Journal of Gerontology: MEDICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci. 2011 April;66A(4):421–429
doi:10.1093/gerona/glq224

Advance Access published on February 2, 2011

Leukocyte Telomere Length and Mortality in the Cardiovascular Health Study

Annette L. Fitzpatrick,[1] Richard A. Kronmal,[2] Masayuki Kimura,[3] Jeffrey P. Gardner,[3] Bruce M. Psaty,[1,4,5,6] Nancy S. Jenny,[7] Russell P. Tracy,[7,8] Sheetal Hardikar,[1] and Abraham Aviv[3]

[1]Department of Epidemiology and [2]Department of Biostatistics, University of Washington, Seattle.
[3]Center of Human Development and Aging, University of Medicine and Dentistry of New Jersey, Newark.
[4]Department of Medicine and [5]Department of Health Services, University of Washington School of Medicine, Seattle.
[6]Group Health Research Institute, Group Health Cooperative, Seattle, Washington.
[7]Department of Pathology and [8]Department of Biochemistry, University of Vermont, Burlington.

Address correspondence to Annette L. Fitzpatrick, PhD, Department of Epidemiology, University of Washington, Collaborative Health Studies Coordinating Center, Building 29, Suite 310, 6200 NE 74th Street, Seattle, WA 98115. Email: fitzpal@u.washington.edu

Background. Leukocyte telomere length (LTL) is related to diseases of aging, but studies of mortality have been inconsistent.

Methods. We evaluated LTL in relation to total mortality and specific cause of death in 1,136 participants of the Cardiovascular Health Study who provided blood samples in 1992–1993 and survived through 1997–1998. LTL was measured by Southern blots of the terminal restriction fragments. Cause of death was classified by a committee of physicians reviewing death certificates, medical records, and informant interviews.

Results. A total of 468 (41.2%) deaths occurred over 6.1 years of follow-up in participants with mean age of 73.9 years (*SD* 4.7), 65.4% female, and 14.8% African American. Although increased age and male gender were associated with shorter LTLs, African Americans had significantly longer LTLs independent of age and sex ($p < .001$). Adjusted for age, sex, and race, persons with the shortest quartile of LTL were 60% more likely to die during follow-up than those within the longest quartile (hazard ratio: 1.61, 95% confidence interval: 1.22–2.12, $p = .001$). The association remained after adjustment for cardiovascular disease risk factors. Evaluations of cause of death found LTL to be related to deaths due to an infectious disease etiology (hazard ratio: 2.80, 95% confidence interval: 1.32–5.94, $p = .007$), whereas a borderline association was found for cardiac deaths (hazard ratio: 1.82, 95% confidence interval: 0.95–3.49, $p = .07$) in adjusted models. Risk estimates for deaths due to cancer, dementia, and ischemic stroke were not significant.

Conclusion. These data weakly corroborate prior findings of associations between LTL and mortality in the elderly.

Key Words: Telomere—Mortality—Cause of death—Cardiovascular disease—Heart failure.

Received March 5, 2010; Accepted October 20, 2010

Decision Editor: Luigi Ferrucci, MD, PhD

TELOMERE shortening has been intrinsically linked to cell division and is a key determinant of replicative senescence of cultured somatic cells (1,2). Leukocyte telomere length (LTL) and its age-dependent shortening, that is LTL dynamics, have been the focus of intense investigations. LTL has been reported to be associated with age (3,4), gender (5,6), and many age-related diseases (7–14) as well as measures of general health (15,16). Several studies have evaluated associations between LTL and mortality with conflicting results; some observing that LTL was inversely related to mortality (17,18,20–24), whereas others have not (16,19,25,26). In addition, the mechanistic connection between LTL and mortality is an enigma and a number of problems have been cited in epidemiological studies (27,28). The question remains whether LTL is an index of aging and longevity or if its dynamics, which may mirror the kinetics of the hematopoietic stem cells (29), are actively engaged in determining end-of-life or age-related pathology (27,30).

The Cardiovascular Health Study (CHS) provides the opportunity to evaluate the association between survival and LTL using a large cohort of adults aged 65 years and older (31). In addition to providing a large sample size, the CHS has been well characterized in terms of health status and incident cardiovascular disease. All participants have been followed to ascertain death and classified by trained clinicians for specific cause of death (32). Over the last several years, LTL was measured in a subset of CHS participants. In this article, we have utilized mortality and other data collected in the CHS to answer the following questions: (a) Is LTL associated with total mortality in adults aged 65 years and older? (b) Do associations between LTL and mortality differ by cause of death? (c) Are associations modified by factors including age, sex, race, or other variables?

Methods

The Cardiovascular Health Study

Participants in the CHS were recruited in 1989–1990 from Medicare eligibility lists in four U.S. communities: Forsyth county, North Carolina; Washington county, Maryland; Sacramento county, California; and Pittsburgh, Pennsylvania (31). The original cohort with a sample of 5,201 adults aged 65 years and older was supplemented with an additional 687 African Americans in 1992–1993. Participants completed an extensive baseline examination consisting of demographics (age, gender, race, education), vital signs (including blood pressure), anthropometry (including height and weight), medical history, health behaviors (including use of tobacco), and a number of other physical and cognitive function measurements (31). Phlebotomy was done several times during the study from which laboratory analysis including lipids and measures of inflammation were completed. DNA was collected from consenting participants and stored for future use. Up to 10 annual examinations were completed through 1998–1999.

Extensive evaluation of cardiovascular events, all hospitalizations, and mortality was done during study follow-up through 2006 (32). Participants' households were telephoned every 6 months to learn of changes in status and health; obituaries, returned newsletters, and use of Centers for Medicare and Medicaid Services data files to confirm hospitalizations were also used as sources for learning of recent deaths. Follow-up completeness in CHS was 97.6% for ascertainment of morbid events and deaths. Data on morbidities and mortality were classified according to prespecified criteria to identify incident cardiovascular events and cause of death. All participants completed an informed consent at baseline and periodically during the study prior to data collection. Consents for collecting medical records were gathered based on Health Insurance Portability and Accountability Act (HIPAA) criteria. Institutional review board approval was collected at all institutions involved in data collection, interpretation, or management of the study.

Measurement of LTL

DNA collected in 1992–1993 from CHS participants and stored at the Central Blood Repository at the University of Vermont was extracted and sent in batches over a 3-year period to the laboratory at the University of Medicine and Dentistry of New Jersey for measurement of LTL. A random sample of about 1,200 participants were selected for LTL measurement who fulfilled the following criteria: completion of the 1992–1993 and 1997–1998 clinic examinations, stored genetic samples collected at these visits, and having a signed consent to use DNA on file. Integrity of the DNA was assessed through electrophoresis on 1.0% agarose gels (200 V for 2 hours) and staining with ethidium bromide. Telomere length was measured by the mean length terminal restriction fragments using the Southern blot method described in detail elsewhere (5,33). Each sample was analyzed twice for telomere length measurement (on different gels on different occasions), and the mean was used for statistical analyses of LTL. The Pearson's Correlation Coefficient for the duplicates of this sample was 0.96, with an average coefficient of variation for pair sets of 1.7%. The laboratory conducting the LTL measurements was blinded to all characteristics of participants.

Covariates and Cause of Death

Data included in these analyses were extracted from the clinical examination completed in 1992–1993, considered "baseline" for these analyses as DNA for LTL measurement was collected at this examination. Height and weight were measured in person at this examination, and body mass index was calculated accordingly. Diabetes status was determined based on American Diabetes Association criteria of fasting glucose concentration greater than or equal to 7·0 mmol/L, and impaired fasting glucose is defined as fasting glucose between 5·8 and 6·9 mmol/L (34). Hypertension was defined as a systolic blood pressure above 140 or diastolic over 90 mm Hg; borderline was calculated as systolic between 130 and 140 mm Hg or diastolic between 80 and 90 mm Hg. Smoking status was self-reported (current, previous, or never). History of myocardial infarction, stroke, and congestive heart failure (CHF) included prevalence of the specific condition at baseline and updated from medical records and other criteria collected as a part of the CHS events system at baseline (32,35). Intima-medial thickness was determined using B-mode carotid ultrasound of the common and interior carotid arteries (36). The ankle–brachial index was assessed by Doppler and computed with a result of less than 0.90 indicating peripheral arterial disease (37). Analyses of fasting insulin, glucose, C-reactive protein, and interleukin-6 were completed centrally at the CHS Central Blood Laboratory at the University of Vermont (38,39).

Upon learning of a participant's death, all relevant data were subsequently collected including hospital medical records, physician questionnaires, nursing home notes, informant interviews, and death certificates. Cause of death was classified independently at in-person meetings by a committee of CHS physicians with expertise in geriatrics using these records (32). Categories of underlying cause of death were initially coded as follows: (a) atherosclerotic coronary heart disease, (b) cerebrovascular disease, (c) atherosclerotic disease other than coronary or cerebrovascular disease, (d) other cardiovascular disease, or (e) noncardiovascular disease. Stroke subtype was classified by a committee of neurologists as either ischemic or

hemorrhagic using magnetic resonance imagings and computed tomographies if available. Associations with ischemic stroke (n = 33) are presented here as the number of hemorrhagic strokes (n = 7) was too small to be analyzed. Mechanism of death for cardiovascular deaths was subsequently coded as being due to CHF, arrhythmias, cardiovascular procedures, or multiple mechanisms. Non cardiovascular deaths were also classified by CHS clinical investigators designating a primary cause of death: trauma, cancer (including primary site), Parkinson's disease, pneumonia, other respiratory disease, infection (excluding sepsis and pneumonia), sepsis, liver disease, gastrointestinal disease, renal failure, metabolic conditions, fractures, dementia, failure to thrive, among the most frequently occurring. For these analyses, infectious disease deaths included those involving pneumonia, sepsis, and other infections. Dementia deaths included those categorized with an underlying cause of dementia or Alzheimer's disease with failure to thrive as a proximate cause (without specific infectious etiology). All cancer sites were included in cancer cause-of-death category.

Statistical Analysis

As data in these analyses were collected as a part of a longitudinal study on LTL attrition, individuals were selected based on the potential to have completed a subsequent clinic examination 5 years after the 1992–1993 visit when the baseline terminal restriction fragment measurement was made for potential use of two DNA samples. Mortality was assessed between July 1997 through June 2006, a maximum of 8.1 and an average of 6.1 (*SD* 2.2) person-years of follow-up. Censoring was done at date of death or June 2006. In analyses of specific cause of death, censoring was done on those deaths other than the cause of interest.

Descriptive statistics were calculated as mean LTL by level of specific participant characteristics. Bivariate associations were tested using t tests or analysis of variance for differences between the means, and linear regression was used to test for trends in variables with more than two categories. Multiple linear regressions were used to evaluate the associations between age, sex, and race with LTL in an adjusted model containing all three variables. Cox proportional hazards regression estimated risk of all-cause mortality, specific cause of death, and mechanism of death associated with mean terminal restriction fragment length both as a continuous variable and categorized into quartiles. In these models, the continuous measure was multiplied by −1 in order to present data reflecting increased risks associated with shorter LTL. The assumption of proportional hazards was evaluated and met using Kaplan–Meier plots and Schoenfeld residual tests. Models were adjusted for age (continuous measure), sex, and race (classified as African American or Other). Hazard ratios (HRs), 95% confidence intervals (CIs), and p values (p) were produced. A second set of models were performed with additional adjustments for baseline hypertension, diabetes, smoking status, coronary heart disease, stroke, CHF, C-reactive protein, and interleukin-6. Tests for interactions between LTL (continuous measure) and age, gender, race, and prevalence of CHF (selected for evaluation based on preliminary observations made on associations between telomere length and morbid heart failure events in earlier work) were also completed.

Results

Data for 1,136 CHS participants with LTL and follow-up data were available for analysis. A total of 468 (41.2%) deaths occurred over an average of 6.1 years of follow-up. Average age of the cohort was 73.9 years (*SD* 4.7), 65.4% were female, and 14.8% were African American (only five individuals were self-reported as "other" not Caucasian or African American). LTL ranged from a minimum of 4.6–8.7 kilobase (kb) pairs, with a mean of 6.3 (*SD* 0.6). In bivariate analyses, factors related to shorter mean LTL included older age, male gender, former smoking status, greater internal carotid intima-medial thickness, and increased interleukin-6 (Table 1). Mean length of telomeres was longer in African Americans and persons with prevalent hypertension. The test for trend in multiple level variables resulted in several additional variables to be associated with shorter LTL, including higher levels of fasting glucose, greater common carotid intima-medial thickness, and lower ankle–brachial index. Education, body mass index, prevalence of diabetes, fasting insulin, and C-reactive protein were not associated with LTL in these cross-sectional analyses.

In this subset of CHS, as shown in Table 2, age, sex, and race were independently associated with LTL. Each increased year of age related to a shortened LTL of 0.03 kb (*SE* 0.004), and men had a shorter LTL than women (beta: −0.171, *SE* 0.03). African Americans had a significantly longer LTL (6.28 in Caucasians vs 6.52 in African American, $p < .001$), and the difference remained significant after adjustment for age and sex.

LTL was significantly associated with total mortality adjusted for age, sex, and race (Figure 1). Measured continuously, each decrease of 1 LTL kb was associated with a 32% increased risk of death over 6.1 years of follow-up (HR: 1.32, 95% CI: 1.10–1.57, $p = .002$) (Table 3). The association remained significant after adjustment for hypertension, diabetes, smoking status, coronary heart disease, stroke, CHF, C-reactive protein, and interleukin-6 (HR: 1.34, 95% CI: 1.11–1.63, $p = .003$). Similarly, individuals with the shortest quartile of LTL were found to have a 61% increased risk of death compared with those in the longest quartile (HR: 1.61, 95% CI: 1.22–2.12, $p = .001$) adjusted for age, sex, and race; this association also remained significant

Table 1. Mean LTL by Selected Characteristics of 1,136 Participants of the Cardiovascular Health Study Aged 65 Years and Older in 1992–1993

Characteristic	LTL			
	N	Mean LTL in kb Pairs (*SD*)	*p**	*p* for Trend†
Age (y)				
<70	169	6.51 (0.67)	<.001	<.001
70–74	547	6.34 (0.55)		
75–79	263	6.25 (0.56)		
≥80	157	6.09 (0.44)		
Sex				
Female	687	6.39 (0.59)	<.001	—
Male	449	6.20 (0.53)		
Race				
African American	168	6.52 (0.65)	<.001	—
White/other‡	968	6.28 (0.55)		
Education				
LT high school	257	6.28 (0.61)	.26	.62
High school/GED	335	6.36 (0.57)		
Some college	267	6.28 (0.55)		
College/postgraduate	276	6.33 (0.56)		
Body mass index quartile				
<24.1	280	6.29 (0.56)	.67	.32
24.2–26.5	274	6.34 (0.57)		
26.6–29.5	283	6.32 (0.60)		
≥29.6	284	6.35 (0.55)		
Diabetes				
No	960	6.33 (0.57)	.10	—
Yes	157	6.25 (0.53)		
Fasting glucose quartiles (mg/dL)§				
<91.6	233	6.38 (0.59)	.14	.02
91.6–98.5	290	6.34 (0.55)		
98.5–109.5	290	6.29 (0.57)		
≥109.6	260	6.28 (0.59)		
Fasting insulin quartiles (mg/dL)§				
<7	195	6.36 (0.61)	.67	.35
7–9.9	292	6.33 (0.54)		
10–13.4	280	6.32 (0.56)		
≥13.5	313	6.29 (0.60)		
Hypertension				
No	660	6.29 (0.57)	.03	—
Yes	471	6.36 (0.58)		
Smoking status				
Never	508	6.36 (0.58)	.03	—
Former	495	6.27 (0.57)		
Current	110	6.36 (0.54)		
History of coronary heart disease (baseline)				
No	920	6.33 (0.57)	.32	—
Yes	216	6.28 (0.55)		
History of stroke (baseline)				
No	1,096	6.32 (0.57)	.47	—
Yes	40	6.25 (0.66)		
History of CHF (baseline)				
No	1,083	6.32 (0.57)	.61	—
Yes	53	6.28 (0.68)		
Common carotid IMT (mm)				
<0.92	290	6.38 (0.57)	.07	.009
0.92–1.01	273	6.35 (0.55)		
1.02–1.15	285	6.31 (0.58)		
≥1.16	276	6.26 (0.59)		
Internal carotid IMT (mm)				
<1.01	280	6.41 (0.60)	.01	.003
1.01–1.26	281	6.32 (0.53)		
1.27–1.68	281	6.27 (0.59)		
≥1.69	280	6.28 (0.56)		
Ankle–brachial index				
GE 1.0	886	6.34 (0.56)	.10	.02
0.90–0.99	103	6.34 (0.62)		
0.80–0.89	39	6.26 (0.45)		
LT 0.80	61	6.16 (0.63)		
C-reactive protein (quartiles)				
<1.26	273	6.34 (0.59)	.73	.90
1.26–2.75	283	6.30 (0.58)		
2,76-6.17	280	6.30 (0.55)		
≥6.18	279	6.34 (0.58)		
Interleukin-6 (pg/mL) (quartiles)				
<1.10	264	6.38 (0.61)	.005	.001
1.10–1.53	263	6.37 (0.57)		
1.54–2.30	265	6.33 (0.55)		
≥2.31	263	6.22 (0.56)		

Notes: CHF = congestive heart failure; GED = general equivalency diploma; IMT = intima-medial thickness; LT = less than; LTL = leukocyte telomere length.

* *p* value for *t* test or analysis of variance.

† *p* value using multiple regression with collapsed categories.

‡ Includes 963 Caucasians, 1 Native American/Alaska Native, and 4 of Other/Mixed Race.

§ Excludes participants taking insulin (*n* = 1,084).

after adjustment for cardiovascular risk factors (HR: 1.58, 95% CI: 1.18–2.12, $p = .002$). In evaluations of specific cause of death, risk of mortality due to infectious disease was related to LTL. Compared with persons in the longest quartile of LTL, those in the shortest quartile had a 2.5-fold increased risk of dying from infectious causes over 6. 1 years of follow-up (HR: 2.47, 95% CI: 1.20–5.08, $p = .01$). After adjusting for cardiovascular risk factors, the HR was increased to 2.80 (95% CI: 1.32–5.94, $p = .007$). A borderline association ($p = .07$) between risk of death due to cardiac causes, and LTL was found in the adjusted model (HR: 1.82, 95% CI: 0.95–3.49 comparing shortest with longest quartile of LTL. Associations were not found for mortality due to cancer, dementia, or ischemic stroke.

As an interaction was found between cause of death due to cardiac causes and baseline prevalence of CHF ($p = .04$, data not shown), we separated cardiac deaths by mechanism, either arrhythmic (not related to heart failure) or due to CHF (Table 4). While not significant, the point estimate indicated individuals with the shortest LTL to be almost

Table 2. Associations Between Leukocyte Telomere Length and Age, Sex, and Race in 1,136 Participants of the Cardiovascular Health Study

Demographic Factor	Unstandardized Coefficients*			
	B	*SE*	*t*	*p*
Age (y)	−0.026	0.004	−7.42	<.001
Male sex	−0.171	0.033	−5.13	<.001
African-American race	0.164	0.046	3.55	<.001

Notes: *All three variables (age, gender, and race) are included in the model.

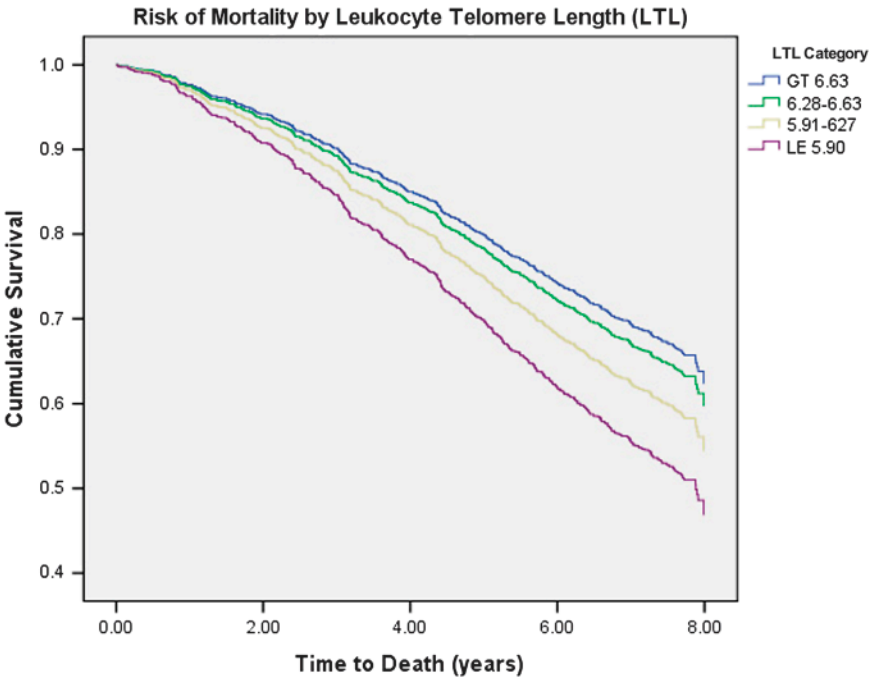

Figure 1. Risk of mortality in 1,136 Cardiovascular Health Study participants by leukocyte telomere length (LTL) quartiles adjusted for age, sex, and race.

twice as likely to die of an arrhythmic cardiac death than were those with the longest LTL (HR: 1.90, 95% CI: 0.90–3.99, $p = .09$). LTL was not associated with death from CHF (HR: 0.56, 95% CI: 0.18–1.77, $p = .32$). It is noteworthy, however, that while not significant, persons dying from a mechanism related to CHF tended to have a longer LTL than those dying from other etiologies.

Discussion

Our results of LTL and mortality corroborate the associations found between shorter LTL and risk of death in several other studies (17,18,20–24). We found that persons in the shortest quartile of LTL had a 60% increased risk of death compared with those with LTL in the longest quartile. In terms of cause-specific mortality, shortened LTL predicted an almost threefold increased risk of death by infectious causes, which was consistent with results reported by Cawthon et al. (17). Although we had hypothesized that, in addition to infectious disease, we would find associations with cardiovascular disease-related deaths based on previous studies and our own preliminary results with cardiovascular risk factors (9), results here were weaker than expected (borderline significance when adjusted for other cardiovascular risk factors) However, aging-related cardiovascular compromise might increase susceptibility and diminish ability to fight infections without necessarily causing death from myocardial infarction, arrhythmia, CHF, or stroke. In addition, as LTL reflects telomere dynamics in the hematopoietic system, shortened LTL might denote aging-related loss of immune function (40,41).

The 60% increased risk of death from any cause between persons in the shortest compared with the longest quartile of LTL found here is similar to other studies in the literature including results from 143 individuals aged 60–97 years (risk ratio 1.7, 95% CI: 0.82–3.53) (13), and in 780 patients with stable coronary artery disease (age-adjusted HR: 1.8, 95% CI: 1.2–2.9) (23). Our results were somewhat weaker than those reported in a study of Swedish twins comparing shortest with longest telomeres (RR: 2.8, 95% CI: 1.1–7.3) (21), in a study of 195 nondemented post-stroke patients reporting an inverse linear relationship with longer LTL (HR: 0.52, 95% CI: 0.28–0.98) (24) and in 204 Danish twin pairs (HR: 0.56, 95% CI: 49–63) (20). Our results differed from several other studies, which did not find associations between LTL and mortality (16,19,25,26). Nor did we duplicate the more than twofold increased risk of cancer mortality recently reported (13), although our number of cases was larger. Differences in methods that measure LTL and demographics of the studies are likely involved. In addition, the null studies generally included small sample sizes.

The lack of an association between LTL and total or cause-specific mortality in the Health ABC Study, which analyzed data from 2,721 participant over 8.2 years of follow-up (16), deserves greater scrutiny. Similar to CHS, the Health ABC Study is a multisite population-based study recruited from Medicare eligibility lists, the outcome ascertainment used similar protocols, and length of follow-up is comparable. However, Health ABC included a much larger percent of African Americans (47%) compared with 15% here. In spite of the greater racial diversity, Health ABC participants appear to be more homogeneous that CHS with a

Table 3. Associations Between Total Mortality and Cause of Death by LTL (continuous and categorical) in 1,136 Participants of the Cardiovascular Health Study

	Deaths (*N*)	HR (95% CI) Adjusted for Demographics*	*p*	HR (95% CI) Fully Adjusted†	*p*
Total mortality					
LTL mean‡	468	1.32 (1.10–1.57)	.002	1.34 (1.11–1.63)	.003
LTL quartiles (kb pairs)					
>6.63	86	1.00	.003		.004
6.28–6.63	101	1.09 (0.81–1.46)	.55	1.02 (0.75–1.39)	.88
5.91–6.27	131	1.28 (0.98–1.70)	.08	1.21 (0.90–1.63)	.21
<5.91	150	1.61 (1.22–2.12)	.001	1.58 (1.18–2.12)	.002
Cancer					
LTL mean‡	108	1.25 (0.88–1.79)	.21	1.16 (0.79–1.70)	.45
LTL quartiles (kb pairs)					
>6.63	24	1.00	.29		.47
6.28–6.63	21	0.87 (0.48–1.56)	.64	0.82 (0.44–1.51)	.52
5.91–6.27	32	1.32 (0.77–2.26)	.32	1.30 (0.74–2.28)	.37
<5.91	31	1.41 (0.81–2.45)	.22	1.17 (0.65–2.10)	.61
Infectious					
LTL mean‡	75	1.63 (1.04–2.56)	.03	1.82 (1.12–2.96)	.01
LTL quartiles (kb pairs)					
>6.63	11	1.00	.07		.02
6.28–6.63	17	1.45 (0.68–3.10)	.34	1.48 (0.66–3.33)	.34
5.91–6.27	19	1.51 (0.71–3.20)	.29	1.38 (0.61–3.11)	.44
<5.91	28	2.47 (1.20–5.08)	.01	2.80 (1.32–5.94)	.007
Dementia					
LTL mean‡	53	0.86 (0.51–1.46)	.59	0.83 (0.48–1.44)	.50
LTL quartiles (kb pairs)					
>6.63	13	1.00	.86		.59
6.28–6.63	12	0.80 (0.36–1.76)	.58	0.67 (0.29–1.52)	.34
5.91–6.27	13	0.74 (0.34–1.63)	.45	0.61 (0.26–1.42)	.25
<5.91	15	0.93 (0.42–3.05)	.86	0.91 (0.41–2.06)	.83
Ischemic stroke					
LTL mean‡	33	1.41 (0.72–2.76)	.31	1.36 (0.66–2.79)	.51
LTL quartiles (kb pairs)					
>6.63	4	1.00	.32		.44
6.28–6.63	11	2.59 (0.82–8.17)	.10	1.96 (0.58–6.56)	.28
5.91–6.27	11	2.33 (0.73–7.47)	.15	2.30 (0.72–7.39)	.16
<5.91	17	1.61 (0.46–5.68)	.46	1.27 (0.34–4.74)	.72
Cardiac (all mechanisms)					
LTL mean‡	105	1.32 (0.90–1.94)	.16	1.47 (0.94–2.29)	.09
LTL quartiles (kb pairs)					
>6.63	19	1.00	.20		.10
6.28–6.63	19	0.90 (0.47–1.70)	.74	0.86 (0.41–1.81)	.70
5.91–6.27	29	1.15 (0.64–2.07)	.65	1.17 (0.59–2.32)	.66
<5.91	38	1.57 (0.88–2.79)	.13	1.82 (0.95–3.49)	.07

Notes: CI = confidence interval; HR = hazard ratio; LTL = leukocyte telomere length.

* Adjusted for age, sex, and African-American race.

† Adjusted for age, sex, African-American race, hypertension, diabetes (ADA), smoking status, history of coronary heart disease, stroke, congestive heart failure, C-Reactive Protein, interleukin-6; *N* reduced to 1,004 due to missing covariate data.

‡ The continuous LTL measure was multiplied by −1 in order to reflect range from longest to shortest corresponding to risk of 1 kb pair shorter LTL.

more restrictive age range (70–79 years) and better health based on exclusions involving both physical and cognitive function. This lack of heterogeneity may have reflected less variation in health that may be needed to discern associations. In addition, standard deviations of LTL were much larger in Health ABC corresponding to the larger coefficient of variation (5.8%) in Q-PCR compared with our results using the Southern blot method (1.7%). Average LTL was actually marginally shorter in African Americans than in whites in Health ABC (4.87 for whites compared with 4.77 for blacks), although in our study LTL was significantly longer in African Americas, which has been reported in other cohorts with LTL measured by the Southern blot method (42). All of these factors may have contributed to the differences in results found between the two studies.

In a preliminary report, we did not find a significant association with total mortality in 419 participants of the CHS cohort (HR: 1.22, 95% CI: 0.91–1.63) (9), only slightly lower than the HR found in the current study. Examination of the two groups revealed similar age and gender distributions; however, there was a slightly larger proportion of African Americans included in the original sample (18.1%) compared with here (14.8%). Additionally, although the sample of CHS participants in the preliminary results were

Table 4. Associations Between LTL and Risk of Cardiac Death by Mechanism of Death, Either Arrhythmia or Congestive Heart Failure, in 1,136 Cardiovascular Health Study Participants

	Arrhythmia			Congestive Heart Failure		
Cardiac Deaths	Deaths	Adjusted HR (95% CI)*	*p*	Deaths	Adjusted HR (95% CI)*	*p*
LTL mean†	61	1.65 (0.99–2.74)	.05	28	0.66 (0.32–1.34)	.25
LTL quartiles (kb pairs)						
>6.63	11	1.00	.09	7	1.00	.41
6.28–6.63	13	1.07 (0.48–2.39)	.87	4	0.49 (0.14–1.69)	.26
5.91–6.27	12	0.86 (0.37–1.96)	.71	11	1.05 (0.40–2.79)	.92
<5.91	25	1.90 (0.90–3.99)	.09	6	0.56 (0.18–1.77)	.32

Notes: CI = confidence interval; HR = hazard ratio; LTL = leukocyte telomere length.

* Adjusted for age, sex, and African-American race.

† The continuous LTL measure was multiplied by −1 in order to reflect range from longest to shortest corresponding to risk of 1 kb pair shorter LTL.

selected at random, those in the follow-up article were required to have survived from 1992 through 1997 in preparation for a longitudinal study. Although this may have affected results, the larger sample size is most likely the reason for significant findings here.

Our finding of an association between LTL and infectious disease-related deaths is reasonable. Data strongly suggest that erosion of telomeres is the result of an accruing burden of oxidative stress and inflammation (27,43), which is known to be enhanced by exposure to infectious and inflammatory diseases (30,44–46). The significant relationship between LTL and interleukin-6, a biomarker of inflammation, found here and in our earlier study (9), supports this finding. The weaker relationship found here with cardiac deaths is more perplexing but may be affected by the different mechanistic factors included in this broad category. Although the number of arrhythmic and CHF-related deaths is small (61 and 28, respectively), the HRs suggest relationships with LTL to be in opposite directions, that is shorter LTL in arrhythmic deaths and longer LTL in those associated with CHF. In some elderly persons, this could potentially be explained by increased left ventricular mass, which is often observed in CHF due to hypertension. In fact, associations between longer LTL and increased left ventricular mass have recently been reported (47,48). However, in this study, we did not have information that would help to precisely discern the causes of CHF. Nevertheless, our findings and the recent reports underscore the complexity involved in the relationships between LTL and cardiovascular indices, and the need for larger and more comprehensive studies to explore them.

Although the focus of this article was on mortality, we examined associations with other morbidities for descriptive purposes. Of greatest importance, associations between LTL and age, sex, and race were found ($p < .001$) in the directions consistent with other reports providing confidence in the data analyzed here. Associations were also found with hypertension, smoking status, glucose, carotid intima-medial thickness, and interleukin-6, but they were not present for body mass index, diabetes, insulin, C-reactive protein, and history of prevalent myocardial infarction, CHF, and stroke. Confirming inconsistencies in the literature, these results may reflect the cross-sectional nature of these data, imprecise measurement, or human variability. It is also possible that LTL reflects the combination of risk and burden of disease rather than associations with individual factors that may reflect "somatic fitness" (49). This has been reported using years of healthy life in Health ABC (16) and physical function in some (15,43) but not in all (50) studies.

The large sample size available for analysis here is a major strength of this study, which allowed us to evaluate individual causes of death in addition to total mortality. Other strengths include the high-quality data reflecting both clinical and subclinical disease that has been collected in the CHS, the detailed standardized evaluation of death events using clinical and other data in addition to death certificates, and the use of Southern blot methodology considered the gold standard for telomere length measurement. However, a limitation in our study is the relatively small number of cases within specific causes of death, which reduced power for analyses and prevented the ability to address other etiologies of death. Although multiple testing was inherent in this investigation and should be considered when evaluating *p* values, these analyses were predicated on previous work and hypotheses developed a priori. Although prospectively collected data were analyzed here, it is unclear whether LTL may influence adverse events in older adults or merely reflects poorer health. Regardless, results showing variability by cause of death confirm that environmental and genetic effects in humans interact differentially with telomere attrition.

This study, one of the largest to date to evaluate associations between LTL and mortality, provides additional evidence that LTL may be a proxy for underlying mechanisms that bring about pathophysiological changes in persons surviving to old age. As oxidative stress alters a number of cellular metabolites during the aging process, LTL may be one of many biomarkers, which as a group can be assessed to determine oxidative status of a person. Study of these mechanisms may help increase knowledge about cell processes involved in disease progression and chronic conditions involving both genetic and environmental influences.

Additional research involving telomere attrition and repair at both the cellular and population level are needed to further elucidate LTL response or influence on the aging process.

Funding

This work was supported by grant numbers 1 R01 HL80698-01 and contract numbers N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, grant number U01 HL080295 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke.

Acknowledgment

A full list of principal Cardiovascular Health Study investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm.

References

1. Wong JM, Collins K. Telomere maintenance and disease. *Lancet*. 2003;362:983–988.
2. Blackburn EH. Telomere states and cell fates. *Nature*. 2000;408:53–56.
3. Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev*. 2008; 88:557–579.
4. Jevapalan JC, Sedivy JM. Cellular senescence and organismal aging. *Mech Ageing Dev*. 2008;129:467–474.
5. Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*. 2001;37:381–385.
6. Nawrot TS, Staessen JA, Gardner JP, et al. Telomere length and possible link to the X-chromosome. *Lancet*. 2004;363:507–510.
7. Samani NH, Boulthy R, Butler R, et al. Telomere shortening in atherosclerosis. *Lancet*. 2001;358:472–473.
8. Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell*. 2006;5:325–330.
9. Fitzpatrick AL, Kronmal RA, Gardner JP, et al. Leukocyte telomere length and cardiovascular disease in the Cardiovascular Health Study. *Am J Epidemiol*. 2007;165:14–21.
10. Brouillette SW, Moore JS, McMahon AD, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet*. 2007; 369:107–114.
11. von Zglinicki T, Serra V, Lorenz M, et al. Short telomere length in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab Invest*. 2000;80:1739–1747.
12. Panossian LA, Porter VR, Valenzuela HF, et al. Telomere shortening in T cells correlates with Alzheimer's disease status. *Neurolbiol Aging*. 2003;24:77–84.
13. Willeit P, Willeit J, Mayr A, et al. Telomere length and risk of incident cancer and cancer mortality. *JAMA*. 2010;304:69–75.
14. Zee RY, Castonguay AJ, Barton NS, Germer S, Martin M. Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study. *Transl Res*. 2010;155:166–169.
15. Terry DF, Nolan VG, Andersen SL, Perls TT, Cawthon R. Association of longer telomeres with better health in centenarians. *J Gerontol A Biol Sci Med Sci*. 2008;63:809–812.
16. Njajou OT, Hsueh WC, Blackburn EH, et al. Association between telomere length, specific causes of death, and years of healthy life in Health, Aging, and Body Composition, a Population-Based Cohort Study. *J Gerontol A Biol Sci Med Sci*. 2009;64A:860–864.
17. Cawthon RM, Smith KR, O'Brien E, et al. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*. 2003:361:393–395.
18. Honig LS, Schupf N, Lee JH, et al. Shorter telomeres are associated with mortality in those with APOE ε4 and dementia. *Ann Neurol*. 2006;60:181–187.
19. Harris SE, Deary IJ, MacIntyre A, et al. The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neurosci Lett*. 2006;406:260–264.
20. Kimura M, Hjelmborg JvB, Gardner JP, et al. Telomere length and mortality: a study of leukocytes in elderly Danish twins. *Am J Epidemiol*. 2008;167:799–806.
21. Bakaysa SL, Mucci LA, Slagvoom PE, et al. Telomere length predicts survival independent of genetic influences. *Aging Cell*. 2007;6: 769–774.
22. Astrup AS, Tarnow L, Jorsal A, et al. Telomere length predicts all-cause mortality in patients with type 1 diabetes. *Diabetologia*. 2010;53: 45–48.
23. Farzaneh-Far R, Cawthon RM, Na B, Browner WS, Schiller NB, Whooley MA. Prognostic value of leukocyte telomere length in patients with stable coronary artery disease: data from the Heart and Soul Study. *Arterioscler Thromb Vasc Biol*. 2008;28:1379–1384.
24. Martin-Ruiz C, Dickinson HO, Keys B, Rowan E, Kenny RA, Von Zglinicki T. Telomere length predicts poststroke mortality, dementia, and cognitive decline. *Ann Neurol*. 2006;60:174–180.
25. Martin-Ruiz CM, Gussekloo J, van Heemst D, et al. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell*. 2005;4:287–290.
26. Bischoff C, Petersen HC, Graakjaer J, et al. No association between telomere length and survival among the elderly and oldest old. *Epidemiology*. 2006;17:190–194.
27. Aviv A. The epidemiology of human telomeres: faults and promises. *J Gerontol A Biol Sci Med Sci*. 2008;63:979–983.
28. Longo DL. Telomere dynamics in aging: much ado about nothing? *J Gerontol A Biol Sci Med Sci*. 2009;64A:963–964.
29. Kimura M, Gazitt Y, Cao X, Zhao X, Lansdorp PM, Aviv A. Synchrony of telomere length among hematopoietic cells. *Exp Hematol*. 2010; 38:854–859.
30. Effros RB. Kleemeier Award Lecture 2008—the canary in the coal mine: telomeres and human health span. *J Gerontol A Biol Sci Med Sci*. 2009;64:511–515.
31. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol*. 1991;1:263–276.
32. Ives DG, Fitzpatrick AL, Bild DE, et al. Surveillance and ascertainment of cardiovascular events: the Cardiovascular Health Study. *Ann Epidemiol*. 1995;5:278–285.
33. Kimura M, Stone RC, Hunt SC, et al. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nat Protoc*. 2010;5:1596–1607.
34. Wahl PW, Savage PJ, Psaty BM, Orfanrd TJ, Robbins JA, Tracy RP. Diabetes in older adults: comparison of 1997 American Diabetes Association classification of diabetes mellitus with 1985 WHO classification. *Lancet*. 1998;352:1012–1015.
35. Psaty BM, Kuller LH, Bild D, et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. *Ann Epidemiol*. 1995;5:270–277.
36. O'Leary DH, Polak JF, Wolfson SK, et al. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study CHS Collaborative Research Group. *Stroke*. 1991;22:1155–1163.
37. Newman AB, Siscovick DS, Manolio TA, et al. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. *Circulation*. 1993;88:837–845.
38. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clin Chem*. 1995;41:264–270.
39. Jenny NS, Tracy RP, Ogg MS, et al. In the elderly, interleukin-6 plasma levels and the –174G>C polymorphism are associated with the development of cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2002;22:2066–2071.
40. Gazit R, Weissman IL, Rossi DJ. Hematopoietic stem cells and the aging hematopoietic system. *Semin Hematol*. 2008;45:218–224.

41. Zimmermann S, Martens UM. Telomeres, senescence, and hematopoietic stem cells. *Cell Tissue Res*. 2008;331:79–90.
42. Hunt SC, Chen W, Gardner JP, et al. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. *Aging Cell*. 2008;7:451–458.
43. Starr JM, Sheils PG, Harris SE, et al. Oxidative stress, telomere length and biomarkers of physical aging in a cohort aged 79 years from the 1932 Scottish Mental Survey. *Mech Ageing*. 2008;129: 745–751.
44. Ilmonen P, Kotrschal A, Penn DJ. Telomere attrition due to infection. *PLoS ONE*. 2009;3(5):e2143. doi:10.1371/journal.pone. 0002143.
45. Pawelee G, Akbar A, Caruso C, et al. Human immunosenescence: is it infectious? *Immunol Rev*. 2005;205:257–268.
46. Kaplan RC, Fitzpatrick AL, Pollak MN, et al. Insulin-like growth factors and leukocyte telomere length: the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2009;65A:1103–1106.
47. Vasan RS, Demissie S, Kimura M, et al. Association of leukoctyte telomere length with echocardiographic left ventricular mass: the Framingham Heart Study. *Circulation*. 2009;120:1195–1202.
48. Kuznetsova T, Codd V, Brouilette S, et al. Association between left ventricular mass and telomere length in a population study. *Am J Epidemiol*. 2010;172:440–450.
49. Aviv A. Telomeres and human somatic fitness. *J Gerontol A Biol Sci Med Sci*. 2006;61:871–873.
50. Mather KA, Jorm AF, Milburn PJ, Tan X, Easteal S, Christensen H. No associations between telomere length and age-sensitive indicators of physical function in mid and later life. *J Gerontol A Biol Sci Med Sci*. 2010;65:792–799.