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Longitudinal Changes in Leukocyte Telomere Length and Mortality in Humans

Laila Bendix,[1,2] Mikael Thinggaard,[1,3] Mogens Fenger,[4] Steen Kolvraa,[1,5] Kirsten Avlund,[1,2,6] Allan Linneberg,[7] and Merete Osler[1,7]

[1]Danish Aging Research Center, University of Southern Denmark, Aarhus University and University of Copenhagen, Odense, Denmark.
[2]Section of Social Medicine, Department of Public Health, University of Copenhagen, Denmark.
[3]Epidemiology, Institute of Public Health, University of Southern Denmark, Denmark.
[4]Department of Biochemistry, Hvidovre Hospital, Denmark.
[5]Department of Clinical Genetics, Vejle Hospital, Denmark.
[6]Center for Healthy Aging, University of Copenhagen, Denmark.
[7]Research Center for Prevention and Health, The Capital Region of Denmark, Glostrup, Denmark.

Address correspondence to Laila Bendix, PhD, MD, Danish Aging Research Center, University of Southern Denmark, JB Winslowsvej 9B, DK-5000 Odense C, Denmark. Email: laila.bendix@slb.regionsyddanmark.dk

Background. Leukocyte telomere length (LTL) ostensibly shortens with age and has been moderately associated with mortality. In humans, these findings have come almost solely from cross-sectional studies. Only recently has LTL shortening within individuals been analyzed in longitudinal studies. Such studies are relevant to establish LTL dynamics as biomarkers of mortality as well as to disentangle the causality of telomeres on aging.

Methods. We present a large longitudinal study on LTL and human mortality, where the 10-year change of LTL is analyzed in 1,356 individuals aged 30–70 years.

Results. We find age, smoking status, and alcohol consumption to be associated with LTL attrition and confirm a strong association with baseline LTL. The latter association might be an epiphenomenon of regression to the mean. We do not find an association of mortality with either absolute LTL or LTL attrition. Further, we show that DNA quality has an impact on TS ratios.

Conclusions. This study establishes that certain lifestyle factors influence LTL dynamics. However, it questions the applicability of LTL dynamics as a predictor of mortality. We suggest cautiousness when assessing actual LTL attrition due to the need for high-quality DNA and the phenomena of regression to the mean.

Key Words: Biomarkers—Longevity—DNA damage and repair—Epidemiology.

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TELOMERES are specialized structures located at the ends of linear chromosomes, which serve as a buffer for the end-replication problem. The consequence of the end-replication problem is that telomeres shorten with each cell division (1). This shortening can be accelerated by direct damage to telomeres by oxidative stress (2). When telomeres reach a certain short length, they signal to the cell to enter a state of senescence. Senescent cells contribute to the aging process by losing the capacity to divide, as well as by changing the local environment (3).

In humans, the shortening rate of telomeres has been found to be influenced by lifestyle factors such as smoking, body mass index, social class, physical activity, and psychological stress (4–6), although the findings have not always been consistent (7). This influence is likely due to these factors increasing the replication need as well as the burden of oxidative stress (8). However, some of the same factors are also thought to influence the level of telomerase, the telomere elongating enzyme (9).

A vast number of cross-sectional studies have reported an age-dependent shortening of leukocyte telomere length (LTL) in humans of 20–40 bp per year (10,11). Contradictory results have been reported from these cross-sectional studies with regards to linking LTL to mortality and morbidity (12–15). Most consistent has been the finding of a weak correlation between LTL and cardiovascular disease (16).

Studies on longitudinal changes of telomeres within individuals are only now emerging (Supplementary Table S1) (17–30). Most of these longitudinal studies unexpectedly found that approximately 25% of individuals apparently exhibited elongation of LTL over time. Different biological explanations for this finding have been raised, such as changes in telomerase activity or changes in stem cell or peripheral white blood cell homeostasis (22,31).

Recently it was suggested, and supported by substantial evidence, that it is mostly due to methodological problems. Namely, a combination of a relatively high coefficient of variation (CV) of the telomere length measures methods used and the well-known problem of "regression to the mean" (18,32).

In the few large studies, inconsistent findings have been reported between the rate of telomere shortening and smoking and body mass index (17–19). However, they have consistently found that longitudinal change is dependent on baseline LTL, suggesting either a feedback loop or influence of regression-to-the-mean (17,18,21,24,27). We know of only two smaller studies that have reported on the association between longitudinal change in LTL and mortality. One found that telomere attrition was not predictive of mortality (27), whereas the other found that the association was only significant for cardiovascular-caused mortality in men and not overall mortality (20).

In the present study, we present data from 1,356 individuals who have had blood drawn on two occasions 10 years apart enabling us to elucidate on the factors influencing telomere dynamics as well as the role of actual telomere length compared with the role of longitudinal change of telomeres in mortality.

Materials and Methods

Participants

The Danish MONICA 1 population (baseline) survey took place at the Research Centre for Prevention and Health in Glostrup, from 1982 to 1984, and included 4,807 individuals born in 1922, 1932, 1942, or 1952 who were randomly selected from 11 municipalities within Copenhagen County. In 1993–1994, 2,656 individuals agreed to participate in MONICA 10 (follow-up). Whole blood from all participants from MONICA 10 was available; however, only 1,862 samples were available from MONICA 1 for DNA extraction. The Danish Civil Registration System provided information on study member's vital status from 1982 to 2011.

The study was approved by the Ethics Committee for Copenhagen County, and all participants gave written consent (33).

Confounders

The participants completed a self-administered questionnaire on sociodemographic variables, health and lifestyle factors, including smoking and physical activity. A general health examination was carried out by trained nurses.

Based on self-reported physical activity, the participants were divided into four groups: participants without physical activity, participants with light physical activity, participants participating in sport activities for more than half an hour each day, and athletes engaged in competitions. Smoking behavior was assessed using the question recommended by World Health Organization to categorize current smokers (Do you smoke: Daily, Sometimes, Never?). Self-rated health was based on responses to a single item ("How do you consider your health in general?"). Socioeconomic status was measured using a validated classification tool developed by the Danish Institute for Social Research on the basis of self-reported information on type of employment, vocational education, and number of subordinates. The participants were classified into five social classes with I as the highest. Marital status was assessed using the question: "What is your marital status: Married, Unmarried, Divorced, Widowed?" Alcohol consumption was assessed on a continuous scale as average drinks per week. Dietary intake was scored based on how often 26 food items were consumed as previously described and divided into three categories (low, medium, and high value) describing the overall diet quality (34). Cholesterol was measured using the CHOD-PAP enzymatic method (Monotest R Cholesterol, Boehringer-Mannheim, Germany). Cardiovascular disease status was self-reported using the question "Has a doctor ever told you that you have: myocardial infarction, other heart diseases?"

DNA Extraction and Normalization

DNA was extracted using ClearGene Chemistry (KBioscience, Hoddesdon, Hertfordshire, England). DNA concentration was standardized prior to polymerase chain reaction (PCR) on a Biomek FX laboratory automation station with a fluorescence reader (Beckman Coulter) using Quant-iT Picogreen (Invitrogen). Samples that failed to fall within a defined range of DNA concentration (1.5–8 ng/μL) were discarded.

Telomere Length Quantification

Telomere length quantification was done with an adaptation of the Q-PCR methods described by Cawthon (35,36). As single copy gene (S), the commercially available TaqMan Copy Number Reference Assay RNase P (Applied Biosystems) was chosen. This was run under recommended conditions. For measurement of telomere repeat copies (T), primers telg—5′-ACACTAAGGTTTGGGTTTGGGTTTG GGTTTGGGTTAGTGT-3′ and telc—5′-TGTTAGGTATC CCTATCCCTATCCCTATCCCTATCCCTAACA-3′ were added at a concentration of 0.5 μM to Ssofast EvaGreen Supermix with low ROX (Bio-Rad). Cycling conditions were 50°C for 2 min, 95°C for 2 min, followed by two cycles of 95°C for 15 s, 52°C for 15 s, and 36 cycles of 95°C for 15 s, 62°C for 15 s, and 71°C for 15 s. A pool of DNA from healthy donors was diluted eightfold and used for the standard curve.

Samples were pipetted by the Biomek FX laboratory automation station. PCR was done on a 7900HT Fast Real-Time PCR System in 384-well plates and analyzed using the 7900HT Sequence Detection System version SDS2.3 (Applied Biosystems). The average slope for the RNAse P run was −3.75 and −3.45 for the telomere PCR. The ratio of telomere repeats copy number to RNase P single copy gene copy number (TS ratio) was calculated from the average T and S of triplicate measurements. The TS ratios reported are thus relative to the telomere length (TL) of the standard used.

Samples were run in triplicate. If at least two of the triplicates for both RNAse P and telomeres varied <10%, the run was accepted; otherwise, the sample was discarded ($N = 79$, approximately 1.6% of total samples run). The overall CVs for triplicates/duplicates included in the analysis were 3.8% for RNAseP and 4.1% for telomeres. Control samples from cell lines were run on each plate, and results were corrected according to these. The CV for TS ratios for the controls across the plates was 7.8%.

Validation.—The validity of the modified method was established firstly by comparing samples run both with qPCR as well as with telomere restriction fragment length (TRF) assay ($N = 51$, $R = .86$, $p < .001$). Secondly, two randomly selected plates were run twice, finding a CV of 7.9% on the raw data and 5.8% on the calibrated data.

Challenges for the longitudinal measurement.—In order to compare baseline and follow-up measurements, it is necessary that the TS ratio technically behaves in the same manner. Because DNA quality is important, we attempted to use a similar extraction method for the two waves; however, the laboratory had optimized the method in the interim time period, including an extra wash step. Further, the storage has not been identical for the two waves because the baseline samples have been stored as whole blood for 13 years longer than follow-up, and the follow-up DNA has been stored at −80°C for 3 years prior to analysis, whereas the baseline samples had just recently been extracted. There is thus a potential difference in the DNA quality in samples from baseline compared with follow-up. As seen in Figure 1, the age-dependent decline is comparable for the two waves. However, the variance is significantly larger in TS for MONICA 10 than for MONICA 1 ($p < .001$ in variance-ratio test).

In order to approach this technical challenge, we analyzed duplicate samples from 121 participants. These 121 duplicate blood samples were all drawn at the time of baseline, but DNA had been extracted on two occasions. That is, both together with the MONICA 10 samples and again with the MONICA 1 samples. The two measurements that should be identical correlated only reasonably ($R = .48$, $p < .001$). We found that the variation of TS ratio in the samples extracted on the two different occasions is on average 19%. Approximately one third of this variation can be explained by the finding that samples being extracted along with the MONICA 10 wave are on average 27% longer than those being extracted along with the MONICA 1 wave. If we calculate the variation after correcting for this difference, the variation falls to 14%. Another third of the variation can also be attributed to the normal variation of the assay, which we have estimated to be approximately 6%. However, it

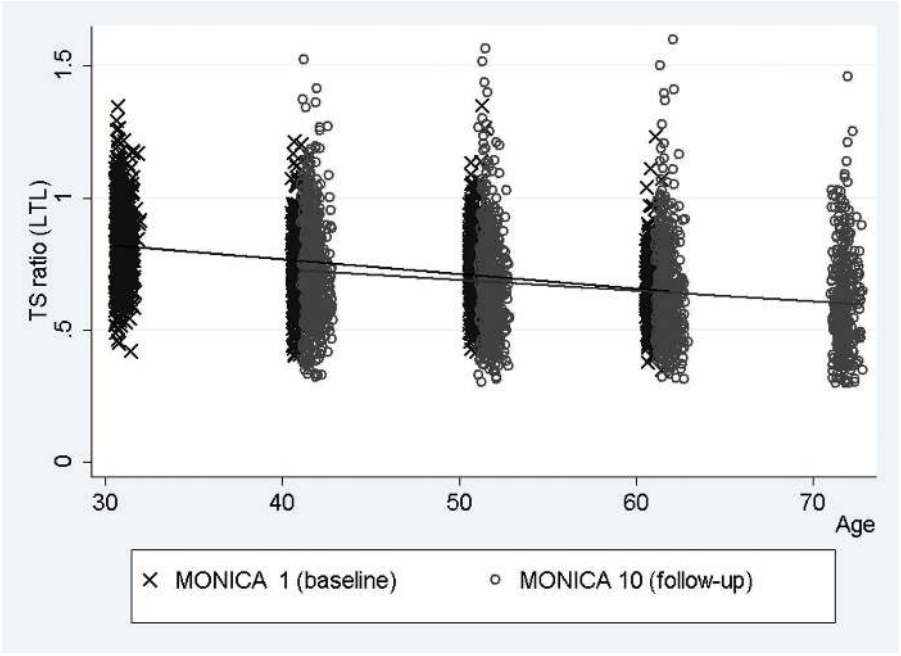

Figure 1. Distribution of leukocyte telomere length (LTL) at baseline (MONICA 1) and follow-up (MONICA 10) as a function of age.

still leaves us with some unexplained variation that could be due to DNA quality falling after longer storage, an effect of blood having been stored longer in the freezer before extraction, or the DNA extraction method.

Based on the earlier discussion, we suggest not using the actual difference, but only the relative difference to the other participants. This means that we cannot estimate the percentage of telomere "shorteners" and "elongaters."

Statistics

Linear regression was applied to evaluate if there was an association between lifestyle factors and LTL at baseline and follow-up when adjusted for age and gender and to establish if these lifestyle factors had an influence on attrition of LTL by letting the follow-up measure acting as outcome adjusting for baseline measure. Age and gender interactions were also analyzed in the models, but no statistical evidence of interactions being present was found.

Survival analysis using Cox's proportional hazard model was performed to study the association between baseline LTL, follow-up LTL, and attrition of LTL. Cox models were also performed to study the association between these measurements adjusting for lifestyle factors. In all the models, gender was adjusted for. Age was used as an underlying time scale and entry time was age of the interview to age of death, migration, or censoring date (July 2011, whichever came first). The proportional hazards assumption, underlying the Cox models, was tested using the Schoenfeld residual test, and no significant violation of the proportional hazard assumption was found.

A p value of .05 was chosen as the significance level for all tests, and the statistical analyses were done using STATA 11.1 (STATACorp, College Station, TX).

Results

LTL results from the qPCR method are expressed as a relative ratio (TS). In this study, TS ratios were near to a normal distribution in both MONICA 1 and MONICA 10. Of the 1,862 samples included at baseline (MONICA 1), 1,765 had their TS ratio successfully measured. The rest were discarded due to problems with DNA concentration or very high interassay CV in the triplicates for either the telomere or RNAse P reaction (this indicates poor DNA quality). A further three samples were discarded as outliers (>±3 *SD*).

Of the 2,576 samples included in the follow-up study (MONICA 10), 2,263 had their TS ratio successfully measured. Again, the remaining samples were discarded due to problems with DNA concentration or very high interassay CV in the triplicates. A further 49 samples were discarded as outliers with an extremely low TS ratio due to almost no amplification of telomeres.

The demographic details on the included participants can be found in Table 1. Further details for the entire population can be found in Supplementary Material. This shows no noteworthy differences between the analyzed samples and the full cohort.

Cross-Sectional LTL Measurements

Mean LTL was significantly associated with sex and age as expected, both at baseline as well as at follow-up (Table 2). The effect of age is smaller at follow-up than at baseline. This is likely due to the population being on average 10.6 years older in this second wave. If we restrict the data to only including the 40–60 year olds, the coefficients become comparable ($\beta = -.00446$ for follow-up and $-.00414$ for baseline, $p < .001$ for both). This age effect could be due to selection bias in older age groups.

Mean LTL at follow-up was further significantly associated with smoking and alcohol consumption. We saw no consistent association with other lifestyle or cardiovascular risk factors.

Longitudinal Changes of LTL

The 1,356 participants who had their TS ratio successfully measured at both baseline and at 10-year follow-up were used for the longitudinal study. Later, we report how baseline LTL as well as lifestyle factors influence the rate of change.

Baseline.—The two LTL measures at baseline and follow-up are correlated (Pearson's $R = .38$, $p < .001$). Age and sex only explain a minor part of this (partial correlation = .32, $p < .001$). Longitudinal change in LTL is correlated to baseline LTL (Pearson´s $R = -.43$, $p < .001$). By regression, we can estimate that baseline LTL accounts for 18% of this variation (Figure 2A).

However, this could also be explained by the phenomenon known as "regression to the mean." In Figure 2B, the longitudinal change in LTL is plotted against the mean LTL (that is the mean of LTL at baseline and follow-up). The correlation between the change in LTL and mean LTL is weaker than to baseline LTL alone (Pearson´s $R = .22$, $p < .001$). This small effect of mean LTL on longitudinal change in LTL suggests that regression to the mean could play a role in the apparent influence of baseline LTL on the rate of change.

Confounders.—We find that age, smoking, and alcohol consumption are associated with longitudinal changes in LTL (Table 3). The association with age is not constant over the age groups, that is, the higher the age, the less is the attrition. This could likely be explained by the fact that the older participants have shorter mean LTL at baseline.

We do not see a significant difference in longitudinal change among the sexes overall or when taking smoking into account. Neither do we see an effect of self-reported cardiovascular disease.

Table 1. Population Characteristics

			MONICA 1 Baseline		MONICA 10 Follow-up	
N			1,762		2,214	
Age	Mean	*SD*	44.7	*10.9*	55.0	*10.7*
Sex	Male	%	915	*51.9*	1104	*49.9*
	Female	%	847	*48.1*	1110	*50.1*
Smoking	Daily	%	900	*51.1*	927	*41.9*
	Occasionally	%	62	*3.5*	88	*4.0*
	Never	%	800	*45.4*	1199	*54.2*
Alcohol	Drinks/wk	*SD*	9.5	*12.1*	10.0	*12.3*
Body mass index	Mean	*SD*	24.4	*3.7*	25.9	*4.2*
Dietary intake	Low	%	893	*51.4*	857	*40.4*
	Medium	%	506	*29.1*	676	*31.9*
	High	%	339	*19.5*	589	*27.8*
Physical activity	Sitting	%	436	*24.7*	456	*20.9*
	Walking	%	940	*53.3*	1240	*56.9*
	Active	%	362	*20.5*	465	*21.3*
	Professional	%	24	*1.4*	18	*0.8*
Socioeconomic status	I	%	78	*4.4*	99	*4.7*
	II	%	252	*14.3*	321	*15.1*
	III	%	508	*28.8*	618	*29.0*
	IV	%	545	*30.9*	668	*31.4*
	V	%	376	*21.4*	421	*19.8*
	Never work	%	2	*0.1*	1	*0.0*
Systolic blood pressure	Mean	*SD*	129.4	*19*	128.9	*19.0*
Diastolic blood pressure	Mean	*SD*	76.4	*11*	81.7	*10.0*
Cholesterol	Mean	*SD*	6.0	*1.2*	6.1	*1.1*
Cardiovascular disease	Yes	%	281	*15.9*	536	*24.2*
	No		1481		1676	
Self-rated health	Very good	%	594	*34.5*	532	*24.1*
	Good	%	857	*49.8*	966	*43.7*
	Average	%	220	*12.8*	610	*27.6*
	Poor	%	44	*2.6*	95	*4.3*
	Very poor	%	7	*0.4*	7	*0.3*
Marital status	Married	%	1274	*72.3*	1541	*69.6*
	Never	%	225	*12.8*	169	*7.6*
	Divorced	%	216	*12.3*	324	*14.6*
	Widow(er)	%	47	*2.7*	180	*8.1*

Note: Distribution is shown as absolute numbers and percentage (italics) for categorical variables and mean and standard deviation (italics) for continuous variables.

We conclude that longitudinal change in LTL is influenced by age, smoking status, and alcohol consumption. These confounders are included along with sex and baseline LTL in the final model (Table 3), confirming that longitudinal change of LTL is associated independently with LTL at baseline, age, smoking, and alcohol consumption.

Mortality

During the 28-year follow-up period for MONICA 1 and the 17-year follow-up period for MONICA 10, 544 (30.9%) and 559 (26.3%) died, respectively. For the 1,356 individuals included in the longitudinal change study, the follow-up period was again 17 years and 352 (26.0%) died.

We find no effect of LTL length on mortality, either at baseline or at follow-up when age and sex has been taken into account (Table 4). None of the effects changed if correcting for smoking status and alcohol (model 2) or any other analyzed confounders (model 3).

Further, we conclude that there is no association between longitudinal change in LTL and mortality. It is seen in Table 4 that the tendency is even contrary to that expected, that is, the less attrition, the higher the hazard ratio for dying. We find no association even after dividing the longitudinal change into quartiles. There is no effect of including the covariates shown to be associated to the rate of change (model 2, Table 4) or when including all the analyzed lifestyle and other risk factors (model 3, Table 4).

To investigate if regression to the mean could play a role in these findings, we have included mean of baseline and follow-up LTL as a possible predictor of mortality in the fourth column in Table 4. This did not have an effect on the coefficients compared with results in the third column where only longitudinal change was included.

Table 2. Associations Between Leukocyte Telomere Length and Covariates

		MONICA 1 (baseline)		MONICA 10 (follow-up)	
		β	*p*	β	*p*
N		1,763		2,214	
Age*		**−.006**	**<.001**	**−.004**	**<.001**
Sex†	Male	Reference		Reference	
	Female	**.015**	**.037**	**.030**	**<.001**
Smoking	Daily	Reference		Reference	
	Occasion	**.042**	**.050**	.023	.293
	No	.003	.689	**.020**	**.012**
Alcohol		−.0001	.717	−.001	.078
Body mass index		.001	.340	.000	.768
Dietary intake	Low	Reference		Reference	
	Medium	−.004	.638	.003	.754
	High	.005	.571	.008	.442
Physical activity	Sitting	Reference		Reference	
	Walking	**−.022**	**.012**	.016	.116
	Active	−.014	.175	.001	.911
	Prof.	−.036	.200	−.020	.599
Socioeconomic status	I	Reference		Reference	
	II	.000	.984	.030	.133
	III	.008	.609	.026	.158
	IV	.019	.239	.032	.081
	V	.001	.964	.022	.258
Systolic blood pressure		−.0001	.786	.000	.168
Diastolic blood pressure		−.000	.934	.000	.228
Cholesterol		.002	.452	.002	.496
Cardiovascular disease	No	Reference		Reference	
	Yes	−.011	.245	−.006	.547
Self-rated health	Very good	Reference		Reference	
	Good	.013	.102	.004	.712
	Average	.012	.316	−.008	.497
	Poor	**.065**	**.010**	−.023	.251
	Very poor	.083	.093	−.055	.239
Marital status	Married	Reference		Reference	
	Never	**.023**	**.050**	−.014	.342
	Divorced	.017	.124	−.017	.140
	Widow(er)	−.012	.616	−.004	.786

Notes: Estimates from a linear regression model for the cross-sectional relationship between covariates and leukocyte telomere length at two time points. Values are corrected for age and sex, except *only corrected for sex and †only corrected for age (Significant findings in bold).

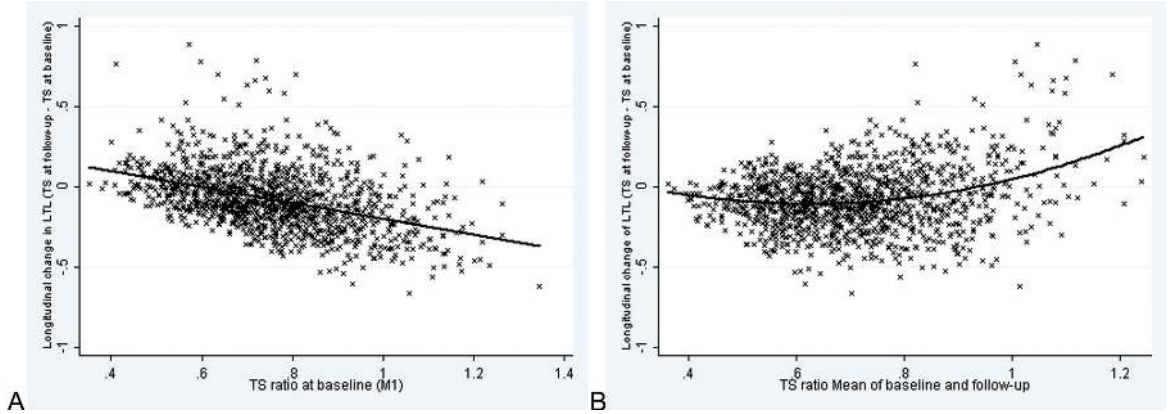

Figure 2. (**A**) Longitudinal change in leukocyte telomere length (LTL) (ie, the difference between follow-up and baseline LTL) is plotted against baseline LTL. It is obvious that the rate of change is dependent on baseline LTL. (**B**) Longitudinal change in LTL is plotted against mean LTL (ie, mean of follow-up and baseline LTL) in a Bland Altman plot (Oldham plot). This suggests that regression-to-the mean is part of the explanation for the baseline length dependency.

Table 3. Associations Between Longitudinal Change in Leukocyte Telomere Length and Covariates

		Model 1 (status at baseline)		Model 2 (status at follow-up)		Final Model	
TS Follow-up		β	*p*	β	*p*	β	*p*
TS baseline		**.460**	**<.001**	**N/A**		**.414**	**<.001**
Age*		**−.002**	**<.001**	**N/A**		**−.002**	**<.001**
Sex†	Male	Reference		**N/A**		Reference	
	Female	.019	.055			.013	.289
Smoking	Daily	Reference		Reference		Reference	
	Occasion.	.028	.300	.033	.220	.036	.188
	No	**.021**	**.040**	**.026**	**.013**	**.024**	**.018**
Alcohol		**−.001**	**.032**	**−.001**	**.005**	**−.001**	**.014**
Body mass index		.001	.330	.001	.312		
Dietary Intake	Low	Reference		Reference			
	Medium	.011	.337	.024	.337		
	High	−.001	.942	.008	.541		
Physical activity	Sitting	Reference		Reference			
	Walking	.016	.651	.004	.762		
	Active	.007	.618	−.011	.467		
	Prof.	.052	.245	−.010	.857		
Socioeconomic status	I	Reference		**N/A**			
	II	.035	.193				
	III	.024	.357				
	IV	.027	.286				
	V	.032	.241				
Systolic blood pressure		.0001	.861	.0002	.504		
Diastolic blood pressure		−.0002	.642	−.0002	.746		
Cholesterol		−.003	.549	−.003	.272		
Cardiovascular disease	No	Reference		Reference			
	Yes	−.005	.704	−.005	.670		
Self-rated health	Very good	Reference		Reference			
	Good	−.011	.282	.018	.443		
	Average	.009	.593	−.005	.700		
	Poor	.038	.274	.001	.989		
	Very poor	−.149	.158	−.064	.437		
Marital status	Married	Reference		Reference			
	Never	−.005	.729	−.006	.729		
	Divorced	−.014	.383	−.010	.432		
	Widow(er)	−.023	.476	−.024	.240		

Notes: Linear regression with TS at follow-up as dependent variable and TS at baseline as confounder. In models 1 and 2, values are corrected for age and sex (except *only corrected for sex, †only corrected for age) and the covariates are evaluated separately. In the final model, age, sex, and baseline leukocyte telomere length, as well as smoking status and alcohol consumption at follow-up, are all included in the regression analysis. Other covariates did not significantly influence the outcome. N/A, not applicable (Significant findings in bold).

Discussion

Previous articles on LTL dynamics have been somewhat inconsistent as to what predicts longitudinal change in LTL, except for baseline LTL. However, these studies have been relatively small (<100), and some have reported a rather high CV (as high as 14.2%). In the present larger study, we find that longitudinal change of LTL is dependent on baseline LTL, age, smoking status, and alcohol consumption. A large part of the effect of baseline LTL on the rate of change was found to be due to regression to the mean.

Finding that LTL attrition, and not only mean LTL, is dependent on lifestyle factors suggests a causal relationship between LTL and lifestyle. Our study, however, lends no support to either mean LTL or LTL attrition being predictors of mortality. We do not find the expected association with cardiovascular disease. This could be due to the fact that the cardiovascular status in the present communication is self-reported.

The study suffers several limitations. In regards to mortality, it is a drawback that we do not know the cause of death and thus we cannot exclude that there could be a cause-specific association with cardiovascular death as previously found. Further, it is a relatively young population for mortality studies, reflected in a rather low number of deaths during the 20-year follow-up period.

We further noticed some technical challenges common to all studies assessing telomere length, but which were accentuated when studying repeated telomere measurements. It is well known that precision of methodology is important (32). In this study, we found a CV of 5.8%. Although this is reasonable compared with other studies, it is still relatively high compared with the expected attrition of LTL. We

Table 4. Leukocyte Telomere Length, Longitudinal Change in Leukocyte Telomere Length, and Mortality

		Baseline (MONICA 1)		Follow-up (MONICA 10)			Longitudinal Change			Longitudinal Change (adjusted for mean LTL)		
		N (death)	HR	*p*	*N* (death)	HR	*p*	*N* (death)	HR	*p*	*N* (death)	HR	*p*
LTL as continuous variable													
Model 1		1,763 (544)	1.22	.51	2,126 (559)	0.91	.69	1,356 (352)	1.07	.84	1,356 (352)	1.04	.91
Model 2			1.21	.54		1.00	1.00		1.21	.54		1.14	.69
Model 3			1.25	.50		1.21	.45		1.16	.66		1.01	.98
LTL divided into quartiles (qrt)													
Model 1	1st qrt		Reference			Reference			Reference			Reference	
	2nd qrt		1.00	.98		0.90	.33		1.17	.32		1.18	.31
	3rd qrt		0.92	.48		1.00	.98		1.31	.08		1.32	.07
	4th qrt		1.14	.31		0.88	.33		1.11	.51		1.09	.62
Model 2	1st qrt		Reference			Reference			Reference			Reference	
	2nd qrt		1.05	.68		0.93	.55		1.07	.69		1.07	.68
	3rd qrt		0.85	.29		1.05	.69		1.36	.05		1.37	.04
	4th qrt		1.17	.24		0.94	.60		1.15	.40		1.11	.55
Model 3	1st qrt		Reference			Reference			Reference			Reference	
	2nd qrt		1.02	.84		0.98	.84		0.98	.90		1.02	.91
	3rd qrt		0.90	.39		1.12	.34		1.30	.11		1.24	.20
	4th qrt		1.13	.38		1.02	.87		1.13	.47		1.15	.43

Notes: Model 1—only adjusted for sex, age is underlying factor in the Cox regression. Model 2—same as model 1, but also adjusted for smoking status and alcohol consumption. Model 3—same as model 1, but also adjusted for all confounders mentioned in Table 3. The longitudinal change in leukocyte telomere length (LTL) is defined as the difference between LTL at baseline and LTL at follow-up. HR = hazard ratio.

expect to find an attrition of 20–40 bp/year, that is, on average 300 bp over 10 years. This attrition is approximately 5% of an average LTL of 6 kb. This problem is further exaggerated in studies with repeated measures because the CV will apply to both measurements.

It is becoming more evident that DNA quality is important for TS measures (37). This is important for cross-sectional studies but is crucial to studies of longitudinal change of LTL if the DNA quality influences the TS ratio differently at baseline compared with follow-up. Due to this challenge, we have refrained from any discussion on elongators/shorteners because we feel that we cannot present a valid answer. This potential source of error might explain some of the discrepancies in the findings between studies using the TRF assay, which is not as sensitive to DNA extraction methods (eg, Chen et al. (18)), and those using qPCR-based assays (eg, Hovatta et al. (24)).

In conclusion, our study finds that longitudinal changes in LTL are associated with lifestyle factors—however, it questions the applicability of both actual LTL as well as LTL attrition as a biomarker for aging, at the individual level, due to the many technical limitations revealed.

Supplementary Material

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

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