Short Telomere Length, Myocardial Infarction, Ischemic Heart Disease, and Early Death

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Objective—We tested the hypothesis that short telomere length is associated with increased risk of myocardial infarction, ischemic heart disease, and early death.

Methods and Results—We measured leukocyte telomere length in 2 prospective studies of 19 838 Danish general population participants from the Copenhagen City Heart Study and the Copenhagen General Population Study. Participants were followed for up to 19 years for incident myocardial infarction (n=929), ischemic heart disease (n=2038), and death (n=4342). Follow-up was 100% complete. Telomere length decreased linearly with increasing age in women and men in both studies ($P=7\times10^{-74}$ to $P=3\times10^{-125}$). Multifactorially adjusted hazard ratios were 1.10 (95% CI 1.01–1.19) for myocardial infarction, 1.06 (1.00–1.11) for ischemic heart disease, and 1.09 (1.05–1.13) for early death per 1000–base pair decrease in telomere length. The multifactorially adjusted hazard ratios for the shortest versus the longest decile of telomere length were 1.49 (1.07–2.07) for myocardial infarction, 1.24 (1.01–1.53) for ischemic heart disease, and 1.25 (1.07–1.46) for early death.

Conclusion—Short telomere length is associated with only modestly increased risk of myocardial infarction, ischemic heart disease, and early death. **(*Arterioscler Thromb Vasc Biol*. 2012;32:822-829.)**

Key Words: acute coronary syndromes ■ ischemic heart disease ■ death ■ myocardial infarction ■ telomere length

Telomeres are protective chromosomal caps at the linear ends of chromosomes consisting of a variable number of TTAGGG repeats.[1] Telomeres shorten with each cell cycle in most cells and therefore reflect organism aging at a cellular level.[1,2] Accordingly, telomere length decreases with increasing age[3,4] but also with male sex, smoking, adiposity, oxidative stress, UV irradiation, and low socioeconomic status.[2,3,5–7] It has therefore been a matter of speculation whether short telomere length is associated with increased risk of cardiovascular disease and early death.[5,8–10]

Previous studies of telomere length and human disease have been limited by cumbersome techniques mainly allowing low-throughput measurements.[11] Furthermore, the majority of studies so far have mainly been smaller case-control studies rather than large prospective studies of unselected individuals from the general population.[12] Accordingly, there is a need for large studies of the general population with extended follow-up, with telomere length measured reliably with a high-throughput method to examine the influence of short telomere length on cardiovascular disease and early death.

We tested the hypothesis that short telomere length is associated with increased risk of myocardial infarction, ischemic heart disease, and early death. For this reason, we developed a high-throughput real-time polymerase chain reaction assay calibrated to measure absolute telomere length. Subsequently, we measured 19 838 individuals from 2 prospective studies of the Danish general population from the Copenhagen City Heart Study and the Copenhagen General Population Study, followed for up to 19 years.

Methods

Study Design

The Copenhagen City Heart Study is a population-based prospective study initiated in 1976 to 1978.[13,14] Participants aged 20 years or above were randomly invited from the Central Population Register and reexamined in 1981 to 1983, 1991 to 1994, and 2001 to 2003, with younger participants added at each reexamination. Whole blood samples from the last 2 examinations were used for DNA isolation. We included 9765 participants with available DNA samples.

The Copenhagen General Population Study is a parallel population-based prospective study initiated in 2003 and still recruiting.[15] Participant selection and data collection were similar to that in

Received on: August 22, 2011; final version accepted on: December 7, 2011.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.111.237271/-/DC1.

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Arterioscler Thromb Vasc Biol **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.111.237271**

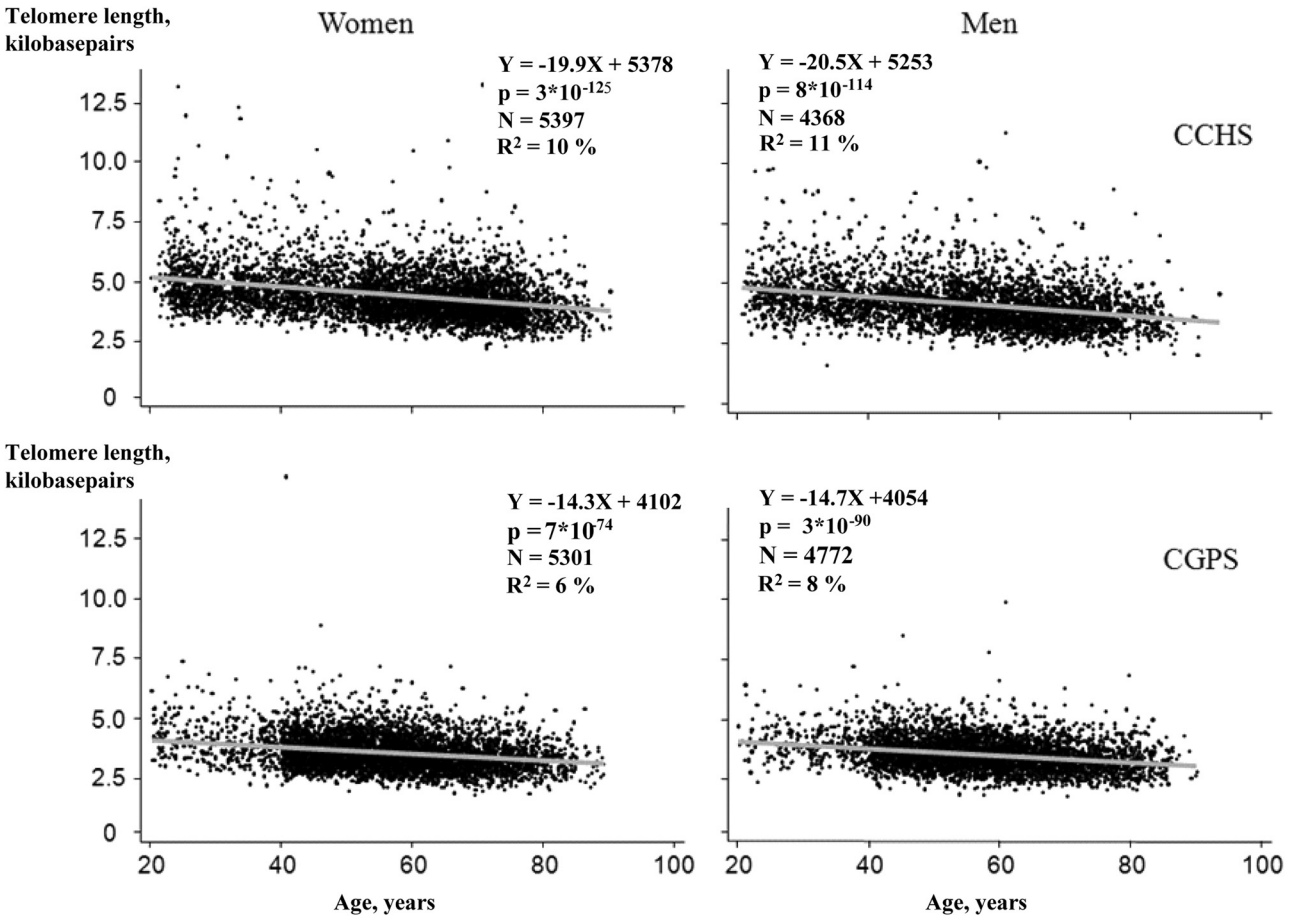

Figure 1. Telomere length in kbp by age in years at blood sampling. Linear regressions are shown in equations and gray lines. N=number of participants. Probability values are for the slope from the linear regression. CCHS indicates Copenhagen City Heart Study; CGPS, Copenhagen General Population Study.

the Copenhagen City Heart Study. We included the first 10 073 participants with available DNA samples.

All participants in both studies were white and of Danish descent. There was no overlap of individuals between the 2 studies, thus permitting independent confirmation of findings in each study.

Covariates

Before examinations, participants filled in self-administered questionnaires concerning present and past lifestyle and health status. This was completed on the day of examination, before physical examination, blood sampling, and measurement of cardiovascular risk factors. The following covariates were obtained or measured[13–15]: age at examination (years), gender (male/female), total cholesterol levels (mmol/L), triglyceride levels (mmol/L), high-density lipoprotein cholesterol levels (mmol/L), high sensitivity C-reactive protein levels (mg/L), use of lipid lowering therapy (no/yes), body mass index (measured weight in kilograms divided by squared measured height in meters), hypertension (no/yes) (yes if participants had systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or reported use of antihypertensive medication), diabetes mellitus (no/yes) (yes if nonfasting blood glucose was >11 mmol/L or if the participant reported having diabetes mellitus or using antidiabetic medication), current smoking (no/yes), heavy alcohol intake (no/yes) (yes if female and male participants reported a weekly alcohol intake above 87.5 and 175 g, respectively), physical inactivity (no/yes) (yes if participants reported <4 hours of leisure time physical activity per week), postmenopausal (no/yes) (yes if women reported being postmenopausal or using hormone replacement therapy or were aged 60 years or above), and use of hormone replacement therapy (yes if women reported use of hormone replacement therapy).

End Points

Using the unique Danish personal identification numbers, date of death was obtained from the Danish Civil Registration System, whereas diagnoses and dates of myocardial infarction and ischemic heart disease were obtained from the national Danish Patient Registry and the national Danish Cause of Death Registry. Diagnoses were classified according to the World Health Organization International Classification of Disease (ICD), 8th revision (ICD-8 codes 410 for myocardial infarction and 410–414 for ischemic heart disease) until 1993, and ICD 10th revision thereafter (ICD-10 codes I21–I22 for myocardial infarction and I20–I25 for ischemic heart disease). Follow-up was 100% complete; that is, we did not lose track of even a single individual.

Telomere Length

We measured telomere length in DNA from leukocytes in peripheral blood, a measurement highly correlated with telomere length in cells from other tissues.[8–10] Telomere length was measured on a CFX384 real-time polymerase chain reaction detection system (Bio-Rad Laboratories), using a modified monochrome multiplex quantitative polymerase chain reaction method.[16,17] Failed samples were measured a second time, and a third if they failed again. Therefore, valid measurements of telomere lengths were available for more than 99.9% of participants. The interassay coefficient of variation of the internal NTERA-2 control was 2% for Ct values at mean values of 17.9 cycles for the telomere assay and 9% for absolute telomere length at the mean level of 2534 base pairs. For a complete

Table 1. Baseline Characteristics of Participants From the General Population by Quartiles of Decreasing Telomere Length

	Copenhagen City Heart Study					Copenhagen General Population Study				
	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	*P* for Trend	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	*P* for Trend
Telomere length, kbp	11.79–4.65	4.64–4.01	4.00–3.49	3.48–1.54		14.81–3.66	3.65–3.19	3.18–2.76	2.75–1.48	
No. of participants	2433	2437	2446	2449		2517	2518	2518	2520	
Age, y	52 (36–64)	56 (41–67)	61 (50–70)	67 (58–74)	3.9×10^{-224}	52 (44–60)	55 (46–64)	57 (48–67)	62 (51–71)	1.4×10^{-134}
Men	997 (41)	1026 (42)	1110 (45)	1235 (50)	2.1×10^{-12}	1075 (43)	1206 (48)	1200 (48)	1291 (51)	1.3×10^{-8}
Total cholesterol, mmol/L	5.8 (5.0–6.7)	5.9 (5.0–6.9)	6.0 (5.3–7.0)	6.1 (5.3–6.9)	1.0×10^{-17}	5.6 (4.9–6.3)	5.6 (4.9–6.4)	5.7 (4.9–6.4)	5.7 (5.0–6.4)	0.001
Triglycerides, mmol/L	1.4 (1.0–2.1)	1.5 (1.1–2.1)	1.6 (1.1–2.3)	1.6 (1.2–2.2)	6.7×10^{-18}	1.4 (1.0–2.1)	1.5 (1.0–2.2)	1.5 (1.0–2.3)	1.6 (1.1–2.3)	2.7×10^{-13}
HDL cholesterol, mmol/L	1.5 (1.2–1.9)	1.5 (1.2–1.8)	1.5 (1.2–1.8)	1.5 (1.2–1.8)	0.03	1.6 (1.3–1.9)	1.6 (1.3–1.9)	1.5 (1.2–1.9)	1.5 (1.2–1.9)	0.01
C-reactive protein, mg/L	1.7 (1.2–3.1)	1.7 (1.2–3.1)	1.9 (1.3–3.5)	2.1 (1.3–3.8)	9.1×10^{-36}	1.3 (0.6–2.9)	1.4 (0.7–3.2)	1.4 (0.7–3.1)	1.7 (0.8–3.7)	1.7×10^{-16}
Lipid-lowering therapy	23 (1.0)	20 (0.8)	29 (1.2)	38 (1.6)	0.02	118 (4.7)	143 (5.7)	196 (7.9)	224 (9.0)	3.6×10^{-11}
Body mass index, kg/m^2	24 (22–27)	25 (22–28)	25 (23–28)	26 (23–29)	6.6×10^{-27}	25 (23–28)	26 (23–29)	26 (24–29)	26 (24–29)	5.9×10^{-9}
Hypertension	1080 (44)	1221 (50)	1372 (56)	1562 (64)	6.9×10^{-46}	1284 (51)	1428 (57)	1485 (59)	1645 (65)	1.3×10^{-24}
Diabetes mellitus	72 (3)	81 (3)	105 (4)	156 (6)	8.3×10^{-10}	98 (4)	107 (4)	112 (4)	137 (5)	0.009
Current smokers	1053 (43)	1137 (47)	1199 (49)	1234 (50)	1.6×10^{-7}	648 (26)	683 (28)	702 (28)	766 (31)	3×10^{-4}
Heavy alcohol intake	990 (41)	909 (37)	919 (38)	876 (36)	9×10^{-5}	426 (17)	469 (19)	445 (18)	453 (18)	0.08
Physical inactivity	938 (55)	800 (54)	696 (56)	547 (62)	0.002	1137 (56)	996 (54)	946 (55)	794 (55)	0.99
Postmenopausal*	760 (53)	857 (60)	998 (70)	1061 (74)	5.1×10^{-94}	776 (54)	815 (63)	858 (66)	900 (74)	3.1×10^{-25}
Use of HRT*	204 (14)	208 (15)	219 (16)	216 (18)	0.007	201 (14)	159 (12)	191 (15)	190 (16)	0.25

Continuous variables are shown as medians (interquartile ranges), and categorical variables are shown as numbers (%). Only measured values are shown; covariates were more than 99% complete. HDL indicates high-density lipoprotein; HRT, hormone replacement therapy.

*Women only.

description of this method, see the Appendix in the online-only Data Supplement.

Statistical Analyses

We used the statistical software package STATA version 11.1 for analysis (StataCorp, College Station, TX). Two-sided $P<0.05$ was significant. We used Cox proportional hazard regression with left-truncated age as the time scale to calculate hazard ratios, which means that the analyses automatically adjusted for age; when we mention in this article that analyses were adjusted for age, we refer to this automatic age adjustment. For all end points, follow-up began at the day of blood sampling. Participants diagnosed with an end point before study entry were excluded from analyses, which is why the number of participants varies between end points. For all end points, follow-up ended at first incident diagnosis, death, emigration, or August 17, 2010, whichever came first. The maximum and median follow-ups were 19 and 17 years, respectively, in the Copenhagen City Heart Study and 7 and 6 years in the Copenhagen General Population Study. Cox regressions were adjusted for age, gender, and study, or multifactorially adjusted for age, gender, study, levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, C-reactive protein, use of lipid lowering therapy, body mass index, hypertension, diabetes mellitus, current smoking, heavy alcohol intake, physical inactivity, postmenopausal status (women only), and use of hormone replacement therapy (women only). Information on covariates was more than 99% complete, and missing continuous covariates were imputed based on age and gender, whereas missing categorical values were assigned to a missing category. For the Copenhagen City Heart Study, data from the 1991 to 1994 and 2001 to 2003 examinations were used as time-dependent covariates for multifactorial adjustment. The proportional hazard assumption was assessed visually by plotting $-\ln(-\ln(\text{survival}))$ versus $\ln(\text{age})$: no major violations were observed. For trend test, participants were categorized according to decreasing telomere length in study-specific quartiles and deciles coded 1 to 4 and 1 to 10, respectively, with the first quartile or decile consisting of participants with the longest telomeres. Interaction between telomere length and each of the covariates adjusted for in the models were investigated by including a 2-factor interaction term and testing them for significance using a Wald test.

Ethical Considerations

The Danish Data Protection Agency, Herlev Hospital, Copenhagen University Hospital, and a local Danish ethical committee approved the studies (KF100.2039/91 and H-KF01-144/01). All participants gave written informed consent.

Results

Telomere Length and Age

Telomere length decreased linearly with increasing age in women and men in both studies of the general population ($P=7\times10^{-74}$ to $P=3\times10^{-125}$) (Figure 1); R^2 between age and telomere length ranged from 6% to 11%. Telomere length decreased by 20 base pairs per year of increase in age in the Copenhagen City Heart Study and by 14.5 base pairs in the Copenhagen General Population Study. In addition, as shown in Table 1, decreasing telomere length was also associated with male gender, increasing levels of total cholesterol, triglycerides, and C-reactive protein, decreasing levels of high-density lipoprotein cholesterol, use of lipid lowering therapy, increasing body mass index, hypertension, diabetes mellitus, current smoking, decreasing heavy alcohol intake, physical inactivity, and for women only postmenopausal status and use of hormone replacement therapy.

Telomere Length and Myocardial Infarction

During follow-up, 929 participants developed myocardial infarction in the 2 studies combined. Per 1000–base pair decrease in telomere length, the multifactorially adjusted hazard ratio of myocardial infarction was 1.10 (95% CI 1.01–1.19) (Table 2). Similar hazard ratios were found in an age-, gender-, and study-adjusted model; in each study alone; and in each gender separately. When stratifying in subgroups of age, gender, and biochemical and lifestyle cardiovascular risk factors, we found no evidence of interaction between

Table 2. Risk of Myocardial Infarction, Ischemic Heart Disease, and Early Death per 1000-bp Decrease in Telomere Length

	Hazard Ratio (95% CI)					
	Combined Studies, Adjustment		Copenhagen City Heart Study, Adjustment		Copenhagen General Population Study, Adjustment	
	Age, Gender, Study	Multifactorially*	Age and Gender	Multifactorially*	Age and Gender	Multifactorially*
All						
Myocardial infarction	1.13 (1.04–1.23)	1.10 (1.01–1.19)	1.11 (1.02–1.21)	1.08 (0.99–1.18)	1.37 (1.07–1.75)	1.31 (1.02–1.67)
Ischemic heart disease	1.08 (1.03–1.14)	1.06 (1.00–1.11)	1.07 (1.01–1.13)	1.04 (0.99–1.10)	1.17 (1.01–1.37)	1.14 (0.98–1.33)
Early death	1.13 (1.09–1.18)	1.09 (1.05–1.13)	1.14 (1.09–1.18)	1.09 (1.05–1.14)	1.11 (0.97–1.26)	1.06 (0.93–1.21)
Women						
Myocardial infarction	1.17 (1.03–1.32)	1.13 (1.00–1.29)	1.14 (1.00–1.30)	1.12 (0.98–1.28)	1.51 (0.98–2.31)	1.49 (0.97–2.29)
Ischemic heart disease	1.06 (0.98–1.14)	1.02 (0.95–1.10)	1.04 (0.96–1.13)	1.00 (0.93–1.08)	1.16 (0.91–1.48)	1.14 (0.89–1.45)
Early death	1.12 (1.07–1.19)	1.08 (1.02–1.14)	1.13 (1.07–1.19)	1.08 (1.02–1.14)	1.09 (0.88–1.33)	1.04 (0.85–1.29)
Men						
Myocardial infarction	1.11 (1.00–1.23)	1.08 (0.97–1.20)	1.08 (0.97–1.21)	1.06 (0.94–1.19)	1.30 (0.96–1.75)	1.23 (0.91–1.67)
Ischemic heart disease	1.11 (1.03–1.20)	1.09 (1.01–1.18)	1.10 (1.01–1.19)	1.08 (1.00–1.18)	1.18 (0.97–1.44)	1.14 (0.93–1.39)
Early death	1.14 (1.08–1.20)	1.10 (1.04–1.16)	1.15 (1.08–1.21)	1.11 (1.05–1.17)	1.11 (0.94–1.32)	1.06 (0.89–1.26)

*Adjusted for age, gender, study, cholesterol, triglycerides, high-density lipoprotein cholesterol, C-reactive protein, use of lipid lowering therapy, body mass index, hypertension, diabetes mellitus, smoking, heavy alcohol intake, and physical inactivity; in women, also adjusted for postmenopausal status and hormone replacement therapy.

these factors and decreasing telomere length on risk of myocardial infarction after Bonferroni correction (Figure 2; required probability value $<0.05/15=0.003$). When divided into study-specific quartiles and deciles of telomere length in the 2 studies combined, we found a multifactorially adjusted hazard ratio for myocardial infarction of 1.18 (0.97–1.43) in the quartile and 1.49 (1.07–2.07) in the decile with the shortest telomeres versus participants in the quartile and decile, respectively, with the longest telomeres (trend tests across quartiles and deciles were $P=0.01$ and $P=0.002$; Figure 3).

Telomere Length and Ischemic Heart Disease

During follow-up, 2038 participants developed ischemic heart disease in the 2 studies combined. Per 1000–base pair decrease in telomere length, the multifactorially adjusted hazard ratio of ischemic heart disease was 1.06 (95% CI 1.00–1.11) (Table 2). Similar hazard ratios were found in an age-, gender-, and study-adjusted model; in each study alone; and in each gender separately. When stratifying in subgroups of age, gender, and biochemical and lifestyle cardiovascular risk factors, we found no evidence of interaction between these factors and decreasing telomere length on risk of ischemic heart disease after Bonferroni correction (Figure 2). When divided into quartiles and deciles of telomere length in the 2 studies combined, we found a multifactorially adjusted hazard ratio for ischemic heart disease of 1.06 (0.93–1.20) in the quartile and 1.24(1.01–1.53) and the decile with the shortest telomeres versus participants in the quartile and decile, respectively, with the longest telomeres (trend tests across quartiles and deciles were $P=0.22$ and $P=0.03$; Figure 3).

Telomere Length and Early Death

During follow-up, 4342 participants died in the 2 studies combined. Per 1000–base pair decrease in telomere length, the multifactorially adjusted hazard ratio of early death was 1.09 (95% CI 1.05–1.13) (Table 2). Similar hazard ratios were found in an age-, gender-, and study-adjusted model; in each study alone; and in each gender separately. When stratifying in subgroups of age, gender, and biochemical and lifestyle cardiovascular risk factors, we found no evidence of interaction between these factors and decreasing telomere length on risk of early death after Bonferroni correction (Figure 2). When divided into quartiles and deciles of telomere length in the 2 studies combined, we found a multifactorially adjusted hazard ratio for early death of 1.18 (1.08–1.29) in the quartile and 1.25 (1.07–1.46) in the decile with the shortest telomeres versus participants in the quartile and decile, respectively, with the longest telomeres (trend tests across quartiles and deciles were $P=4\times10^{-4}$ and $P=6\times10^{-5}$; Figure 3).

Discussion

After measuring telomere length using a high-throughput real-time polymerase chain reaction method in 19 838 individuals from the general population followed for up to 19 years, we observed that short telomere length was associated only modestly with increased risk of myocardial infarction, ischemic heart disease, and early death. We cannot exclude that these modest risk estimates could be explained by residual undetected confounding.

The mechanism behind these findings could be that short telomere length is a marker of a systemic degenerative phenotype arising after years of exposure to cardiovascular risk factors, causing cellular damage and resulting in an increasing frequency of tissue mitosis and thereby shorter telomere length.[18] A systemic degenerative phenotype could then lead to the observed increased risk of myocardial infarction, ischemic heart disease, and early death.

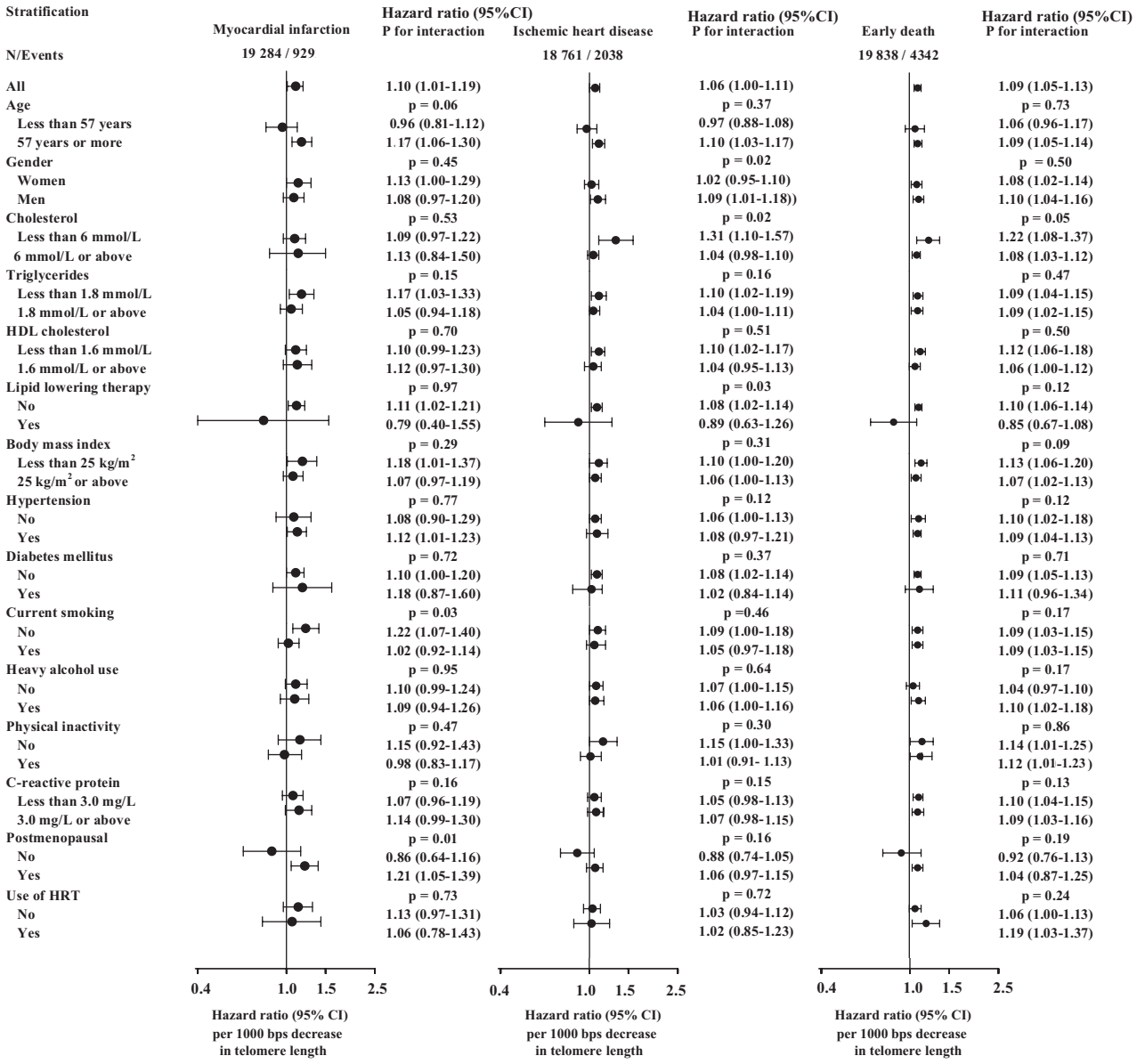

Figure 2. Risk of myocardial infarction, ischemic heart disease, and early death per 1000-bp decrease in telomere length for both studies combined. Black dots indicate hazard ratios and horizontal lines indicate 95% confidence intervals. HDL indicates high-density lipoprotein; HRT, hormone replacement therapy.

We confirmed previous findings of a strong linear correlation between decreasing telomere length and increasing age[4,7,19–25] demonstrating the validity of our high-throughput assay. In our studies of the general population, telomere length decreased by 14.5 to 20 base pairs per year. This is similar to the finding in previous reports of 14 to 29 base pairs lost per year,[3,20,23] but less than that in other reports of up to 7 base pairs lost per year.[22–24,26,28] These study differences may reflect interpopulation variation,[29] or they may be a result of interassay variation because some former assays included subtelomeric regions.[30]

For myocardial infarction, the present observation of association between decreasing telomere length and increased risk is in accordance with previous studies,[4,20,27,31] all smaller than the present one. Following 19 284 individuals prospectively for up to 19 years, we detected 929 incident myocardial infarctions and a hazard ratio for myocardial infarction of 1.18 (95% CI 0.97–1.43) for individuals in the shortest versus longest telomere quartile, whereas the Bruneck Study, a prospective study of 800 individuals followed for 10 years, observed 43 incident myocardial infarctions and a hazard ratio of 3.58 (1.32–9.70) for individuals in the shortest versus the longest telomere tertile.[4] Also, the Cardiovascular Health Study, a prospective study of 388 individuals followed for 7 years, reported 36 incident myocardial infarctions and a hazard ratio for myocardial infarction of 1.55 (0.85–2.83) per 1000–base pair decrease in telomere length,[27] whereas our comparable hazard ratio was 1.10 (1.01–1.19). Finally, a case-control study from Leicester of 203 cases and 180 controls reported an odds ratio for myocardial infarction of 2.79 (1.53–5.11) for individuals in the shortest versus the longest telomere length quartile.[20] Taken together, previous

Decreasing telomere length	Participants no.	Events no.	Incidence rate per 10,000 person-years	Hazard ratio (95% CI)	Hazard ratio (95% CI)	P-trend
Quartiles						
Myocardial infarction						
1st	4864	163	33(28-39)		1.00	p = 0.01
2nd	4853	187	39(34-46)		0.95 (0.77-1.17)	
3rd	4801	253	56(50-63)		1.10 (0.90-1.34)	
4th	4766	326	80(72-89)		1.18 (0.97-1.43)	
Ischemic heart disease						
1st	4779	393	83(75-91)		1.00	p = 0.22
2nd	4739	444	98(89-107)		0.95 (0.83-1.09)	
3rd	4673	549	128(117-139)		1.02 (0.89-1.16)	
4th	4570	652	170(158-184)		1.06 (0.93-1.20)	
Early death						
1st	4950	705	139(129-150)		1.00	p = $4x10^{-4}$
2nd	4955	871	178(167-190)		1.00 (0.90-1.10)	
3rd	4964	1173	248(234-262)		1.08 (0.98-1.19)	
4th	4969	1593	369(351-387)		1.18 (1.08-1.29)	
				0.5 1.0 1.5		
Deciles						
Myocardial infarction						
1st	1946	50	25(19-32)		1.00	p = 0.002
2nd	1945	81	42(34-52)		1.48 (1.04-2.11)	
3rd	1946	59	31(24-39)		0.95 (0.65-1.39)	
4th	1933	78	41(33-51)		1.20 (0.84-1.71)	
5th	1947	82	44(35-54)		1.19 (0.84-1.69)	
6th	1937	89	48(39-59)		1.18 (0.83-1.67)	
7th	1905	104	58(48-71)		1.34 (0.95-1.88)	
8th	1918	118	68(57-82)		1.43 (1.03-2.00)	
9th	1907	120	72(60-86)		1.39 (1.00-1.95)	
10th	1900	148	95(81-112)		1.49 (1.07-2.07)	
Ischemic heart disease						
1st	1918	134	68(57-80)		1.00	p = 0.03
2nd	1901	164	88(75-102)		1.12 (0.89-1.40)	
3rd	1911	160	86(74-101)		0.99 (0.79-1.24)	
4th	1898	175	96(83-111)		1.03 (0.82-1.30)	
5th	1890	204	114(100-132)		1.17 (0.94-1.46)	
6th	1891	201	112(98-129)		1.03 (0.83-1.29)	
7th	1848	222	131(115-150)		1.13 (0.91-1.40)	
8th	1854	236	144(127-164)		1.12 (0.91-1.39)	
9th	1829	243	155(137-176)		1.14 (0.92-1.42)	
10th	1821	299	207(185-232)		1.24 (1.01-1.53)	
Early Death						
1st	1976	235	112(99-128)		1.00	p = $3x10^{-5}$
2nd	1981	288	144(128-162)		1.03 (0.86-1.23)	
3rd	1988	329	166(148-184)		1.12 (0.94-1.33)	
4th	1982	347	177(159-197)		1.04 (0.87-1.24)	
5th	1978	377	195(177-216)		1.08 (0.91-1.28)	
6th	1993	433	223(203-245)		1.06 (0.89-1.25)	
7th	1974	482	258(236-282)		1.19 (1.01-1.40)	
8th	1984	516	282(259-307)		1.15 (0.97-1.35)	
9th	1987	591	335(309-364)		1.23 (1.05-1.44)	
10th	1995	744	449(419-483)		1.25 (1.07-1.46)	
				0.5 1.0 1.5 2.5		

Figure 3. Risk of myocardial infarction, ischemic heart disease, and early death by decreasing telomere length in quartiles and deciles for both studies combined. Black dots indicate hazard ratios, and horizontal lines indicate 95% confidence intervals. Probability values for trend are for decreasing telomere length in quartiles or deciles.

and present studies all support that decreasing telomere length is associated with increased risk of myocardial infarction, whereas the present study, with the most statistical power, suggests that the magnitude of the risk estimate is more modest than originally thought.

For risk of ischemic heart disease, the present study detected 2038 events and a hazard ratio of 1.06 (0.93–1.20) for individuals in the shortest versus longest telomere quartiles, a modest effect size compared with that in a previous study.[32] Here, in the West of Scotland Primary Prevention Study, a nested case-control study of 484 cases and 1058 controls, an odds ratio for ischemic heart disease of 1.44 (1.10–1.90) was observed for individuals in the shortest versus the longest telomere tertile.[32] However, as participants in that study were randomly assigned to treatment with statins or placebo, these results not be directly comparable to those of our study.

For risk of early death, our observed 4342 deaths and a hazard ratio of 1.09 (1.05–1.13) per 1000–base pair decrease in telomere length is similar to that of 1.22 (0.91–1.63) observed in the Cardiovascular Health Study, a prospective study of 419 individuals followed for 7 years with 156 deaths.[27] However, a prospective study of 143 individuals aged 60 years or older followed for a maximum of 20 years, reported 101 deaths and a 1.86 (1.22–2.83)-fold mortality rate for individuals in the shortest versus the longest half of telomere length.[33] In contrast to our findings and that of the other reports mentioned above,[27,33] a prospective study of 812 individuals aged 73 years or older observed 412 deaths but no association between telomere length and overall survival.[34] Taken together, the combined evidence supports that short telomere length is associated with a modest increased risk of early death.

Strengths of the present study include the large study size of 19 838 participants from the general population followed prospectively for up to 19 years. Second, telomere length was measured with a new, high-throughput assay, validated through inverse association with age. Third, telomere length was measured on more than 99.9% of available participants because of several rounds or reruns. Fourth, because of the national Danish Civil Registration System and the national Danish Patient Registry, we had complete information on incident myocardial infarction, ischemic heart disease, and death during follow-up. Fifth, using 2 independent studies, we were able to confirm findings across studies, minimizing the risk of chance findings. Sixth, we determined the absolute telomere length of our calibrator to 5290 base pairs, which was comparable to previous reports for this cell line of 5360 and 6500 base pairs.[35,36]

Limitations of the present study include that we examined risk of cardiovascular disease and early death in whites only, and our results may therefore not necessarily be applicable to other ethnicities. Also, we measured telomere length in leukocytes from peripheral blood only, and not in all cell types in the body; however, leukocyte telomere length is highly correlated with that in cells from other tissues.[5,8–10] Finally, there is a question of whether a variable such as telomere length that is closely related with age can be a strong "age-independent" determinant of another variable, such as morbidity and mortality. However, although decreasing telomere length was associated with increasing age, age explained only 6% to 11% of the variation in telomere length, and we adjusted for age in all our analyses.

In conclusion, short telomere length is associated only modestly with increased risk of myocardial infarction, ischemic heart disease, and early death.

Acknowledgments

We thank laboratory technician Anja Jochumsen for assisting with the large-scale telomere measurements. We are indebted to staff and participants of the Copenhagen City Heart Study and the Copenhagen General Population Study for their important contributions.

Sources of Funding

This work was supported by Chief Physician Johan Boserup and Lise Boserup's Foundation, the Copenhagen County Foundation, and the Danish Heart Foundation, all of which are nonprofit organizations with no right to approve or disapprove of the work.

Disclosures

Dr Cawthon stands to profit if Telome Health, Inc, sells telomere length measurements by his assay. For this study, Dr Cawthon helped his coauthors develop a new assay for high throughput. Dr. Cawthon did not do any of the telomere length measurements on participants in the present study and declares that his involvement in the study cannot have biased any of the associations found.

References

1. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet*. 2005;6:611–622.
2. von Zglinicki T, Martin-Ruiz CM. Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med*. 2005;5:197–203.
3. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, Kimura M, Lu X, Spector TD, Aviv A. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med*. 2008;168:154–158.
4. Willeit P, Willeit J, Brandstatter A, Ehrlenbach S, Mayr A, Gasperi A, Weger S, Oberhollenzer F, Reindl M, Kronenberg F, Kiechl S. Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. *Arterioscler Thromb Vasc Biol*. 2010;27:1649–1655.
5. Butt HZ, Atturu G, London NJ, Sayers RD, Bown MJ. Telomere length dynamics in vascular disease: a review. *Eur J Vasc Endovasc Surg*. 2010;40:17–26.
6. Cherkas LF, Aviv A, Valdes AM, Hunkin JL, Gardner JP, Surdulescu GL, Kimura M, Spector TD. The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell*. 2006;5:361–365.
7. Han J, Qureshi AA, Prescott J, Guo Q, Ye L, Hunter DJ, De V, I. A prospective study of telomere length and the risk of skin cancer. *J Invest Dermatol.* 2009;129:415–421.
8. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev* 2000; 119:89–99.
9. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. *Pediatr Res*. 2002;52:377–381.
10. Wilson WR, Herbert KE, Mistry Y, Stevens SE, Patel HR, Hastings RA, Thompson MM, Williams B. Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. *Eur Heart J*. 2008;29:2689–2694.
11. Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, Harley CB, Aviv A. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nat Protoc*. 2010;5:1596–1607.
12. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci*. 2011;66: 202–213.

13. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA*. 2008;300:2142–2152.
14. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298:299–308.
15. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med*. 2008;359:1897–1908.
16. *Users Bulletin #2*. Available at: www3.appliedbiosystems.com/CMS/groups/mcb_support/documents/generaldocuments/CMS_040980.pdf. Accessed December 27, 2011.
17. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res*. 2009;37:e21.
18. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J, Horner JW, Maratos-Flier E, DePinho RA. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011;469:102–106.
19. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med*. 2006;12:1133–1138.
20. Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol.* 2003;23:842–846.
21. Butler MG, Tilburt J, DeVries A, Muralidhar B, Aue G, Hedges L, Atkinson J, Schwartz H. Comparison of chromosome telomere integrity in multiple tissues from subjects at different ages. *Cancer Genet Cytogenet*. 1998;105:138–144.
22. Farzaneh-Far R, Lin J, Epel E, Lapham K, Blackburn E, Whooley MA. Telomere length trajectory and its determinants in persons with coronary artery disease: longitudinal findings from the heart and soul study. *PLoS One*. 2010;5:e8612.
23. Maubaret CG, Salpea KD, Jain A, Cooper JA, Hamsten A, Sanders J, Montgomery H, Neil A, Nair D, Humphries SE. Telomeres are shorter in myocardial infarction patients compared to healthy subjects: correlation with environmental risk factors. *J Mol Med*. 2010;88:785–794.
24. Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. *Lancet*. 2001;358:472–473.
25. Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstatter A, Kronenberg F, Kiechl S. Telomere length and risk of incident cancer and cancer mortality. *JAMA*. 2010;304:69–75.
26. Ehrlenbach S, Willeit P, Kiechl S, Willeit J, Reindl M, Schanda K, Kronenberg F, Brandstatter A. Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. *Int J Epidemiol*. 2009;38:1725–1734.
27. Fitzpatrick AL, Kronmal RA, Gardner JP, Psaty BM, Jenny NS, Tracy RP, Walston J, Kimura M, Aviv A. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol*. 2007;165:14–21.
28. Huda N, Tanaka H, Herbert BS, Reed T, Gilley D. Shared environmental factors associated with telomere length maintenance in elderly male twins. *Aging Cell*. 2007;6:709–713.
29. Eisenberg DT, Salpea KD, Kuzawa CW, Hayes MG, Humphries SE. Substantial variation in qPCR measured mean blood telomere lengths in young men from eleven European countries. *Am J Hum Biol*. 2011;23:228–231.
30. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30:e47.
31. Zee RY, Michaud SE, Germer S, Ridker PM. Association of shorter mean telomere length with risk of incident myocardial infarction: a prospective, nested case-control approach. *Clin Chim Acta*. 2009;403:139–141.
32. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ. 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.
33. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*. 2003;361:393–395.
34. Bischoff C, Petersen HC, Graakjaer J, Andersen-Ranberg K, Vaupel JW, Bohr VA, Kolvraa S, Christensen K. No association between telomere length and survival among the elderly and oldest old. *Epidemiology*. 2006;17:190–194.
35. Wang Y, Fang MY. Effect of ginseng saponin, arsenic trioxide, β-elemene combined with CTX on telomere-telomerase system in K562 cell line. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2006;14:1089–1095.
36. Fehrer C, Voglauer R, Wieser M, Pfister G, Brunauer R, Cioca D, Grubeck-Loebenstein B, Lepperdinger G. Techniques in gerontology: cell lines as standards for telomere length and telomerase activity assessment. *Exp Gerontol*. 2006;41:648–651.