Leukocyte telomere length and all-cause, cardiovascular and cancer mortality: Results from individual participant data meta-analysis of two large prospective cohort studies

Running head: Leukocyte telomere length and mortality

Ute Mons*, Aysel Müezzinler*, Ben Schöttker, Aida Karina Dieffenbach, Katja Butterbach, Matthias Schick, Anne Peasey, Immaculata De Vivo, Antonia Trichopoulou, Paolo Boffetta, Hermann Brenner

* These authors contributed equally to this work.

Author for correspondence

Dr. Ute Mons, M.A.
German Cancer Research Center (DKFZ)
Division of Clinical Epidemiology and Aging Research
Im Neuenheimer Feld 581
69120 Heidelberg, Germany
Phone: +49 6221-42-1344
Fax: +49 6221-42-1302
E-mail: u.mons@dkfz.de

Word count: 199 (abstract), 2986 (main text)

Abbreviations – CHANCES, Consortium on Health and Ageing: Network of Cohorts in Europe and the United States; CI, confidence interval; CVD, cardiovascular disease; ESTHER, Epidemiological Study on the Chances of Prevention, Early Recognition, and Optimised Treatment of Chronic Diseases in the Older Population; HR; hazard ratio; PCR, polymerase chain reaction, LTL, leukocyte telomere length; NHS, Nurses' Health Study.

Abstract

We studied the associations of LTL with all-cause, cardiovascular and cancer mortality in 12,199 adults participating in two population-based prospective cohort studies from Europe (ESTHER) and the US (Nurses' Health Study). Blood samples were collected in 1989-1990 (Nurses' Health Study), and in 2000-2002 (ESTHER), respectively. LTL was measured by quantitative polymerase chain reaction. Z-scores of LTL were calculated to standardise LTL measurements across the cohorts. Cox Proportional Hazard regression models were used to calculate relative mortality according to continuous levels and quintiles of z-scores of LTL. The hazard ratios obtained from each cohort were subsequently pooled by meta-analysis. Overall, 2882 deaths were recorded over follow-up (Nurses' Health Study: 1989-2010, ESTHER: 2000-2015). LTL was inversely associated with age in both cohorts. After adjustment for age, a significant inverse trend of LTL with all-cause mortality was observed in both cohorts. In random effects meta-analysis, age-adjusted hazard ratios (95% confidence intervals) for shortest LTL quintile compared to longest LTL quintile were 1.23 (1.04-1.46) for all-cause mortality, 1.29 (0.83-2.00) for cardiovascular mortality, and 1.10 (0.88-1.37) for cancer mortality. In this study population with an age range of 43-75, we corroborate previous evidence suggesting that LTL predicts all-cause mortality beyond its association with age.

Keywords: telomere length; leukocyte; all-cause mortality; cancer; CVD; aging; cohort study

Telomeres are special chromatin structures that are found at the ends of chromosomes, which are comprised of a stretch of repetitive DNA (TTAGGG) and a variety of specifically bound proteins. Depending on the age, type of the tissue, chromosomes and replicative history of cells, the length of telomeres can vary between 0.5 to 15 kilobase pairs, with ~30 to 200 base pairs lost after each mitotic cell division in somatic cells (1, 2). This leads to a gradual telomere shortening with age (3, 4), due to incomplete replication of linear chromosomes by DNA polymerases (5). Critically short telomere length leads to cell senescence and apoptosis (6).

A number of epidemiological studies, with varying sample sizes and characteristics, yielded inconsistent results with regards to the link between telomere length in leukocytes (LTL) and mortality (3). Regarding cause-specific mortality such as cardiovascular disease (CVD) mortality and cancer mortality, results have also been inconsistent. The associations reported between LTL and CVD indicate a modest inverse link (7-10), but are heterogeneous with regards to CVD mortality (11-13). The associations of LTL with cancer seem to be even more complex. A number of studies have found increased risk of cancer incidence (14-18) with short LTL and mixed results on cancer mortality (11, 12, 18). However, more recent studies investigating cancer-specific associations have also correlated longer LTL with increased risk of certain malignancies, including pancreatic cancer, hepatocellular carcinoma, melanoma, sarcoma and lung adenocarcinoma (19-24). With regards to all-cause mortality, several recent prospective cohort studies could not establish an association between LTL and all-cause mortality (12, 25, 26), while the by far largest study comprising a cohort of nearly 65.000 Danish individuals found linear graded associations of LTL with all-cause, CVD and cancer mortality (27).

Considering the inconsistent findings in the literature, which could be due to a lack of statistical power in most of the studies, we aimed to further investigate the association of LTL with all-

cause, CVD and cancer mortality in two large independent cohorts in the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES).

METHODS

Study design and participants

Data from Nurses' Health Study (NHS) and the ESTHER study (Epidemiological Study on the Chances of Prevention, Early Recognition, and Optimised Treatment of Chronic Diseases in the Older Population) were used. Both are ongoing prospective cohort studies from the USA and Germany, respectively, and participate in the CHANCES (Consortium on Health and Ageing: Network of Cohorts in Europe and the United States; www.chancesfp7.eu) consortium (28).

The NHS is a prospective cohort study of 121,700 female registered nurses in 11 US states who were 30–55 years of age at enrolment. In 1976 and biennially thereafter, self-administered questionnaires gather detailed information on lifestyle, menstrual and reproductive factors, and medical history. Self-reports of major chronic diseases are confirmed by medical records and pathology report reviews, telephone interviews or supplemental questionnaires. From 1989 to 1990, 32,826 women provided blood samples. The details of blood collection methods have been previously described (29). The current analysis included data from 8633 women who were selected to participate in different nested case-control studies. Genomic DNA was extracted from peripheral blood leukocytes using QIAmp (Qiagen, Chatsworth, CA, USA) 96-spin blood control. The study was approved by the institutional review board of Brigham and Women's Hospital (Boston, MA).

The ESTHER study (Epidemiological Study on the Chances of Prevention, Early Recognition, and Optimised Treatment of Chronic Diseases in the Older Population) is an ongoing cohort study with the main objective of improving the prevention, early diagnosis and therapy of chronic age-related diseases. Overall, 9,949 men and women aged 50-75 years were recruited between July 2000 and December 2002 during a general health check-up in Saarland, south-west Germany, by their general practitioners and followed with respect to incidence of major diseases and deaths since then. Information on age, sex, socio-demographic characteristics, medical history, health status, family history and lifestyle factors were obtained by detailed questionnaires in a standardised manner. Whole blood samples were obtained from all participants from peripheral blood. The current analysis is based on 3,566 participants for whom measurements of LTL in baseline blood samples were available. They are a representative sub-sample of the entire study population whose extracted DNA became available first (a comparison of this sub-sample with the overall ESTHER sample is given in Web Table 1). Genomic DNA was extracted by high salt method and stored at -20°C. The ESTHER study has been approved by the ethics committees of the medical faculty of the University of Heidelberg and of the medical board of the state of Saarland. Informed consent was obtained from all participants.

Measurements

LTL measurements were performed independently in different laboratories. In both studies, DNA concentration was quantified using Quant-iT PicoGreen® (Invitrogen) and relative telomere lengths, in genomic DNA extracted from peripheral blood leukocytes, were measured by quantitative PCR (polymerase chain reaction) (30). This method assesses the ratio of telomere

repeat copy number to number of single copy gene (T/S ratio) in experimental samples relative to a reference sample. T/S ratio is proportional to average telomere length as amplification is proportional to the number of primer binding sites in the first cycle of the PCR reaction and relative telomere length was calculated as the exponentiated T/S ratio. Two quality-control samples were inserted into each PCR plate in order to assess the coefficients of inter- and intra-plate variability. The quantitative PCRs were performed on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA) in the NHS and on the LightCycler 480 System (Roche Diagnostics, GmbH, Germany) in the ESTHER study.

Terminal restriction fragment analysis was additionally performed in a sub-sample (N=20) of the ESTHER study population to validate our results from the quantitative PCR measurements and obtain absolute LTL in base pairs. Briefly, 3.5 µg of genomic DNA were digested overnight at 37 °C with restriction enzymes HphI and Mnl I (Thermo Scientific GmbH Schwerte, Germany) and loaded onto 0.7% agarose gel with DIG-labelled marker (DIG-labelled Marker VII, Roche, Diagnostics GmbH Mannheim, Germany). Then, DNA was processed as previously described (31). Detection of DIG-labelled probe and marker was performed using Anti- Digoxigenin-AP, Fab fragments and CDP-Star (Roche Diagnostics GmbH Mannheim, Germany). Image analysis was done with ImageJ Analysis Software (Version 1.44) (32).

Statistical analyses

The correlation between relative LTL and absolute LTL measurements was 0.622 (p=0.005). The coefficients of variation for the telomere assay of quality control samples were 6.5% and 5.3% in the ESTHER cohort, and were less than 4% for triplicates in the NHS cohort, respectively.

In order to standardise LTL measurements across cohorts a z-transformation was applied. This means that measurements were transformed in a way that the mean is zero and the standard deviation is one, i.e. after transformation LTL measurements are expressed in units of the standard deviation. The differences in quintiles of z-scores with age, lifestyle factors and health-related outcomes were tested for statistical significance by analysis of variance (ANOVA) tests.

Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations of LTL with all-cause, CVD and cancer mortality both in the whole cohort and stratified by sex and age group (50-59 years and ≥60 years). A shared frailty model was fit with measurement batch as a random effect in order to account for within-batch correlations. Continuous z-score levels, quintiles of z-scores and dichotomised z-scores (z-score < median and z-score ≥ median) were used as exposure variables in regression models.

Regression models with four different levels of adjustment were used. Model 1 was the crude model without any covariates in the model and only adjusting for batch effect; model 2 adjusted for batch and age (and also sex in the ESTHER cohort); model 3 additionally adjusted for potential further confounders including smoking status (never, former, current), body mass index defined as weight (kg)/squared height (m^2) (≤18.5, 18.6-24.9, 25-29.9, ≥30), alcohol consumption (abstainer, low intake, medium intake, high intake, which were defined as follows: men: 0.1-39.9 g/week, 40-59.9 g/week, ≥60 g/week, women: 0.1-19.9 g/week, 20-39.9 g/week, ≥40 g/week, respectively (33)), physical activity (inactive, active, which were defined as follows: 0 hours of vigorous physical activity/week; >0 hours of vigorous physical activity/week) and years of education. Model 4 further adjusted for systolic blood pressure (mmHg), total cholesterol (mmol/l), presence of diabetes, history of myocardial infarction, cancer and stroke.

The additional variables included in model 4 might not necessarily be considered as potential confounders of the association between LTL and mortality, but could be considered potential mediators of LTL-mortality associations. Following the estimation of study-specific HRs, meta-analyses were carried out to calculate summary HRs across the cohorts. In a conservative approach the random effects estimates were taken as "main results" to allow for variation of true effects across studies. Random effects estimates were derived using the DerSimonian–Laird method (34). All variables used for the project were created by each cohort according to the pre-agreed harmonisation rules of the CHANCES consortium.

All analyses were conducted with SAS 9.3 and statistical tests were two-sided using a 5 % significance level. R v.3.0.2 and the package 'meta' were used to carry out the meta-analysis (35).

RESULTS

The general characteristics of the study populations according to age-adjusted quintiles of LTL z-scores are shown in Table 1. There were 8633 and 3566 eligible participants from the NHS and ESTHER cohorts, respectively. The age range in the NHS was 42.7-70.2, with a mean age of 59.0 (SD 6.6) years. The age range in the ESTHER cohort was 50-75 with a mean age of 61.9 (6.6). After a mean follow-up duration of 18.4 (SD 3.8) years, 2149 deaths were recorded in the NHS. After 12.5 (SD 2.9) years of follow-up, there were 733 deaths in the ESTHER cohort. Age was inversely associated with LTL in both cohorts ($p<0.0001$).

Results of the survival analyses from the individual cohorts are provided in Web Tables 2, 3 and 4 for all-cause, CVD and cancer mortality, respectively. In both cohorts, the HRs from the crude

model (only adjusted for batch effect) showed an inverse association between LTL and all-cause mortality, with especially marked graded associations in ESTHER (Web Table 2). After additionally adjusting for age, associations were clearly attenuated, but still a significant inverse trend of telomere length with all-cause mortality was observed in both cohorts. However, after adjusting for further covariates (models 3 and 4), the associations did no longer reach statistical significance. Comparable patterns were observed for CVD and cancer mortality.

Results of the random effects meta-analyses are summarised in Table 2 for the whole study population, and summary-estimates of sex- and age-stratified models are shown in Figure 1. Looking at the z-score quintiles, gradients were observed for all-cause, CVD and cancer mortality in the crude model, and for all-cause mortality also after age-adjustment. The HR for all-cause mortality in the quintile with the shortest telomere length was 1.66 (1.09-2.53) in the crude model, 1.23 (1.04-1.46) in the age-adjusted model, and 1.10 (0.97-1.25) in the model adjusted for age and further covariates. The summary trend estimate for the association of the continuous z-score with all-cause mortality was 0.82 (0.68-0.99) in the crude model, indicating an 18% decrease in all-cause mortality per increase by one standard deviation of relative telomere length. After adjustment for age, the summary estimate was attenuated to 0.92 (0.85-1.00; p-value=0.052), and to 0.96 (0.93-1.00; p-value=0.065) after adjustment for further covariates. Again, patterns were overall similar for CVD and cancer mortality, but no summary estimate from the random effects meta-analyses reached statistical significance. No big differences across sex or age-groups were seen in stratified models for the outcome all-cause mortality, but associations tended to be somewhat stronger in men and in those aged 60 and older (Figure 1). Tests for interaction however indicated no significant effect modification by age or by sex for any outcome (details not shown).

Results of fixed effects meta-analyses are presented in Web Table 5, showing consistent associations which were somewhat attenuated but of higher precision. Heterogeneity was high especially for models with smaller numbers of covariates (details not shown), warranting the use of random effects meta-analyses.

DISCUSSION

In this study including 12,199 adults in total with 2882 deaths over a mean follow-up duration of 16.7 years, LTL was significantly associated with all-cause mortality. After adjustment for age, the associations were clearly attenuated, but the quintile with the shortest LTL still showed significantly increased mortality hazards (HR: 1.23, 1.04-1.46). Comparable patterns were seen for CVD and cancer mortality, but the summary estimates did not reach statistical significance. Mortality patterns were generally consistent across sex and age, but associations tended to be somewhat stronger among men and above the age of 60. Associations were stronger in the ESTHER study, which was smaller than NHS but included both men and women and had a slightly narrower and older age-range. Nevertheless, patterns were generally quite consistent across both cohorts.

In the present study, we observed that when adjusting for age, the association of LTL with mortality attenuated, reflecting the association of age with all-cause mortality. However, even after age adjustment the mortality hazard ratio of the shortest LTL quintile was still significantly increased, indicating a relationship beyond age. The associations further attenuated after adjustment for lifestyle-related variables, possibly reflecting associations of lifestyle with mortality. But even after these adjustments, LTL was still inversely and borderline significantly

associated with mortality (HR per one standard deviation in LTL: 0.96, 0.93-1.00). Hence, we provide further evidence that LTL predicts mortality beyond its association with age and possibly also beyond associations with lifestyle, and thus could be an indicator of biological fitness in adults in the general population.

In the last decade telomere biology and dynamics in populations have drawn substantial attention in medical and epidemiological fields. While it is established that LTL shortens with age (4), results from studies investigating the association of LTL with health outcomes as well as lifestyle-related factors, such as smoking, obesity, physical activity and stress, have not been as definite (36-39). Most of the studies hypothesised that lifestyle and environmental factors that lead to heightened oxidative stress would lead to higher telomere attrition rates, hence shortened LTL, which would impair survival in the long run (40). However, inconsistent results yielded from highly heterogeneous studies suggest that telomere dynamics might be a rather more complex trait than initially thought. There are multiple factors contributing to this heterogeneity, and limitations of measurement methods are especially important. The LTL measurement methods used show substantial heterogeneity between the studies, impairing the comparability of studies.

There was also large variation in the size and age-range of previous studies assessing the association between LTL and mortality. Whereas some earlier, relatively small studies had reported strong inverse association between LTL and mortality (17, 41, 42), much weaker associations or no association at all was seen in a number of larger, mostly more recent studies (11-13, 25, 26, 43). However, in the so far by far largest study from Denmark with 64,637 participants, with 7607 deaths during 22 years of follow-up, linear graded associations and modestly increased hazards for the shortest versus longest deciles of LTL were seen for all-cause

mortality (HR: 1.40, 1.25-1.57), CVD mortality (1.36, 1.12-1.66) and cancer mortality (1.35, 1.11-1.65) (27). Our study confirms these findings in a meta-analysis of two large prospective cohort studies from Germany and the USA. Even though in our study not all summary estimates reach statistical significance (especially after multivariate adjustment and for cancer and CVD mortality), possibly due the smaller sample size, the mortality patterns are quite consistent with the Danish study.

Interestingly, two recent US American studies noted racial differences in the association of LTL with mortality. A study in postmenopausal women found a significant association of the shortest LTL quartile with increased all-cause and cardiovascular mortality in white women, but no association in African American women (43). In contrast, a study using data from the National Health and Nutrition Examination Survey (NHANES) found a strong association between LTL and cardiovascular mortality only among African American participants (11). As both cohorts used in this present study consist mostly of white participants, we were not able to study ethnic differences. Our findings however support an association of LTL with mortality among white Europeans and Americans.

Our study had specific strengths and limitations. As the relative LTL data were derived from different cohorts, z-scores were calculated to be able to pool data together and carry out meta-analysis. As a result, only magnitude of the associations could be shown and the corresponding T/S ratios or absolute LTL, in base pairs, could not be quantified. As in any observational study, measurement error in self-reported variables is inevitable. Although the analyses were controlled for multiple covariates which could possibly act as confounders, the possibility of residual confounding remains. The LTL measurement yielded acceptable, but not excellent, coefficients of variation for the quality control samples, which are in the usual range reported in the

literature. This variation may render it more difficult to detect true associations. Then again, high correlation coefficients achieved between the terminal restriction fragment analysis, which is regarded as the gold-standard, and quantitative PCR measurements, document the quality of LTL measurements in the ESTHER study. The large sample size with a follow-up time longer than 10 years in both studies makes this study one of the largest in the field thus far. The inclusion of two independent cohorts from Europe and the US suggest a broad generalizability of our results to older white populations. Although there was also some heterogeneity in data collection across the studies, this heterogeneity was minimized by major efforts of data harmonization, which is not commonly possible in meta-analyses of published data.

Altogether our findings support previous evidence suggesting that LTL predicts all-cause mortality even beyond its association with age and could also be inversely associated with CVD and cancer mortality. Hence, LTL could serve as an indicator of biological fitness in the general population.

Acknowledgements

Author affiliations: Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany (Ute Mons, Aysel Müezzinler, Ben Schöttker, Aida Karina Dieffenbach, Katja Butterbach, Hermann Brenner); Network Aging Research (NAR), University of Heidelberg, Heidelberg, Germany (Aysel Müezzinler, Ben Schöttker, Hermann Brenner); German Cancer Consortium (DKTK), Heidelberg, Germany (Aida Karina Dieffenbach, Hermann Brenner); Genomics and Proteomics Core Facility, German Cancer Research Center (DKFZ), Heidelberg, Germany (Matthias Schick); Department of Epidemiology and Health Care, University College London, London, UK (Anne Peasey); Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Immaculata De Vivo); Program in Genetic Epidemiology and Statistical Genetics, Harvard School of Public Health, Boston, Massachusetts (Immaculata De Vivo); Hellenic Health Foundation, Athens, Greece (Antonia Trichopolou, Paolo Boffetta); Department of Hygiene, Epidemiology and Medical Statistics, University of Athens, Athens, Greece (Antonia Trichopoulou); Institute for Translational Epidemiology and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, New York (Paolo Boffetta)

Data used throughout the present study are derived from the CHANCES project. The project was coordinated by the Hellenic Health Foundation, Greece. The project was supported by the Seventh Framework Programme for Research and Technological Development of the Directorate-General for Research and Innovation in the European Commission (Grant Agreement number: HEALTH-F3-2010-242244). The Nurses' Health Study is supported by the National Cancer Institute, National Institutes of Health (1R01 CA134958, 2R01 CA082838, P01 CA087969, R01 CA49449, CA065725, CA132190, CA139586, CA140790, CA133914, CA132175, CA163451, HL088521, HL60712, U54 CA155626, R01 AR059073, HL34594). The ESTHER study was funded in part by grants from the Baden Württemberg Ministry of Science, Research and Arts, and the Federal Ministry of Education and Research. The work of Aysel Müezzinler was supported by a scholarship from the Klaus Tschira Foundation. The study sponsors

played no role in the study design, data collection, analyses, or interpretation of results, the preparation of the manuscript, or the decision to submit the manuscript for publication.

Conflict of interest: None declared.

References

1. Lansdorp PM, Verwoerd NP, van de Rijke FM, et al. Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet* 1996;5(5):685-691.
2. Martens UM, Zijlmans JM, Poon SS, et al. Short telomeres on human chromosome 17p. *Nat Genet* 1998;18(1):76-80.
3. Mather KA, Jorm AF, Parslow RA, et al. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci* 2011;66(2):202-213.
4. Muezzinler A, Zaineddin AK, Brenner H. A systematic review of leukocyte telomere length and age in adults. *Ageing Res Rev* 2013;12(2):509-519.
5. Olovnikov AM. [Principle of marginotomy in template synthesis of polynucleotides]. *Dokl Akad Nauk SSSR* 1971;201(6):1496-1499.
6. Blackburn EH. Telomere states and cell fates. *Nature* 2000;408(6808):53-56.
7. Brouilette S, Singh RK, Thompson JR, et al. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 2003;23(5):842-846.
8. Farzaneh-Far R, Cawthon RM, Na B, et al. 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(7):1379-1384.
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(1):14-21.
10. Weischer M, Bojesen SE, Cawthon RM, et al. Short telomere length, myocardial infarction, ischemic heart disease, and early death. *Arterioscler Thromb Vasc Biol* 2012;32(3):822-829.
11. Needham BL, Rehkopf D, Adler N, et al. Leukocyte telomere length and mortality in the National Health and Nutrition Examination Survey, 1999-2002. *Epidemiology* 2015;26(4):528-535.
12. Svensson J, Karlsson MK, Ljunggren O, et al. Leukocyte telomere length is not associated with mortality in older men. *Exp Gerontol* 2014;57:6-12.
13. Fitzpatrick AL, Kronmal RA, Kimura M, et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci* 2011;66(4):421-429.
14. Ma H, Zhou Z, Wei S, et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PLoS One* 2011;6(6):e20466.
15. Prescott J, Wentzensen IM, Savage SA, et al. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. *Mutat Res* 2012;730(1-2):75-84.
16. Wentzensen IM, Mirabello L, Pfeiffer RM, et al. The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2011;20(6):1238-1250.
17. Willeit P, Willeit J, Kloss-Brandstatter A, et al. Fifteen-year follow-up of association between telomere length and incident cancer and cancer mortality. *JAMA* 2011;306(1):42-44.
18. Willeit P, Willeit J, Mayr A, et al. Telomere length and risk of incident cancer and cancer mortality. *JAMA* 2010;304(1):69-75.
19. Fu X, Wan S, Hann HW, et al. Relative telomere length: a novel non-invasive biomarker for the risk of non-cirrhotic hepatocellular carcinoma in patients with chronic hepatitis B infection. *Eur J Cancer* 2012;48(7):1014-1022.
20. Han J, Qureshi AA, Prescott J, et al. A prospective study of telomere length and the risk of skin cancer. *J Invest Dermatol* 2009;129(2):415-421.
21. Lan Q, Cawthon R, Shen M, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of non-Hodgkin lymphoma. *Clin Cancer Res* 2009;15(23):7429-7433.

22. Lynch SM, Major JM, Cawthon R, et al. A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. *Int J Cancer* 2013;133(11):2672-2680.
23. Sanchez-Espiridion B, Chen M, Chang JY, et al. Telomere length in peripheral blood leukocytes and lung cancer risk: a large case-control study in Caucasians. *Cancer Res* 2014;74(9):2476-2486.
24. Xie H, Wu X, Wang S, et al. Long telomeres in peripheral blood leukocytes are associated with an increased risk of soft tissue sarcoma. *Cancer* 2013;119(10):1885-1891.
25. Bendix L, Thinggaard M, Fenger M, et al. Longitudinal changes in leukocyte telomere length and mortality in humans. *J Gerontol A Biol Sci Med Sci* 2014;69(2):231-239.
26. Weischer M, Bojesen SE, Nordestgaard BG. Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. *PLoS Genet* 2014;10(3):e1004191.
27. Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. *J Natl Cancer Inst* 2015;107(6):djv074.
28. Boffetta P, Bobak M, Borsch-Supan A, et al. The Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES) project--design, population and data harmonization of a large-scale, international study. *Eur J Epidemiol* 2014;29(12):929-936.
29. Prescott J, Du M, Wong JY, et al. Paternal age at birth is associated with offspring leukocyte telomere length in the nurses' health study. *Hum Reprod* 2012;27(12):3622-3631.
30. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002;30(10):e47.
31. Figueroa R, Lindenmaier H, Hergenhahn M, et al. Telomere erosion varies during in vitro aging of normal human fibroblasts from young and adult donors. *Cancer Res* 2000;60(11):2770-2774.
32. Kimura M, Hjelmborg JV, Gardner JP, et al. Telomere length and mortality: a study of leukocytes in elderly Danish twins. *Am J Epidemiol* 2008;167(7):799-806.
33. Rehm J, Room R, Monteiro M, et al. Chapter 12: Alcohol use. In: Ezzati M, Lopez AD, Rodgers A, et al., eds. *Comparative quantification of health risks: global and regional burden of disease attribution to selected major risk factors*. Geneva, Switzerland: WHO, 2004.
34. Normand SL. Meta-analysis: formulating, evaluating, combining, and reporting. *Stat Med* 1999;18(3):321-359.
35. Schwarzer G. *Package ‚meta': General package for meta-analysis*. URL: https://cran.r-project.org/web/packages/meta/meta.pdf; 2015.
36. Cassidy A, De Vivo I, Liu Y, et al. Associations between diet, lifestyle factors, and telomere length in women. *Am J Clin Nutr* 2010;91(5):1273-1280.
37. Müezzinler A, Mons U, Dieffenbach AK, et al. Smoking habits and leukocyte telomere length dynamics among older adults: Results from the ESTHER cohort. *Exp Gerontol* 2015;70:18-25.
38. Müezzinler A, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. *Obes Rev* 2014;15(3):192-201.
39. Zhu H, Wang X, Gutin B, et al. Leukocyte telomere length in healthy Caucasian and African-American adolescents: relationships with race, sex, adiposity, adipokines, and physical activity. *J Pediatr* 2011;158(2):215-220.
40. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 2000;908:99-110.
41. 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(9355):393-395.
42. Ehrlenbach S, Willeit P, Kiechl S, et al. 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(6):1725-1734.

43. Carty CL, Kooperberg C, Liu J, et al. Leukocyte Telomere Length and Risks of Incident Coronary Heart Disease and Mortality in a Racially Diverse Population of Postmenopausal Women. *Arterioscler Thromb Vasc Biol* 2015;35(10):2225-2231.

Figure 1. Summary Estimates* of Association of LTL with All-cause Mortality (Nurses' Health Study (1989-2010) and ESTHER (2000-2015)), Stratified by Age-group and Sex

* Estimates represent summary estimates from meta-analyses; except estimates for men, which represent estimates for the male subgroup of ESTHER

Table 1. Population Characteristics by Quintile of Age-adjusted Telomere Length in the Nurses' Health Study (1989-2010) and ESTHER (2000-2015)

Characteristic	Quintile of *z-score*															*P* value[a]
	1			2			3			4			5			
	Mean (SE)	**N**	**%**	**Mean (SE)**	**N**	**%**	**Mean (SE)**	**N**	**%**	**Mean (SE)**	**N**	**%**	**Mean (SE)**	**N**	**%**	
Nurses' Health Study (N=8633)																
N (%)		1726	20.0		1727	20.0		1727	20.0		1726	20.0		1727	20.0	
Age[b], years	60.0 (6.3)			59.3 (6.6)			59.1 (6.5)			58.6 (6.7)			58.1 (6.9)			**<0.001**
Sex, % male		0	0		0	0		0	0		0	0		0	0	-
Body mass index	25.6 (4.9)			25.6 (4.6)			25.4 (4.7)			25.2 (4.8)			25.4 (4.6)			0.09
Smoking Status, % Current		264	19.5		286	21.1		297	21.9		275	20.3		235	17.3	0.08
Vigorous physical activity, %		789	19.1		836	20.3		822	19.9		829	20.1		852	20.6	0.62
Education, years	14.8 (1.4)			14.8 (1.4)			14.8 (1.4)			14.8 (1.4)			14.9 (1.4)			0.45
Alcohol, g/day	5.4 (9.7)			5.7 (9.6)			6.0 (10.7)			5.7 (10.3)			5.2 (8.6)			0.13
Total cholesterol, mmol/l	5.4 (1.2)			5.4 (1.2)			5.5 (1.2)			5.5 (1.2)			5.4 (1.2)			0.30
Systolic blood pressure, mmHg	127.6 (13.8)			128.2 (13.9)			127.0 (13.5)			127.7 (14.0)			128.2 (14.0)			0.08
Cancer History, %		72	20.1		69	19.3		79	22.1		74	20.7		64	17.9	0.78
Diabetes History, %		104	24.6		88	20.9		83	19.7		69	16.4		78	18.5	0.09
CHD History, %		41	22.9		38	21.2		35	19.6		31	17.3		34	19.0	0.80
Stroke History, %		14	28.6		12	24.5		9	18.4		12	24.5		2	4.1	0.06
Cancer Death, %		158	19.9		157	19.7		169	21.2		153	19.2		159	20.0	0.93
CVD Death, %		116	23.6		88	17.9		85	17.3		93	18.9		109	22.2	0.11
Death of any cause, %		460	21.4		425	19.8		420	19.5		432	20.1		412	19.2	
ESTHER (N=3566)																
N (%)		713	20.0		713	20.0		712	20.0		712	20.0		716	20.0	
Age[b], years	62.3 (6.7)			62.4 (6.4)			62.3 (6.5)			61.9 (6.4)			60.5 (6.6)			**<0.001**
Sex, % male		367	18.6		373	18.9		381	19.3		407	20.6		449	22.7	**0.02**
Body mass index	28.0 (4.6)			27.6 (4.2)			27.7 (4.3)			27.8 (4.7)			27.4 (4.1)			0.16
Smoking Status, % Current		140	22.2		121	19.2		126	20.0		124	19.7		120	19.0	0.72
Vigorous physical activity, %		300	20.1		299	20.0		300	20.1		297	19.9		299	20.0	1.00
Education, years	9.5 (1.1)			9.4 (1.0)			9.4 (0.9)			9.4 (1.0)			9.4 (0.9)			0.54
Alcohol, g/day	11.4 (16.9			10.6 (13.6)			10.2 (14.0)			9.2 (11.7)			9.2 (13.6)			**0.02**
Total cholesterol, mmol/l	5.5 (1.4)			5.4 (1.5)			5.1 (1.6)			5.0 (1.6)			5.1 (1.6)			**<0.001**

Systolic blood pressure, mmHg	140.4 (18.7)			140.2 (19.4)			141.3 (20.6)			140.8 (20.9			138.8 (19.2)			0.18
Cancer History, %		48	20.8		43	18.6		45	19.5		45	19.5		50	21.7	0.95
Diabetes History, %		86	21.2		82	20.2		83	20.4		81	20.0		74	18.2	0.91
CHD History, %		34	17.4		42	21.5		51	26.2		39	20.0		29	14.9	0.13
Stroke History, %		28	26.2		19	17.8		26	24.3		15	14.0		19	17.8	0.24
Cancer Death, %		43	18.1		52	21.9		51	21.5		46	19.4		45	19.0	0.86
CVD Death, %		34	17.1		37	18.6		44	22.1		41	20.6		43	21.6	0.78
Death of any cause, %		100	16.2		135	21.8		127	20.5		133	21.5		124	20.0	0.17

SE, standard error

[a] based on ANOVA

[b] z-score not adjusted for age

Table 2. Summary Estimates of Association of LTL with Mortality From Random-Effects Meta-Analysis (Nurses' Health Study (1989-2010) and ESTHER (2000-2015))

			All-cause mortality								
Telomere Length	**n**	**Cases**	**HR[a]**	**95% CI**	***P* value**	**HR[b]**	**95% CI**	***P* value**	**HR[c]**	**95% CI**	***P* value**
Quintiles of *z-score*											
1 (shortest)	2425	627	1.66	1.09, 2.53	0.018	1.23	1.04, 1.46	0.017	1.10	0.97, 1.25	0.156
2	2462	616	1.50	0.96, 2.36	0.078	1.18	0.94, 1.48	0.151	1.10	0.88, 1.37	0.417
3	2414	568	1.26	0.96, 1.66	0.098	1.06	0.94, 1.20	0.345	1.02	0.89, 1.17	0.810
4	2470	572	1.17	1.02, 1.34	0.025	1.08	0.96, 1.22	0.219	1.06	0.93, 1.21	0.349
5 (longest)	2427	499	Ref.			Ref.			Ref.		
z-score (continuous)	12199	2882	0.82	0.68, 0.99	0.043	0.92	0.85, 1.00	0.052	0.96	0.93, 1.00	0.065
z-score* < *median	6106	1508	1.27	0.97, 1.67	0.081	1.10	0.94, 1.28	0.244	1.04	0.89, 1.21	0.629
z-score* ≥ *median	6093	1374	Ref.			Ref.			Ref.		
			CVD mortality								
Telomere Length	**n**	**Cases**	**HR[a]**	**95% CI**	***P* value**	**HR[b]**	**95% CI**	***P* value**	**HR[c]**	**95% CI**	***P* value**
Quintiles of *z-score*											
1 (shortest)	2374	182	1.84	0.85, 3.97	0.120	1.29	0.83, 2.00	0.258	1.05	0.82, 1.34	0.707
2	2411	153	1.38	0.62, 3.11	0.431	1.06	0.62, 1.81	0.825	0.93	0.60, 1.44	0.742
3	2371	137	1.30	0.60, 2.82	0.501	1.08	0.62, 1.88	0.793	1.01	0.54, 1.90	0.967
4	2415	152	1.26	0.84, 1.89	0.270	1.09	0.86, 1.39	0.486	1.03	0.76, 1.41	0.826
5 (longest)	2390	144	Ref.			Ref.			Ref.		
z-score (continuous)	11962	800	0.82	0.62, 1.08	0.153	0.94	0.81, 1.08	0.381	0.99	0.91, 1.08	0.844
z-score* < *median	5984	402	1.22	0.78, 1.90	0.379	1.02	0.76, 1.37	0.897	0.97	0.75, 1.25	0.813
z-score* ≥ *median	5978	398	Ref.			Ref.			Ref.		

Table 2 continues on the following page.

		Cancer mortality									
Telomere Length	**n**	**Cases**	**HR[a]**	**95% CI**	***P* value**	**HR[b]**	**95% CI**	***P* value**	**HR[c]**	**95% CI**	***P* value**
Quintiles of *z-score*											
1 (shortest)	2345	233	1.42	0.88-2.27	0.149	1.10	0.88-1.37	0.416	1.04	0.84-1.27	0.736
2	2370	225	1.27	0.85-1.90	0.245	1.03	0.85-1.26	0.744	1.01	0.77-1.31	0.967
3	2334	235	1.19	0.98-1.44	0.077	1.10	0.91-1.32	0.319	1.08	0.89-1.33	0.425
4	2375	211	1.05	0.87-1.28	0.605	1.00	0.82-1.22	0.994	1.01	0.82-1.23	0.953
5 (longest)	2358	207	Ref.			Ref.			Ref.		
z-score (continuous)	11783	1111	0.88	0.74-1.05	0.164	0.98	0.92-1.04	0.494	0.99	0.92-1.06	0.764
z-score < median	5900	573	1.22	0.87-1.73	0.251	1.09	0.85-1.38	0.504	1.05	0.79-1.40	0.718
z-score ≥ median	5883	538	Ref.			Ref.			Ref.		

HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference category

[a] crude model, only adjusted for batch effect (random effect)

[b] like model 1, but additionally adjusted for age (and sex in ESTHER)

[c] like model 2, but additionally adjusted for smoking status, body mass index, physical activity, alcohol consumption and education