerase chain reaction using appropriate controls and periodical reproducibility experiments; and the detailed statistical analysis, which used a hazard model that treats age rather than duration of study as the “clock,” tested sensitivity to the exclusion of outliers and to specification of neutrophils, and reflected an integrative interpretation of the processes depicted in Figure 1. The main weaknesses include one-time biomarker measurements; limited number of deaths for cause-specific analyses; and lack of telomere measurements by WBC type.

In conclusion, we have demonstrated that shorter telomeres predict mortality independently of the distribution of WBC, but this association is tied to inflammation. The complex pathways linking short telomeres, senescence, and inflammation may underlie current controversies regarding the role of LTL as a biomarker of aging. The conceptual framework presented here could help to design and interpret future studies that assess the role of LTL in aging and mortality.

**Supplementary Material**

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

**Funding**

This work was supported by the National Institute on Aging (grant numbers R01AG16790, R01AG16661) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant number R24HD047879) within the National Institutes of Health. SEBAS was funded by the Division of Behavioral and Social Research of the National Institute on Aging (grant numbers R01 AG16790, R01 AG16661). The Bureau of Health Promotion (BHP, Department of Health, Taiwan) provided additional financial support for SEBAS 2000.

**Acknowledgments**

We are grateful to Julie Malicdem for technical support with telomere length measurements and Dr. Peter Rabinovitch for his comments and suggestions on the manuscript. We also acknowledge the hard work and dedication of the staff at the Center for Population and Health Survey Research (BHP), who were instrumental in the design and implementation of the Social Environment and Biomarkers of Aging Study and supervised all aspects of the fieldwork and data processing.
References


