

Association of Telomere Length in Older Men With Mortality and Midlife Body Mass Index and Smoking

Timo E. Strandberg,¹ Outi Saijonmaa,² Reijo S. Tilvis,³ Kaisu H. Pitkälä,⁴ Arto Y. Strandberg,³
Tatu A. Miettinen,³ and Frej Fyhrquist²

¹Unit of General Practice, Institute of Health Sciences/Geriatrics, University of Oulu and University Hospital, Finland.

²Minerva Institute for Medical Research and University of Helsinki, Finland.

³Department of Medicine, Geriatric Clinic, University of Helsinki, Finland.

⁴Unit of General Practice, Helsinki University Central Hospital, Finland.

Address correspondence to Timo E. Strandberg, MD, PhD, Unit of General Practice, Institute of Health Sciences/Geriatrics, University of Oulu and University Hospital, PO Box 5000, FIN 90014 Oulun yliopisto, Finland. Email: timo.strandberg@oulu.fi

Background. Leukocyte telomere length has been taken as a measure of biological age but several inconsistencies exist.

Methods. We investigated associations between leukocyte telomere length in old age, midlife risk factors, and mortality. The Helsinki Businessmen Study (a cohort of mainly business executives, born 1919–1934) had baseline assessments of cardiovascular risk factors including body mass index between 1964 and 1973 at a mean age of 40. Leukocyte telomere length and proportion of short telomeres were measured from DNA samples collected in 2002–2003 ($n = 622$, mean age 78 years). Body mass index and smoking in old age were assessed from questionnaires. Total mortality was verified from registers through January 2010. Main outcome measures were relationships between telomeres, body mass index, smoking, and mortality.

Results. Leukocyte telomere length and notably proportion of short telomeres (<5kb) in old age were significantly ($p = .008$ after full adjustments) and in a graded manner associated with midlife overweight and smoking. The associations were independent of age and cardiovascular risk factors including postload glucose. Associations with body mass index and smoking were nonsignificant in old age, and telomere length did not predict 7-year total mortality.

Conclusions. We conclude that smoking and overweight in midlife, irrespective of glucose, cholesterol and blood pressure, are related to shorter leukocyte telomeres in old men. Telomere length in old age did not predict total mortality possibly due to competing causes.

Key Words: Telomeres—Obesity—Smoking—Mortality.

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TELOMERES are specialized nucleoprotein structures at the end of chromosomes, which preserve chromosome stability and integrity. Telomeres shorten with each cell division finally leading to permanent growth arrest and senescence of the cell (1). Telomerase enzyme activity repairs telomere shortening, and genetic variation in telomerase has been correlated with longevity (2). Because telomeric DNA is, on the other hand, prone to oxidative damage (3,4), telomere shortening could serve as an overall indicator of genetic factors, replicative history and cumulative genomic damage of somatic cells. Therefore, the telomere length has been considered a marker of biological age (5–7). Leukocyte telomere length (LTL) has previously been associated with chronic diseases, such as cardiovascular diseases (8–11), and diabetes (12–16), as well as cardiovascular risk factors and lifestyle (17–25). However, there are many conflicting reports, and the relation between LTL and mortality is equivocal (26–31). Also, it is still obscure whether shorter

LTL is a cause or consequence of senescence. For example, diabetes and its complications may shorten telomeres due to oxidative stress and lead to premature senescence or shortening of telomeres (due to telomerase deficiency) may lead to disturbances in pancreatic cell replication and insulin secretion leading to diabetes (14). In the Helsinki Businessmen Study (32–34), we have information about cardiovascular risk from early midlife until old age. In the present report, we have related LTL measured in late life to both earlier cardiovascular risk factors and follow-up mortality in this cohort.

METHODS

This follow-up study with genetic testing has been approved by the Ethics Committee of the Helsinki University Hospital, Department of Medicine (the most recent application by the code HUS 429/13/03/01/09).

Examinations at Baseline in the 1960s and During Follow-Up

In a long-term outcome study, a cohort of 3,490 healthy Finnish men, born 1919–1934 (the Helsinki Businessmen Study) and aged 30–45 years at baseline, has been prospectively followed from the 1960s up to the present day for cardiovascular risk factors (smoking, body mass index [BMI], blood pressure, and cholesterol; measured between 1964 and 1973), mortality, and quality of life. All participants were Caucasian men from the highest social class being businessmen or executives with similar socioeconomic and job status. The study population and examinations have been described in detail earlier (32–34). Part of the cohort participated in a multifactorial prevention study during the 1970s (32), but exclusion of the intervention group ($n = 612$) did not affect the present results and therefore have been retained in the analyses to improve statistical power.

The cohort has been followed-up with mailed questionnaires (between 1985 and 2010) including questions about current diseases, body weight, and smoking. In 2002–2003, a random sample of men were invited for clinical and laboratory tests whereupon venous blood samples were taken for genetic analyses ($N = 622$); in addition, fasting plasma samples were obtained from 480 men for routine laboratory data (including plasma glucose).

Total mortality of the study cohort through January 2010 was retrieved from the Central Population Register, which keeps registry of all Finnish citizens. According to the Register, assessment of vital status is very reliable for people having their permanent place of residence in Finland (more than 95% of the present cohort) irrespective whether they die in Finland or abroad. Moreover, the assessment of the vital status is also quite reliable for Finnish citizens living permanently abroad. Causes of death were only available through December 2007, and the numbers at that time were too small to draw reliable conclusions about cause-specific mortality.

Telomere Measurement

Telomere length was measured from blood samples as previously described (14) using TeloTAGGG Telomere length assay kits (Roche Molecular Biochemicals, Basel, Switzerland). Briefly, an aliquot (1 μ L) of DNA was digested with HinfI and RsaI (20 U/ μ L DNA each) (Roche) at 37°C for 2 hours. Separation of digested DNA was done by 0.8% agarose gel electrophoresis at 5 V/cm in 0.04 M Tris–acetate, 0.001 M EDTA, pH 8.0, buffer for 2–3 hours.

After electrophoresis, the DNA fragments were transferred by Southern blotting to a positively charged nylon membrane (Hybond N+; Amersham, Little Chalfont, UK) at room temperature using 3 M NaCl and 0.3 M sodium citrate, pH 7.0. The transferred DNA was then fixed on the blotting membrane by ultraviolet cross-linking (UV Stratilinker 1800; Stratagene, La Jolla, CA).

The blotted DNA fragments were hybridized to a digoxigenin-labeled probe specific for telomeric repeats in a hybridization oven (Techne, Burlington, NJ) at 42°C for 3 hours.

The membrane was incubated with a digoxigenin-specific antibody covalently coupled to alkaline phosphatase and then visualized by virtue of alkaline phosphatase–metabolizing CDP-Star, a highly sensitive chemiluminescence substrate. The membrane was then exposed to a hyperfilm ECL (Amersham). Films were analyzed using Adobe PhotoShop and Science Lab 99 Image gauge software (Fuji Photo Film Co Ltd).

Mean size of the telomere restriction fragment (λ) was estimated using the formula $\{\sum\}(OD_i - \text{background}) / \{\sum\}(OD_i - \text{background}/Li)$, where OD_i is the chemiluminescent signal and Li is the length of the telomere restriction fragment at Position i . Interassay coefficient of variation was 3.70% when calculated from an internal control DNA sample in 96 assays.

There is an increasing evidence suggesting that regardless of mean telomere length, one critically short telomere may cause a cell to enter senescence (35–37). Therefore, using the same films as for mean telomere restriction fragment analysis, we also calculated the percentage of short telomeres, shorter than 5 kb, in each telomeric sample. This value was chosen as it was the lowest cutoff limit providing reliable results.

Briefly, the total chemiluminescence intensity of each sample was measured and the signal intensity below molecular size marker 5 kb was quantitated. Percentage of short telomeres = (intensity of chemiluminescence signal below 5 kb – background) \times 100/(total signal intensity – background).

Statistical Methods

LTL and proportion of short telomeres showed a Gaussian distribution and were studied both as continuous variables and divided in tertiles. BMI was divided conventionally in three groups: <25, 25–29, and ≥ 30 kg/m². Spearman's rank correlation and analysis of covariance were used to study the relationship between LTL and risk factors. The cumulative mortality and its associations with LTL and risk factors were assessed with the Kaplan–Meier method using the log-rank test to calculate the significance of differences and the Cox proportional hazards method. Statistical analyses were performed with NCSS 2004 (NCSS, Kaysville, UT). Significance was defined as two-sided $p < .05$.

RESULTS

Mean telomere length was 8.2 kb (SD 0.4), and the mean proportion of short telomeres (<5 kb) was 11.8% (SD 2.4). Mean age at the time of telomere measurement in 2003 was 75.7 years (SD 3.9), median BMI was 25.2 kg/m² (interquartile range 23.4–27.3, $n = 487$), and median plasma glucose was 5.3 mmol/L (interquartile range 5.0–5.9, $n = 492$).

Table 1. Characteristics of the Study Group (N = 622)

Variable	
At start of follow-up in 1964–1973	
Age, y	40.5 (4.3)
Body mass index, median (IQ range), kg/m ²	25.2 (23.8–27.0)
Blood pressure, mmHg	—
Systolic	133.2 (14.4)
Diastolic	85.1 (9.6)
Cholesterol, mmol/L	6.5 (1.1)
Triglycerides (n = 235), mmol/L	1.5 (0.7)
One-hour blood glucose (n = 484), median (IQ range) mmol/L	5.7 (4.6–6.9)
Ever-smokers, n (%)	357 (57.4)
In 2002–2003	
Age, y	75.7 (3.9)
Body mass index, median (IQ range), kg/m ²	25.2 (23.4–27.3)
Plasma glucose (n = 492), mmol/L	5.5 (1.1)
Current smokers, n (%)	35 (7.1)
Telomere length, median (IQ range), kb	8.1 (7.9–8.5)
Proportion of short telomeres, median (IQ range), %	11.8 (10.0–13.4)

Note: Mean (SD) unless otherwise stated. IQ = interquartile range.

Of the men, 7.1% (n = 35 of 492 reporting) were active smokers, 14.6% (n = 91) had diabetes, and 7.3% (n = 36) were obese. Clinical data at baseline of the follow-up (between 1964 and 1973, median 1968) are shown in Table 1.

LTL and the proportion of short telomeres were not significantly correlated with age, serum lipids nor with glucose measured in 2002–2003. Neither there were significant correlations with serum cholesterol, blood pressure nor with blood 1-hour postload glucose measured during the 1960s. There were weak positive correlations between the proportion of short telomeres and BMI measured during the 1960s (r = .09, p = .04), the respective r with BMI in 2002–2003 was 0.07 (p = .13). LTL was not associated with weight change between 1960 and 2003 (r = .003, p = .94). In contrast, age-adjusted telomere length was significantly longer among life-long never-smokers as compared with past or present smokers (8.24 kb, SE 0.03 vs 8.14 kb, SE 0.02, p = .0004), whereas the difference in the proportion of short telomeres tended to be significant (11.56%, SE 0.15 vs 11.95%, SE 0.13, p = .05).

Telomere length according to BMI classes at baseline in the 1960s and in 2002–2003 are shown in Tables 2 and 3, respectively. Especially, the proportion of short telomeres in old age was related to midlife overweight, and the association became stronger after adjusting for cardiovascular risk factors including glucose. The same trend was observed in cross-sectional analyses in 2002–2003, but the differences in LTL between BMI classes were not significant.

The age-adjusted combined effect of BMI and smoking in midlife on LTL in old age is shown in the Figure 1 (Panel A: telomere length and Panel B: proportion of short telomeres) that both midlife risk factors were age independently and in a graded manner associated with shorter LTL in old age. Further adjustment for glucose, cholesterol and blood pressure strengthened the associations (p values .002 and .006, respectively).

During the 7-year follow-up, 130 men (20.9%) died. There was a 3.2-fold increased (p < .001) mortality among current smokers in 2002–2003 as compared with nonsmokers, whereas a U-shaped association was observed with BMI in 2002–2003 and mortality. With BMI 25–29 kg/m² as reference, mortality was 2.3-fold increased (p = .02) among those with BMI ≥30 kg/m², and the 1.3-fold increase among those with BMI <25 kg/m² was not significant (p = .23). Association between total mortality and LTL tertiles was flat (age-adjusted log-rank p = .95). The relationship between tertiles of proportion of short telomeres and total mortality was U-shaped but not significant. In age-adjusted Cox analyses with the second tertile as reference, p values were .11 and .06 for the lowest and highest tertiles, respectively.

DISCUSSION

Although LTL in old age was only weakly associated with current degree of overweight as well as current smoking, we observed a strong and graded association between overweight and smoking in midlife and LTL in late life. To our knowledge, this has not been previously reported. The

Table 2. Telomere Data in Old Age According to Body Mass Index Classes at Start of Follow-Up During the 1960s

Variable	Body Mass Index Class			p Value Between Groups
	<25, n = 224	25–29, n = 221	≥30, n = 35	
Telomere length, kb				
Unadjusted	8.22 (0.03)	8.15 (0.03)	8.11 (0.07)	.13
Adjusted for age	8.22 (0.03)	8.16 (0.03)	8.10 (0.07)	.13
As above + postload glucose (log transformed)	8.23 (0.03)	8.15 (0.03)	8.07 (0.08)	.06
As above + smoking	8.23 (0.03)	8.15 (0.03)	8.07 (0.08)	.08
As above + cholesterol and systolic blood pressure	8.23 (0.03)	8.15 (0.03)	8.06 (0.08)	.06
Proportion of short telomeres, %				
Unadjusted	11.50 (0.15)	11.98 (0.14)	12.42 (0.40)	.017
Adjusted for age	11.50 (0.15)	11.97 (0.14)	12.47 (0.40)	.015
As above + postload glucose (log transformed)	11.45 (0.17)	12.03 (0.16)	12.71 (0.44)	.008
As above + smoking	11.46 (0.17)	12.01 (0.16)	12.76 (0.44)	.008
As above + cholesterol and systolic blood pressure	11.44 (0.17)	12.03 (0.17)	12.75 (0.44)	.008

Note: Mean (SE) unless otherwise stated.

Table 3. Telomere Data in Old Age According to Body Mass Index Classes in 2002–2003

Variable	Body Mass Index Class			<i>p</i> Value Between Groups
	<25, <i>n</i> = 224	25–29, <i>n</i> = 221	≥30, <i>n</i> = 35	
Telomere length, kb				
Unadjusted	8.23 (0.03)	8.15 (0.03)	8.18 (0.07)	.14
Adjusted for age	8.23 (0.03)	8.14 (0.03)	8.17 (0.07)	.10
As above + glucose (log transformed) and smoking	8.22 (0.03)	8.11 (0.03)	8.16 (0.08)	.07
Proportion of short telomeres, %				
Unadjusted	11.52 (0.16)	11.96 (0.17)	12.00 (0.41)	.14
Adjusted for age	11.50 (0.16)	11.97 (0.17)	12.04 (0.41)	.10
As above + glucose (log transformed) and smoking	11.57 (0.19)	12.13 (0.20)	12.01 (0.47)	.13

Note: Mean (SE) unless otherwise stated.

weaker nonsignificant association at old age might be explained by several other factors contributing to telomere attrition in older people, for example, frailty, telomerase insufficiency, and oxidative stress. The strong association of LTL with overweight and smoking in midlife may therefore relate more closely and specifically to obesity and smoking per se, especially as this association was independent of concomitant cholesterol, blood pressure, and glucose. LTL did not predict total mortality during 10 years of follow-up in old age.

During recent years, shorter LTL has in general been linked to age, several monogenic and polygenic diseases, and cardiovascular risk factors including smoking and obesity (8–25). Intermediate mechanisms are related to either disturbances of cell division or increased oxidative stress, but the order of events is not always clear. Consequently, there are several contradictory reports. For example, both obesity and smoking were associated with shorter telomeres in a cross-sectional study of 18- to 76-year-old women (17). No such relationship was observed in a cohort of 35- to 55-year-old men and women (18) and in another study only among women (19). That obesity nevertheless induces shorter LTL is supported by a study where shorter LTL was associated with obesity in adults but not in children (20), by a study where LTL was inversely associated with weight gain (21), and by a study where telomere length of adipose tissue cells was longer among never-obese individuals (23). Glucose and diabetes were not associated with LTL in our cohort and also elsewhere the relationship has been controversial (12–16,18). Age was not associated with LTL in our study probably due to relatively narrow age range and measurement in old age.

In our cohort, both obesity and smoking in old age predicted total mortality, but the association with BMI was not linear. In contrast, LTL measured in old age did not predict total mortality—reliable conclusions about cause-specific mortality could not be made due to small numbers. Previous

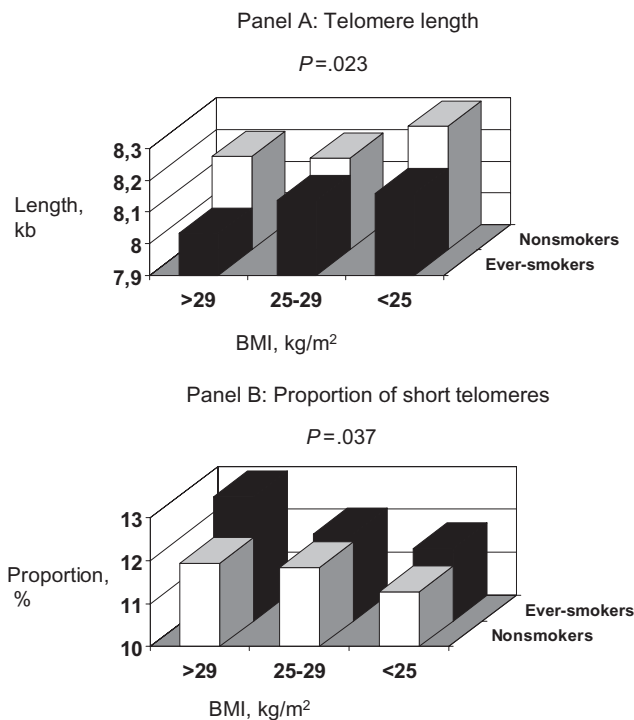


Figure 1. Age-adjusted telomere characteristics in old age according to body mass index (BMI) and smoking in midlife. (A) Telomere length. (B) Proportion of short telomeres.

studies examining LTL and mortality have been contradictory. LTL has predicted cardiovascular disease and also cardiovascular death in unselected men and women aged 45–84 years during a 10-year follow-up (9), all-cause mortality in coronary patients (27), in a cohort of older twins (28), and in the Cardiovascular Health Study (31). On the other hand, our results are in accordance with some earlier studies (26) and most recently with the Zutphen Elderly Study (30), where LTL did not predict mortality among older men. According to “obesity paradox”, lower BMI is associated with higher mortality in older people, frailty being a possible intermediating factor (34). Therefore, also the value of LTL length as a (noncardiovascular) mortality predictor among older people may be diminished because of contrasting associations of age and body weight with mortality. In sum, although LTL is of value as a marker of biological age in a cohort with a wide age range, intervening factors may disturb this association in old age.

Limitations

There are potential limitations in the present study. The cohort consists of Caucasian men with high socioeconomic status, which obviously limits generalizability but may give a cleaner look at underlying mechanisms. Prevalence of obesity (BMI ≥ 30 kg/m²) was fairly low at baseline reflecting the general condition in the population during the 1960s. BMI in 2002–2003 was based on self-report, which, however,

is common in epidemiological studies. LTL was measured in old age, and because of this “survival bias”, impact of shorter LTL in earlier life is probably underestimated. The differences in telomere characteristics, although statistically significant, were small and their clinical significance is unknown.

In conclusion, our results support the notion that smoking and overweight in long-term shorten telomeres and that this effect is independent of age and other cardiovascular risk factors. The relationship between telomeres and mortality in old age is inconsistent, possibly because telomere attrition is affected by clustering of various mortality predictors in later life.

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CONFLICT OF INTEREST

No disclosures to report.

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