Physical activity and telomeres in old age – a longitudinal 10-year follow-up study

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Abstract

Background: Telomeres are crucial parts of chromosomes that protects the genome. They shorten every time the cell replicates and shorter telomeres have been associated with increasing age and with many health behaviours. There is inconclusive evidence of the association between physical activity and telomere length.

Objectives: To examine how leisure-time physical activity (LTPA) is associated with telomere length and telomere attrition during 10 years of follow-up in elderly people.

Design: This study is 10-year prospective follow-up study.

Method: For this prospective study**,** we examined 1014 subjects (mean age at baseline 60.8 years) from the Helsinki Birth Cohort Study (HBCS). Relative LTL was measured with a quantitative real-time PCR and LTPA with a validated questionnaire. Multiple linear regression analyses were used to assess the association between sex-specific LTPA quartiles and LTL at baseline and change in LTL over 10 years. The analyses were adjusted for age, educational attainment, smoking, body fat percentage, estrogen exposure in women and for follow-up time when applicable.

Results: At baseline, volume of LTPA was not associated with LTL in men (p=0.66) or in women (p=0.33). Among women, however, higher volume of LTPA at baseline was associated with greater shortening of LTL (p for linearity 0.040) during the 10-year follow-up. No association was found among men (p for linearity 0.75).

Conclusions: Our findings suggest that PA has a sex-specific role in regulation of telomere length in the aging process as in our study a high volume of LTPA in elderly women, but not in men, was associated with more rapid telomere attrition.

Keywords: Prospective study, physical activity, healthy active aging.

Introduction

Telomeres are repetitive sequences of DNA at the end of eukaryotic chromosomes. They stabilize the chromosome during DNA replication and allow the chromosomal DNA to be replicated completely without loss of it {1}. Telomeres shorten every time the cell replicates and eventually become critically short, causing cells to become senescent or die. Shorter telomeres have been associated with increasing age {2}. Telomeres have been proposed to be a biomarker of aging, but the supporting evidence is still equivocal {3}. Shorter telomeres have been associated with premature mortality and age-related diseases such as cardiovascular disease, hypertension and type 2 diabetes. There is also evidence of the association of shorter telomeres with health behaviours such as smoking, unhealthy diet and stressful life events {4, 5}. Leucocyte telomere length (LTL) is partly heritable {6}. Furthermore, oxidative stress has been associated with shorter telomeres and telomere shortening can be accelerated by factors that induce oxidative stress and inflammation {7} and may be protected by factors with antioxidant effects like moderate physical activity (PA). Albeit PA can suppress inflammatory pathways, the effects can depend on the intensity, frequency, and duration of exercise {8}.

There is inconclusive evidence of the association between PA and LTL. A few observational and cross-sectional studies among adults have shown an association between higher PA and longer LTL {9, 10}, but there are studies that have not reported any significant association {11}. Longitudinal studies have also reported mixed findings concerning the association between PA and related markers with LTL. In the study of Soares-Miranda et al. an increase in leisure-time PA (LTPA) was associated with a trend toward less shortening in LTL during five years follow-up, but no association was reported between walking pace, walking distance and walking score and change in LTL {12}. There are also 6-12 months randomised controlled PA intervention trials that have examined the potential influence of PA on telomere length, but these studies have not fully established such a relationship {13}.

As the significance of PA on telomere length remains uncertain and there are only few prospective studies {12} in older adults we examined how self-reported LTPA is associated with LTL and with change in LTL during a 10-year follow-up period in a well characterized study population.

Materials and Methods

Study population

The study population belongs to the Helsinki Birth Cohort Study (HBCS) that consists of 13,345 singletons born in Helsinki between 1934 and 1944 and who were alive in 1971 when all residents of Finland received a unique personal identification number. In the year 2000, a total of 2,902 people were randomly selected from the sample of 8,760 subjects from HBCS who were born at Helsinki University Central Hospital, and invited to participate in a clinical examination conducted between the years 2001 and 2004. The target was to reach 2000 participants for the clinical examination. From this clinical study cohort (n=2003), 1404 people who were alive and living within 100 km distance from the study clinic in Helsinki were invited to participate in a clinical follow-up in 2011. A total of 1094 participants attended the clinical examination between 2011 and 2013.

The present study includes 1014 individuals. To be included into this study, participants were required to have information on physical activity in 2001-2004 and telomere length both in 2001-2004 and in 2011-2013.

The clinical study protocol was approved by the Ethics Committee of Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from each participant before any study procedure was initiated.

Assessment of LTPA

LTPA was assessed in the first clinical examination in 2001-2004. LTPA was assessed by using a validated exercise questionnaire KIHD (Kuopio Ischaemic Heart Disease Risk Factor Study) 12-month LTPA history {14}. The KIHD questionnaire has been modified from the Minnesota leisure time activity questionnaire and the questionnaire presents a list of different PA types, including conditioning LTPA (e.g. running, skiing, swimming), non-conditioning LTPA (e.g. household work, gardening, shoveling snow), physical activity from commuting to work (walking or cycling) and an additional category for "other" physical activities specified by the participant. The subjects were asked to fill in frequency (occasions per month), average duration and intensity (0=recreational, 1=conditioning, 2=brisk conditioning and 3=competitive, strenuous exercise) of each activity performed during the previous 12 months. For each activity and intensity class a metabolic equivalent of task, MET, value was used based on a synthesis of available empirical data, the compendium of Ainsworth et al. {15}. MET is the ratio of metabolic rate during activity to the metabolic rate at rest (1 MET = 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ or 1 $kcal \cdot kg^{-1} \cdot h^{-1}$). To calculate the volume of LTPA in MET-hours (METh), MET values were multiplied with the average duration and frequency of activities. The total volume of LTPA is expressed in METh per week.

Telomere length measurements

Telomere length was measured twice, in both clinical examinations in 2001-2004 and in 2011-2013. Relative telomere length was determined from whole blood leukocyte DNA by quantitative real-time polymerase chain reaction (PCR) as previously described {16}. Genomic DNA was extracted from EDTA-anti-coagulated whole peripheral blood by using commercially available kits according to manufactures' instructions (QIAamp Bllod Maxi Kit and DNeasy blood and tissue kit; Qiagen srl). DNA concentration and purity were assessed by comparing ultraviolet absorbance at wavelengths 260 nm to absorbance at 230 nm and

280 nm. Samples with ratios ranging between 1.7 to 2.1 were considered pure and suitable for following steps. DNA integrity was tested by electrophoresis.

At the first examination, relative telomere length was determined as ratio of telomere DNA to β-haemoglobin single-copy gene signal intensities, as previously described {17}. The plate effect was taken into account by normalizing the telomere signal and reference gene signal to the corresponding mean of four control samples that were analysed for every qPCR plate before taking the T/S ratio (telomere reaction and β-haemoglobin reaction ratio).

At the second examination, the relative telomere length measurement was performed with the use of multiplex quantitative real-time PCR method described in detail by Guzzardi et al. {16}. The multiplex method provides a relative telomere length (T/S) ratio expressed as ratio between the amplification of the telomere sequence (T) and that of a single copy gene (S), measured for each sample in the same well and PCR run, and normalized using a common reference DNA sample.

Covariates

The participants were measured for weight and height in the clinical examination at baseline. Body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters (kg/m^2). Lean body mass (LBM) and body fat were assessed with bioelectrical impedance by using the InBody 3.0 eight-polar tactile electrode system (Biospace Co., Ltd., Seoul, Korea). Data on reproductive history, including age at menarche and menopause, were assessed by questionnaires. Duration of reproductive life in years, computed as the difference between age at menopause and menarche, was used as a surrogate for the length of endogenous estrogen exposure. Participants' smoking habits were also assessed by questionnaires at baseline. Smoking status was expressed as years of smoking. Individually linked data on educational attainment (years of studying) was obtained from Statistics Finland. All measurements were done by trained study nurses.

Statistical analyses

Data were examined in men and women separately, because the length and dynamics of telomeres are gender dependent {18}. Results are expressed as means with standard deviations. For analyses both men and women were divided into quartiles according to baseline volume of LTPA. Significances between LTPA groups at baseline was evaluated with analyses of variance. Comparisons within men and women of repeated measurements were performed by paired t test.

We applied multiple linear regression analyses to assess the association between LTPA quartiles and LTL at baseline and the relative and residual change in LTL during the 10-year follow-up. To calculate the residual change we first performed linear regression analyses between the follow-up measurement and the baseline measurement of LTL. In the second stage, predicted values from the prior regression model were subtracted from the observed follow-up measurement of LTL. All analyses were adjusted for age, educational attainment (years of studying), smoking (years of smoking), percentage of body fat and in women for estrogen exposure. Estrogen exposure was divided in two groups (≤35 years and >35 years). The models including relative and residual change in LTL as an outcome were additionally adjusted for follow-up time. In the case on violation of the assumptions (e.g. non-normality) a bootstrap-type test with 2,500 replications were employed.

The statistical analyses were carried out with Stata/SE 14.2 (StataCorp LP, College Station, TX, USA).

Results

The study cohort included 445 men and 569 women. Mean age of the 1014 participants was 61 years at the first clinical examination (range 56-69 years). Mean follow up time was 9.8 years (range 7.9-11.5 years). Table 1 shows the characteristics of the study population at the first clinical examination divided into four groups according to LTPA for women and men

separately. Both in women and men, participants were older in the upper LTPA quartiles (p for trend <0.001). In women, body fat percentage was lower in those with greater volume of LTPA (p for trend 0.004). Otherwise, there were no statistically significant differences in the characteristics between the LTPA groups either in men or women. During the follow-up time LTL shortened in men -0.57 (CI -0.60 to -0.54, unadjusted p<0.0001) and in women -0.53 (CI -0.56 to -0.50, unadjusted p<0.0001).

Figure 1 shows the association between LTPA quartiles and leucocyte telomere length at baseline. LTPA quartiles were not associated with LTL in men (p=0.66) or in women (p=0.33) after adjustment for age, educational attainment, smoking years, body fat percentage and length of reproductive life in women. Further adjustment for hormone therapy use did not attenuate the findings in women.

Figure 2 shows the association between baseline LTPA quartiles and change in leucocyte telomere length during the 10-year follow-up in men and women separately. The change in LTL is shown separately for relative and residual change. There were no significant associations between baseline LTPA quartiles and relative change in LTL over ten years in men (p for linearity 0.21) or in women (p=0.071) (Figure 2A). In women, a higher volume of LTPA at baseline was associated with greater absolute shortening of LTL during the 10-year follow-up (p for linearity 0.04) (Figure 2B). In men, there were no linear relationship between LTPA quartiles and residual change in LTL (p for linearity 0.75).

Discussion

In this prospective study in an aging birth cohort we investigated the association between LTPA and telomere length at baseline and telomere attrition during 10 years of follow-up. At baseline the volume of LTPA was not associated with LTL in either sex. In men, LTPA was not significantly associated with either the relative or residual change of LTL during ten years follow-up. However, in women, higher baseline volume of LTPA was associated with greater

shortening of LTL during the follow-up. This relationship was stronger in analyses that used residual change of LTL, which takes into account the fact that rate of LTL attrition is proportional to baseline LTL {19}.

Only a few cross-sectional studies have explored the association between PA and LTL among elderly people. The findings from these studies have been conflicting. Our findings are partly in line with a study in older people showing that cross-sectionally only greater reported walking distance was associated with longer LTL but not LTPA or reported walking pace {12}. In longitudinal analyses no significant association was found between baseline PA and 5-year change in LTL {12}. Also, Woo et al. {11} found no association between PA and telomere length in an elderly Chinese population. One randomized controlled intervention trial performed in older people has reported that after six months of individualized PA prescription no significant association between changes in steps per day and changes in LTL were noted, but in the intervention group reduced sitting time was significantly associated with telomere lengthening {13}. The fact that we observed an inverse relationship between LTPA and change in LTL among women contradicts with a recent study consisting of elderly women that reported a positive association between greater amounts of total LTPA and moderate-to-vigorous PA and longer LTL {9}. On the other hand, in the same study group, when PA was measured objectively only women with over 150 min per week of MVPA had longer LT while light PA was not significantly associated with LTL {20}. But in these studies women were older (64-95 years) than in our study and the settings were cross-sectional.

There are several mechanisms that could explain how PA may alter LTL shortening. PA has an anti-inflammatory and an anti-oxidative effect {21} that can protect LTL against inflammation and oxidative stress, which are known accelerators of LTL shortening {22}. An inverted U-shaped relationship between PA and LTL has been suggested {23}. This could be related to oxidative stress. In our study, we did not see a U-shaped relationship among women, but our results support that also high volume of PA is related to shorter LTL. This could be due to the differences in the measurements or dose (type, intensity, frequency and

duration) of PA. PA can also stimulate telomerase, a reverse transcriptase enzyme, activity, which is responsible for maintaining telomere length {24}. A recent study reported that one single session of high intensity interval cycling increased hTERT gene expression in young and older men but not in women {25}. hTERT is a functional protein of telomerase that acts as a limiting factor in functional telomerase activity and can therefore be used as an index of telomerase function. The difference between men and women in the hTERT gene expression after a physical activity session can be one explanation to the gender differences reported here {25}.

We identified gender differences, which could be explained by gender dependency of the telomere dynamics. Women have longer telomeres {18} and the longer telomeres in women imply a slower rate of telomere attrition in women than men. Also, in our study group women had longer LTL than men, but the attrition rate of LTL did not differ between gender. This is inconsistent with a study exploring the LTL dynamics around menopause, showing that LTL attrition rate was higher in men than in women at postmenopausal age {26}. Estrogen, which has antioxidant effects and promotes telomerase enzyme expression and activity {27}, can also explain gender dependent differences in telomere dynamics.

The strengths of our study include a large, well-characterized study cohort. The study was longitudinal and we had a long follow-up time. We used a validated KIHD questionnaire in assessing self-reported PA. The KIHD collects LTPA data from the previous 12 months and it also provides information about the type, intensity and duration of activity. We measured telomere length from leucocytes using real-time quantitative PCR method and the telomere signal was normalized to the signal for single copy gene to generate T/S ratio which is proportional to the absolute quantification of LTL in base pair. Although this may not be the gold-standard for assessment of telomere length, it is the most common method used {28}.

There are possible confounding factors that may account for the result in this study. First, there might be a survival effect. Shorter telomeres have been associated with cardiovascular diseases and premature mortality, which might have resulted in the survival and the inclusion

in our study of subjects with longer LTL. Second, there might also be a selection bias, because only subjects who were in better fit might have attended the follow-up examination. Furthermore, those with a better health status may be able to engage more in PA. The limitations of HBCS have been previously discussed. The participants may not represent all older people in Finland, because they were both born and attended child welfare clinics in the city of Helsinki. The information of physical activity was obtained by self-reports that are associated with recall bias. Moreover, we did not take into account the exogenous estrogen exposure, which could increase LTL among women. Albeit, the role of hormone therapy on LTL remains inconclusive {29}. Although a range of covariates were available and evaluated as potential confounders, there may be some unmeasured ones, e.g. stress. A previous study has actually suggested that vigorous PA protected those experiencing high stress by buffering its relationship with LTL {30}. Furthermore, our results may only be generalized to leukocyte LTL, but leukocyte telomere length correlate highly with that in cells from other tissues {31}.

In conclusion, our results showed that the association between PA and LTL is still contradictory. At baseline LTPA was not associated with LTL. In men, the volume of LTPA at baseline was not associated with change in LTL during ten-year follow-up. In contrast in women, an inverse relationship between LTPA and change in LTL was observed. Our findings suggest that PA has a sex-specific role in the regulation of telomere length during the aging process.

Acknowledgement

The authors would like to acknowledge the efforts of the participants who voluntarily gave their time to participate in the study

Statement of Ethics

The clinical study protocol was approved by the Ethics Committee of Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from each participant before any study procedure was initiated.

Disclosure

The authors have no conflicts of interest to declare.

Statement Funding Sources

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Author Contributions

JE is the overall Principal Investigators. HJ, NW and HK take responsibility for the analysis design, the integrity of the data, the accuracy of the data analysis and the critical inter-pretation of the data. MAG and IO take responsibility of the analyses of telomere length. All authors contributed to the final version of the paper and have read, as well as, approved of the final manuscript.

Table 1 Baseline characteristics of the participants according to the LTPA levels.

	LTPA quartiles				
	I	II	III	IV	P for trend
Women					
Number	142	142	142	143	
Total Volume of LTPA (METh/wk), median (IQR)	13 (9, 17)	29 (25, 32)	46 (40, 51)	83 (68, 108)	
Age (years), mean (SD)	60 (3)	60 (3)	61 (3)	62 (3)	<0.001
Weight (kg), mean (SD)	72.7 (14.0)	70.9 (12.3)	71.5 (12.4)	70.9 (13.2	0.31
Height (cm), mean (SD)	162 (6)	162 (5)	163 (5)	162 (6)	0.38
Body mass index (kg/m2), mean (SD)	27.7 (4.9)	26.9 (4.7)	26.8 (4.3)	26.9 (4.5)	0.14
Lean body mass (kg), mean (SD)	47.8 (5.5)	47.4 (5.4)	47.7 (4.8)	47.6 (5.6)	0.89
Percent body fat (%), mean (SD)	26.0 (9.4)	24.5 (8.9)	23.6 (8.2)	23.7 (8.7)	0.004
Years of fulltime studying, mean (SD)	12.2 (3.4)	12.6 (3.7)	12.2 (3.5)	11.8 (3.2)	0.27
Smoking years, mean (SD)	8.2 (14.1)	7.9 (14.1)	7.7 (12.9)	7.1 (12.6)	0.47
Duration of reproductive life in years, mean (SD)	38.0 (4.7)	37.2 (5.0)	36.2 (5.5)	37.2 (4.7)	0.065
Men					
Number	110	112	111	112	
Total Volume of LTPA (METh/wk), median (IQR)	13 (8, 18)	27 (24, 32)	46 (41, 55)	88 (73, 107)	
Age (years), mean (SD)	60 (2)	61 (3)	61 (3)	61 (2)	<0.001
Weight (kg), mean (SD)	83.5 (13.3)	81.1 (10.5)	84.6 (13.3	84.6 (14.4)	0.21
Height (cm), mean (SD)	176 (6)	176 (6)	176 (5)	177 (6)	0.20
Body mass index (kg/m2), mean (SD)	27.9 (3.9)	26.4 (2.9)	27.1 (3.3)	27.4 (3.6)	0.66
Lean body mass (kg), mean (SD)	65.4 (7.6)	64.2 (6.3)	65.8 (7.2)	66.7 (8.2)	0.085
Percent body fat (%), mean (SD)	21.3 (8.1)	18.6 (5.6)	19.6 (6.7)	20.2 (7.4)	0.15
Years of fulltime studying, mean (SD)	12.8 (3.7)	13.0 (3.7)	13.7 (4.1)	12.7 (3.8)	0.75
Smoking years, mean (SD)	16.3 (16.4)	15.4 (15.8)	13.9 (15.1)	14.3 (15.0)	0.26

Abbreviations: LTPA, leisure-time physical activity; MET, metabolic equivalents of task; IQR, interquartile range; SD, standard deviation

Figure 1 The correlation between leucocyte telomere length at baseline and change in leucocyte telomere length during follow-up period in women and men.

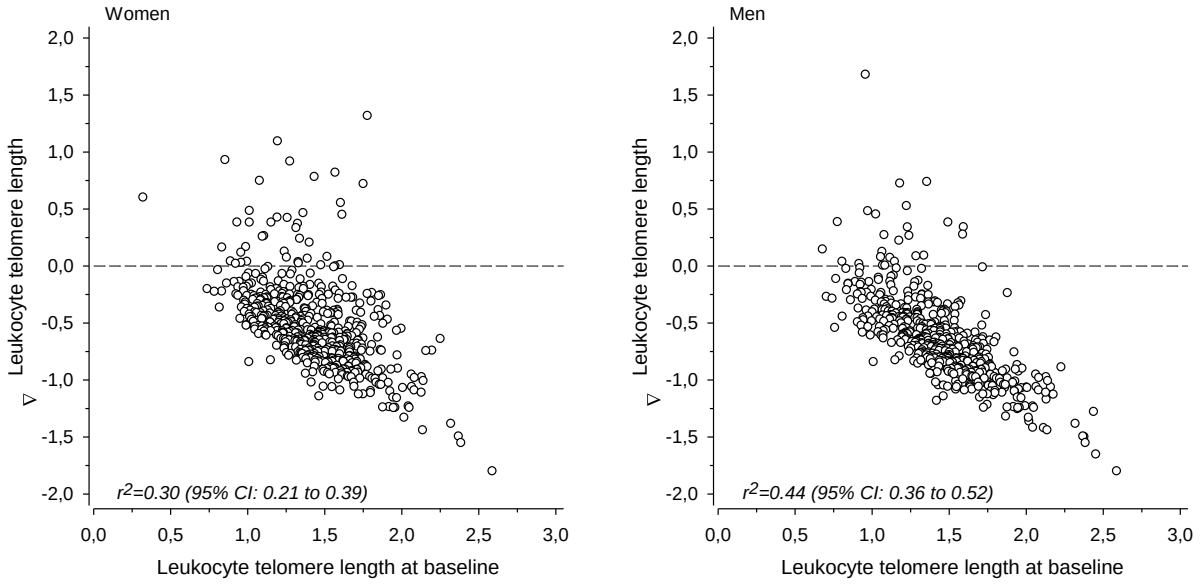


Figure 2 The association between leisure-time physical activity (LTPA) and leucocyte telomere length at baseline in women and men. Analyses are adjusted for age, educational attainment, smoking years, body fat percentage and in women with estrogen exposure categorized in two groups (≤35 years and >35 years). Error bars indicate 95% confidence interval. P-value indicates linearity.

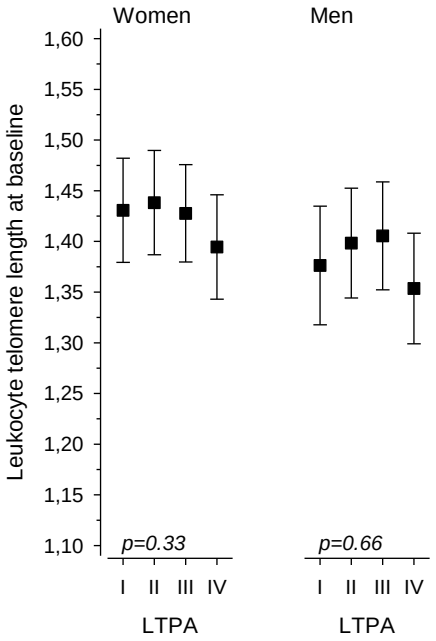
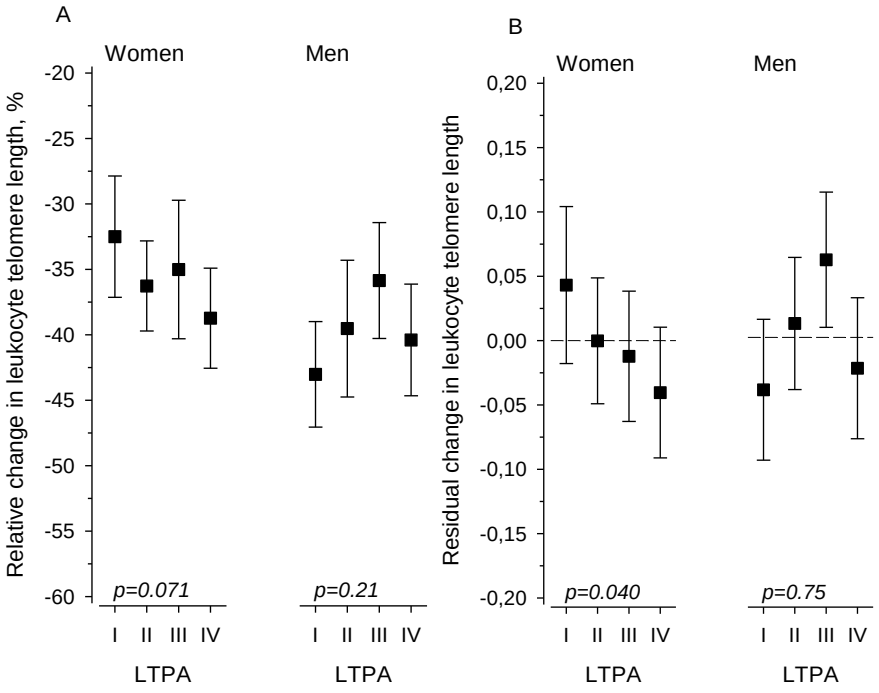


Figure 3 Relative change in leukocyte telomere length according to baseline leisure-time physical activity levels (LTPA) in women and men (A). Residual change in leucocyte telomere length according to baseline LTPA in women and men (B). Values were adjusted for age, educational attainment, smoking years, body fat percentage, follow-up time and in women with estrogen exposure categorized in two groups (≤35 years and >35 years). Error bars indicate 95% confidence intervals. P-value indicates linearity.



References

1. Blackburn EH. Telomeres: structure and synthesis. J Biol Chem. 1990;265(11):5919-21.

2. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345(6274):458-60.

3. 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(2):202-13.

4. Puterman E, Lin J, Krauss J, Blackburn EH, Epel ES. Determinants of telomere attrition over 1 year in healthy older women: stress and health behaviors matter. Mol Psychiatry. 2015;20(4):529-35.

5. Huzen J, Wong LS, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, et al. Telomere length loss due to smoking and metabolic traits. J Intern Med. 2014;275(2):155-63.

6. Hjelmborg JB, Dalgard C, Moller S, Steenstrup T, Kimura M, Christensen K, et al. The heritability of leucocyte telomere length dynamics. J Med Genet. 2015;52(5):297-302.

7. Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. Telomere length assessment: biomarker of chronic oxidative stress? Free Radic Biol Med. 2008;44(3):235-46.

8. Sallam N, Laher I. Exercise Modulates Oxidative Stress and Inflammation in Aging and Cardiovascular Diseases. Oxid Med Cell Longev. 2016;2016:7239639.

9. Shadyab AH, LaMonte MJ, Kooperberg C, Reiner AP, Carty CL, Manini TM, et al. Leisure-time physical activity and leukocyte telomere length among older women. Exp Gerontol. 2017;95:141-7.

10. Tucker LA. Physical activity and telomere length in U.S. men and women: An NHANES investigation. Prev Med. 2017;100:145-51.

11. Woo J, Tang N, Leung J. No association between physical activity and telomere length in an elderly Chinese population 65 years and older. Arch Intern Med. 2008;168(19):2163-4.

12. Soares-Miranda L, Imamura F, Siscovick D, Jenny NS, Fitzpatrick AL, Mozaffarian D. Physical Activity, Physical Fitness, and Leukocyte Telomere Length: The Cardiovascular Health Study. Med Sci Sports Exerc. 2015;47(12):2525-34.

13. Sjogren P, Fisher R, Kallings L, Svenson U, Roos G, Hellenius ML. Stand up for health--avoiding sedentary behaviour might lengthen your telomeres: secondary outcomes from a physical activity RCT in older people. Br J Sports Med. 2014;48(19):1407-9.

14. Lakka TA, Salonen JT. Intra-person variability of various physical activity assessments in the Kuopio Ischaemic Heart Disease Risk Factor Study. Int J Epidemiol. 1992;21(3):467-72.

15. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011;43(8):1575-81.

16. Guzzardi MA, Iozzo P, Salonen M, Kajantie E, Eriksson JG. Rate of telomere shortening and metabolic and cardiovascular risk factors: a longitudinal study in the 1934-44 Helsinki Birth Cohort Study. Ann Med. 2015;47(6):499-505.

17. Kajantie E, Pietilainen KH, Wehkalampi K, Kananen L, Raikkonen K, Rissanen A, et al. No association between body size at birth and leucocyte telomere length in adult life--evidence from three cohort studies. Int J Epidemiol. 2012;41(5):1400-8.

18. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. 2014;51:15-27.

19. Verhulst S, Aviv A, Benetos A, Berenson GS, Kark JD. Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for 'regression to the mean'. Eur J Epidemiol. 2013;28(11):859-66.

20. Shadyab AH, LaMonte MJ, Kooperberg C, Reiner AP, Carty CL, Manini TM, et al. Association of Accelerometer-Measured Physical Activity With Leukocyte Telomere Length Among Older Women. J Gerontol A Biol Sci Med Sci. 2017.

21. Bogdanis GC, Stavrinou P, Fatouros IG, Philippou A, Chatzinikolaou A, Draganidis D, et al. Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans. Food Chem Toxicol. 2013;61:171-7.

22. von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci. 2002;27(7):339-44.

23. Savela S, Saijonmaa O, Strandberg TE, Koistinen P, Strandberg AY, Tilvis RS, et al. Physical activity in midlife and telomere length measured in old age. Exp Gerontol. 2013;48(1):81-4.

24. Gilson E, Geli V. How telomeres are replicated. Nat Rev Mol Cell Biol. 2007;8(10):825-38.

25. Cluckey TG, Nieto NC, Rodoni BM, Traustadottir T. Preliminary evidence that age and sex affect exercise-induced hTERT expression. Exp Gerontol. 2017;96:7-11.

26. Dalgard C, Benetos A, Verhulst S, Labat C, Kark JD, Christensen K, et al. Leukocyte telomere length dynamics in women and men: menopause vs age effects. Int J Epidemiol. 2015;44(5):1688-95.

27. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, et al. Estrogen activates telomerase. Cancer Res. 1999;59(23):5917-21.

28. Ehrlenbach S, Willeit P, Kiechl S, Willeit J, Reindl M, Schanda K, 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):1725-34.

29. Lin J, Kroenke CH, Epel E, Kenna HA, Wolkowitz OM, Blackburn E, et al. Greater endogenous estrogen exposure is associated with longer telomeres in postmenopausal women at risk for cognitive decline. Brain Res. 2011;1379:224-31.

30. Puterman E, Lin J, Blackburn E, O'Donovan A, Adler N, Epel E. The power of exercise: buffering the effect of chronic stress on telomere length. PLoS One. 2010;5(5):e10837.

31. 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(3):89-99.