Physical activity, physical fitness and leukocyte telomere length.

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Abstract

Introduction-The influence of physical activity (PA) and physical fitness (PF) at older ages on changes in telomere length (TL), repetitive DNA sequences that may mark biologic aging, is not well-established. Few prior studies have been conducted in older adults, these were mainly cross-sectional, and few evaluated PF.

Methods-We investigated cross-sectional and prospective associations of PA and PF with leukocyte TL among 582 older adults (age 73±5 y at baseline) in the Cardiovascular Health Study, having serial TL measures and PA and PF assessed multiple times. Cross-sectional associations were assessed using multivariable repeated-measures regression, in which cumulatively averaged PA and PF measures were related to TL. Longitudinal analyses assessed cumulatively averaged PA and PF against later changes in TL; and changes in cumulatively averaged PA and PF against changes in TL.

Results-Cross-sectionally, greater walking distance and chair test performance, but not other PA and PF measures, were each associated with longer TL (*p*-trend=0.007, 0.04 respectively). In longitudinal analyses, no significant associations were observed between PA and PF with change in TL. In contrast, changes in leisure-time activity and chair test performance were each inversely associated with changes in TL.

Conclusions-Cross-sectional analyses suggest that greater PA and PF are associated with longer TL. Prospective analyses show that changes in PA and PF are associated with differences in changes in TL. Even so, even later in life, changes in certain PA and PF measures are associated with changes in TL, suggesting that leisure-time activity and fitness could reduce leukocyte telomere attrition among older adults.

Key words: elderly, exercise, fitness, biological aging, DNA

Introduction

Telomere length (TL), repetitive sequences of DNA placed at the ends of eukaryotic chromosomes that act as "caps" protecting genomic integrity and stability (46), has received attention as a potential marker of biologic aging. (4, 20) Leukocyte TL in humans has been associated with age-related diseases, disease biomarkers and mortality.(4, 5, 9, 11, 12, 30, 32) For example, in the Cardiovascular Health Study, shorter TL was associated with higher risk of CVD, with age-related disease burden and mortality. (5, 11, 12, 32)

Shortening of TL may be predominantly influenced by oxidative stress and inflammation.(33) It has been hypothesized that higher levels of physical activity (PA) and physical fitness (PF) may delay TL shortening, potentially through anti-inflammatory and anti-oxidative mechanisms.(22, 41) Greater PA and PF are consistently associated with lower morbidity and mortality from chronic diseases (1), supporting a potential "anti-aging" effect. Yet, only limited epidemiologic evidence supports an influence on PA or PF on TL. Among prior studies, some observational(7, 8, 17, 18, 21, 28, 34, 48) and 1 intervention (26) studies suggested favorable roles of PA or PF for TL profiles, but other observational (6, 47) and intervention (36, 42) studies did not. In addition, all of the observational studies assessed only cross-sectional associations of a single PA or PF measure, limiting conclusions on long-term, cumulative PA or PF. The 2 interventional studies were also of short duration (3-6 months), limiting inference on effects of long-term PA or PF. No prior studies separately measured both PA and PF and assessed whether each of PA and PF was independently associated with TL. For example 2 of 4 prior studies had small samples sizes (N<65), limiting statistical power to detect associations; and none separately evaluated both PA and PF to determine their potential independent associations with TL. Finally, few of these prior studies were conducted in older adults (18, 28, 34, 47), a particularly relevant population in which to study aging since old age is associated with a high prevalence of chronic diseases and consequently a possibly accelerated rate of telomere shortening. A recent 6-month randomized controlled physical activity trial in 68-year-old, sedentary and overweight subjects, suggested that reduced siting time, but not greater time spent exercising, was associated with telomere lengthening. (38) However, this study had a small sample size (N=49). (38)

To address these issues and determine whether long-term PA and PF are associated with TL and TL attrition later in life, we investigated the cross-sectional and prospective associations of PA and PF with TL in a community-based cohort study of older US adults.

Methods

Population

The design and recruitment of the Cardiovascular Health Study have been described.(13, 44) Briefly, 5,201 ambulatory, non-institutionalized men and women ≥65 years of age were randomly selected and enrolled from Medicare eligibility lists in 4 US communities in 1989-90; and an additional 687 black participants were similarly recruited and enrolled in 1992. The institutional review committee at each center approved the study, and all participants provided informed consent. From 1989-90 to 1998-99 participants were followed by annual study visits. Standardized evaluations included physical examination, diagnostic testing, laboratory evaluation, and questionnaires on health status, medical history, and cardiovascular risk factors.(13, 27, 44) Blood was collected and stored during most visits, and DNA collected from those participants that provided consent to use genetic material. Individuals from each enrollment phase were included in the present study if they consented the use of their DNA, had at least 12 mg DNA available, had stored leukocytes for additional DNA preparation, and had measures of PA and PF info at baseline. Characteristics of individuals included in this analysis were generally similar to the whole cohort.

Assessment of PA and PF

PA was assessed at multiple serial visits (Supplementary Figure 1, SDC, Timeline). Usual leisure-time activity was assessed using a modified, validated Minnesota Leisure-Time Activities questionnaire, which has been associated with risk of multiple disease outcomes in this cohort. (23) The questionnaire evaluated frequency and duration of 15 different activities during the prior 2 weeks, including gardening, mowing, raking, swimming, hiking, aerobics, tennis, jogging, racquetball, walking, golfing, bicycling, dancing, calisthenics, and exercise cycling.(37) Each activity was defined as having an intensity value in metabolic equivalent task (MET) units,(43) and participant responses regarding types, frequency, and duration of each activity were used to calculate weekly energy expenditure (kcal/week) from leisure-time activity. Usual exercise intensity was also assessed, with responses including no exercise or low, medium, or high intensity of exercise.(37) Usual walking habits, including average walking pace (gait speed), and distance walked, were assessed annually at each follow-up visit. We evaluated these metrics in pre-specified categories, including: usual pace walked (<2, 2-3 and >3 mph), blocks walked (quintiles), exercise intensity (none, low, medium and high) and leisure-time activity (quintiles). A previously defined walking score was also evaluated based on the combination of walking pace and walking distance.(23)

PF was also assessed at multiple serial visits (Supplementary Figure 1, SDC, Timeline), including based on 15-ft walk (sec), grip strength (kg) and chair stands (sec). In the 15-ft walk, a trained examiner measured the time needed for each participant to walk a 15-ft course (4.5m) at his or her usual pace. Grip strength was measured in the dominant hand using a hand-held JAMAR dynamometer, recording the force in kg for the best of 3 attempts at maximal squeeze. For the chair stand, a trained examiner recorded how quickly each participant performed 5 consecutive chair stands (standing up, with arms folded across the chest, from a seated position on a 45-cm-tall chair), timed to the nearest tenth of 1 sec. We evaluated each PF measure separately and, similar to the walking score, also constructed a summary measure based on all 3 PF measures (each in quintiles) to better capture the full variation of PF within the cohort.

Measurement of telomere length

TL (kilo base pairs, kbp) was measured as the mean length of the terminal restriction fragments in peripheral leukocytes.(4, 11, 25) A total of 582 older adults consented for DNA preparation and use, had at least 12 μg of available DNA, and had stored leukocytes for additional DNA preparation in both 1992-93 and 1997-98 and were included in the present analysis of TL change. TL was measured using the Southern blot method as previously described.(3, 25) Each sample was analyzed twice on different gels on different occasions, with mean value used for statistical analyses. The Pearson correlation coefficient for these duplicates was 0.97, with mean CV for pair sets of 1.5%. The laboratory conducting the TL measurements was blinded to all participant characteristics.

DNA integrity was assessed through electrophoresis of 0.5 μg of DNA on 1.0 ethidium bromide. These measures suggested some degradation, which would attenuate the ability to detect differences in TL changes over time, especially over only 5 years (1992-93 to 1997-98).

Covariates

Information on a wide range of covariates was obtained during study visits, including demographics, education, income, detailed smoking habits, alcohol use, usual dietary habits, body mass index (BMI), medication use, hypertension, diabetes and presence or absence of coronary heart disease, congestive heart failure.(13) Body mass index was calculated as weight (kg)/height $(m)^2$. Hypertension status was defined as either not present (systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg and no use of antihypertensive medication), borderline (systolic pressure 140–

159 mmHg or diastolic pressure 90–94 mmHg and no use of antihypertensive medication), or definite (systolic pressure ≥160 mmHg or diastolic pressure ≥95 mmHg or use of antihypertensive medication). Diabetes mellitus was classified using the American Diabetes Association criteria (21) as not present, impaired fasting glucose, or definite diabetes. Myocardial infarction was diagnosed using an algorithm including cardiac symptoms as chest pain, abnormal cardiac enzyme concentrations, and serial electrocardiogram changes. Fatal CHD included deaths not meeting criteria for myocardial infarction if occurring within 72 h of chest pain or with previous history of ischemic heart disease. CHD includes MI, angina, angiplasty, bypass and death due to atheroscleriotic. Strokes were classified as ischemic if there was evidence of focal brain deficit without evidence of primary hemorrhage; hemorrhagic if there was bloody spinal fluid on lumbar puncture or evidence of blood in the subarachnoid space, ventricles, or parenchyma on brain imaging or at surgery or autopsy that did not appear consistent with hemorrhage into an infarction; or unknown type if information was insufficient for classification.(19) CVD was defined as combined incident stroke, fatal and nonfatal MI and coronary heart disease death.

Statistical Analysis

Cross-sectional associations of PA and PF with TL were assessed using multivariable repeated-measures linear regression, utilizing measures of TL in both 1992-93 and 1997-98 and accounting for within-person correlation. To minimize misclassification (measurement error) and also better represent long-term effects of habitual PA and PF, we took advantage of repeated measures of PA to PF to perform cumulative updating (averaging of serial values) (Supplementary Figure 1, SDC, Timeline). When PA or PF were missing, the existing values were carried forward.

Cumulatively averaged PA and PF measures from 1989-93 were related to TL in 1992-93; and cumulatively averaged PA and PF from 1993-98 were related to TL in 1997-98. PA measures were assessed as categorical (indicator) variables; with tests for trend evaluated by entering PA categories as ordinal variables.

Longitudinal analyses of PA and PF with TL change were assessed using multivariable linear regression. Cumulatively averaged PA and PF from 1989-93 were related to the subsequent change in TL between 1992-93 and 1997-98; and changes in cumulatively averaged PA and PF between 1989-93 and 1993-98 were related to changes in TL between 1992-93 and 1997-98. The TL rate of change was calculated in bp/year, as ($LTL_{1997\text{-}98} - LTL_{1992\text{-}93}$)/follow-up years.

To minimize confounding, we adjusted models for major demographic factors including age, sex, race, study enrollment site, education, income, smoking status, and usual dietary habits, including consumption of total energy, omega-3 polyunsaturated fatty acids, omega-6 polyunsaturated fatty acids, and dietary fiber.(6, 10) We also evaluated factors which could be plausible biologic intermediates (i.e., on the putative causal pathway between PA and TL), including, body-mass index, waist circumference, fasting glucose, insulin, inflammatory markers, prevalent diseases, including T2DM and CVD.

In additional analyses, we evaluated both PA and PF measures in the same model to assess their independent associations with TL. To minimize the possibility of reverse causation (poor health causing low PA/PF), we performed sensitivity analyses restricted to participants reporting only good, very good, or excellent overall health and also having no limitation in activities of daily living or instrumental activities. Because in some participants (45%) the measured change in TL was positive (potentially representing measurement error, given that TL is not generally expected to increase), we also performed sensitivity analyses evaluating change in TL as a binary variable (any attrition, yes/no) and as a continuous variable but with any observed increases recoded as 0 (no change). We assessed potential interaction by age, sex, race and BMI by including a cross-product term of each potential modifier and each PA/PF measure in the regression model, evaluating significance of interaction using the Wald test. Analyses were performed using Stata 10.0 (College Station, Tx), two-tailed alpha=0.05.

Results

At baseline, mean age was 73±5 years, and 62 % of participants were women (Table 1). About 1 in 5 participants had prevalent CHD, and 1 in 7 had prevalent diabetes. Participants spent an average of 1045±1446 kcal per week on leisure-time activities and 35% engaged in moderate intensity PA. On average, participants walked 41±65 blocks per week, with 67% having a pace above 2 mph. The mean time needed to complete a distance of 15 ft and 5 chair stands was 5.5±2.0 and 14.8±4.9 seconds, respectively. Additionally, the mean hand grip strength was of 27.5±9.8 kg.

Overall at baseline, TL ranged from 5.1 to 8.6 kb, with mean±SD of 6.3±0.6 kb and median 6.3 kb. Mean TL change, calculated as $TL_{1997\text{-}98}$-$TL_{1992\text{-}93,}$ was -0.012±0.18 kb between 1992-93 and 1997-98, an annualized attrition of -2.44 bp/year.

Cross-sectional analysis of PA and PF and TL

In cross-sectional multivariable-adjusted analyses, greater reported walking distance and a better chair test performance were associated with longer TL (*p*-trend=0.007 and 0.04 respectively) (Table 2). Additionally, a better overall fitness score was associated with a trend toward longer TL (*p*-trend=0.09). In contrast, walking pace, leisure-time activity, time to complete a 15-ft walk, and hand grip strength were not significantly associated with TL. Analysis included only participants with excellent, very good and good health status and those with no limitations in activities of daily living or instrumental activities generated similar results.

Longitudinal analysis of PA and PF and change in TL

In multivariable longitudinal analyses, no significant associations were observed between PA and PF from 1989-93 and subsequent 5-year change in TL (Table 3). Results including only participants with good or better health status and without limitations in activities of daily living or instrumental activities were generally similar. In secondary analyses evaluating change in TL as a binary variable (attrition, yes/no) or as a continuous variable but with any observed increases re-coded as 0, no significant associations were observed between PA and PF from 1989-93 and subsequent 5-year change in TL (Supplementary Table 1 and 2, SDC, additional statistical analyses).

Longitudinal analysis of changes in PA and PA and change in TL

When we evaluated changes in PA and PF and changes in TL, change in leisure-time activity was associated with a trend toward less shortening in TL (*p*-trend=0.07), and change in chair test performance was associated with less shortening in TL (*p*-trend=0.04). For example, each 1000 kcal/week of increased leisure-time activity was associated with a trend toward 2.2 bp/year less attrition (95%CI: -0.18, 4.6); and each one second change in the time needed to complete 5 chair stands was associated with 0.9 bp/year less attrition in TL (95% CI: 0.04 1.8). Other PA measures such as walking pace, walking distance and walking score, and other PF measures such as the walk test, hand grip test, and overall PF score, were not significant associated with change in TL. When we excluded participants with poor self-reported health status or having any limitations in activities of daily living or instrumental activities, associations of changes in leisure-time activity and chair test performance with change in TL were strengthened in magnitude (2.8 bp/year and 1.2 bp/year, respectively) and statistical significance (*p*-trend=0.04 and 0.02, respectively). Results were generally similar in sensitivity analyses recoding any observed increases in TL to no change (Supplementary Table 3, SDC, additional statistical analyses).

Results were not appreciably altered in several sensitivity analyses, including further adjustment for both PA and PF measures to assess their independent associations with TL or further adjustment for baseline characteristics that could be either confounders or mediators of these relationships (see Methods). Additionally, we performed cumulative averaging with 50% weight given to most recent PA/PF measure, with similar results to the equal weight cumulative averaging (data not shown).

Discussion

In this large prospective study among older adults, average age 73 years at their first measurement of TL, cross-sectional analyses suggested that greater walking distance as well as chair test performance are associated with longer TL. Furthermore, prospective analyses have shown that changes in leisure-time activity and in chair test performance are associated with differences in change in TL. The lack of prospective associations of other PA and PF metrics could be due to measurement error in TL due to DNA degradation, which would have diminished the ability to detect changes. Even so, even later in life, changes in certain PA and PF are associated with TL, suggesting that greater leisure-time activity and fitness could reduce leukocyte attrition among older adults.

Telomeres are cap-like nucleoproteins at chromosome ends, which protect genome from degradation and interchromosomal fusion(16, 35). In the normal cellular process, a small portion of telomeric DNA is lost with each cell division, when a limit length is achieved cell undergoes apoptosis.(35) Normally with aging chromosomes become increasingly impaired due to DNA damage, eventually leading to apoptotic signals and cell death; however, telomeres can prevent or delay such damage.(16) It has been hypothesized that certain lifestyles factors may accelerate telomere shortening and consequently affect health, healthy aging, and longevity.(35) Shorter TL is associated with several age-related diseases, (39) including cardiovascular diseases and type 2 diabetes.(11) Our observed findings of longer telomeres with some measures of greater PA and PF at baseline and less telomere attrition with some measures of changes in PA and PF longitudinally suggest that PA and PF could influence pathways related to TL. Such an effect could, for example, partly account for the beneficial associations of PA and PF with many age-related diseases. (39) (35)

Biologic plausibility of our findings is supported by the putative pathways of telomere loss, which are thought to be related to cumulative burdens of oxidative stress and inflammation (2, 14), and the pathways of benefits of regular PA, which include upregulation of antioxidant defense systems (15) and reduced chronic systemic inflammation. (41) By these and other pathways, PA may reduce oxidative DNA damage; (33, 39) for example, duration of exercise has been inversely correlated with biomarkers for DNA and telomere damage and with p16 expression, a biomarker for cellular aging.(39) Interestingly, a bout of acute exercise increases production of free radicals, dependent on intensity and duration.(15) This pro-oxidant response may be necessary for activation of beneficial anti-oxidant and other cellular defense systems (29), by means of which habitual, long-term PA, such as we evaluated in this study, may lead to beneficial physiological adaptations.(15)

Another possible explanatory pathway might be through an upregulation of telomerase reverse transcriptase that seems to occur after exercise. (14) For example, mechanisms for beneficial effects of omega-3 fatty acids and PA on survival after acute myocardial infarction could relate to elevation in telomerase expression, resulting in higher regeneration potential (31, 45). Although controversial, some evidence suggests that leucocyte TL could actually elongate over a decade (24); however, others believe that apparent elongation is mainly due to measurement error (40). No consensus seems to exist concerning this potential for lengthening of telomeres; further studies on this topic are needed.

In the present work we observed similarities and differences in cross-sectional versus prospective analyses as for example, walking distance but not leisure-time activity in cross-sectional analyses was associated with longer TL; conversely in prospective analyses leisure-time activity but not walking distance was associated with differences in change in TL. Interestingly, chair test was associated with both cross-sectional and prospective analyses. The reasons for these specific associations are unknown and our novel findings highlight the need for further investigation of how different types of PA and different measures of PF may influence TL.

The American College of Sports Medicine and American Heart Association recommend that older adults engage in at least in 30 min of moderate PA on most days of the week.(1) Our results support these general guidelines by suggesting that long-term PA may influence telomere dynamics later in life.

Previous studies of PA and TL have provided inconsistent results; and only 4 were conducted in older adults. (18, 28, 34, 47) Of these, one cross-sectional study among 2,006 older Chinese participants reported no association between PA and TL(47); the other 3 studies, also cross-sectional but conducted in much smaller samples (N=32 to 204), found positive associations between PA and TL.(18, 28, 34) Our results are consistent with these latter 3 cross-sectional studies and also with other cross-sectional studies, conducted among middle age and younger participants, linking higher PA to longer TL.(7, 8, 17, 18, 21, 28, 34, 48) Our findings build upon and expand these previous results by evaluating both cross-sectional and longitudinal associations of PA, PF and TL, including changes in both, in a well-established cohort of older US adults.

Our analysis had several strengths. Information on PA, PF, TL and other risk factors was prospectively assessed using standardized methods. Participants were randomly selected and enrolled from Medicare eligibility lists in several US communities, providing a community-based sample of older adults. Serial measures of PA allowed evaluation of cumulatively updated PA, reducing misclassification and providing a better measure of longer-term PA. Serial measures also allowed the novel evaluation of how changes in PA relate to changes in TL. Prospective analyses as well as sensitivity analyses excluding less healthy participants reduced the potential for reverse causation, and adjustment for a wide range of covariates minimized the potential impact of confounding.

Potential limitations were also present. Measurement error in TL, and in particular TL change, would diminish the ability to detect associations, which would cause underestimation of the magnitude and statistical significance of our findings. Additionally, the TL quantification technique used is a less sensitive method to identify subtle differences between individuals and requires high-quality DNA. We evaluated several different PA and PF indices, increasing the possibility of chance findings. However, several of our findings are consistent with other studies; and one could consider each PA or PF and TL association a separate hypothesis. Borderline *p* values should be interpreted with caution, with careful attention to both internal consistency and biological plausibility. PA measures were obtained from self-report, and may appropriately reflect relative ordering (ranking) of participants but not precise quantitative levels of energy expenditure. Although a range of covariates were available and evaluated as potential confounders and findings were similar in sensitivity analyses, residual confounding due to unknown or incompletely measured factors cannot be excluded. The assessments of PA, PF, and TL were subject to random error and biological variability, which would attenuate findings toward the null. The prospective associations of cumulatively updated PA with TL could also partly reflect the effects of PA earlier in life; in contrast, the associations of changes in PA with TL would not be confounded by PA at younger ages. Different participants had different number of exposure measures and thus possible different precision of the exposure. Results were attained from older, predominantly white

Americans and may not be directly generalizable to other populations. Furthermore, our results may only be generalized to leukocyte TL, since it may not reflect TL dynamics in other tissues. Conversely, leukocyte TL is the most commonly measured TL metric, and has been associated with diverse exposures and disease endpoints in prior studies.

In sum, our results suggest that greater walking distance and chair test performance are cross-sectionally associated with longer TL; and that changes in leisure-time activity and in chair test performance are associated with differences in change in TL. These results suggest that PA and PF may have a role in the regulation of telomere length during the aging process.

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Conflict of Interest Disclosures

None of the authors have a conflict of interest in relation to this manuscript.

Supplemental Word Content 1. pdf

References

1. American College of Sports Medicine, Chodzko-Zajko WJ, Proctor DN et al. American College of Sports Medicine position stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc*. 2009;41(7):1510-30.
2. Aviv A. Telomeres and human aging: facts and fibs. *Science of aging knowledge environment : SAGE KE*. 2004;2004(51):pe43.
3. Benetos A, Gautier S, Lafleche A et al. Blockade of angiotensin II type 1 receptors: effect on carotid and radial artery structure and function in hypertensive humans. *J Vasc Res*. 2000;37(1):8-15; discussion 68-70.
4. Benetos A, Okuda K, Lajemi M et al. Telomere length as an indicator of biological aging: The gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*. 2001;37(2):381-5.
5. Burnett-Hartman AN, Fitzpatrick AL, Kronmal RA et al. Telomere-associated polymorphisms correlate with cardiovascular disease mortality in Caucasian women: the Cardiovascular Health Study. *Mech Ageing Dev*. 2012;133(5):275-81.
6. 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):1273-80.
7. Cherkas LF, Hunkin JL, Kato BS et al. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med*. 2008;168(2):154-8.

8. Du M, Prescott J, Kraft P et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. *Am J Epidemiol*. 2012;175(5):414-22.
9. Epel ES, Merkin SS, Cawthon R et al. The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging*. 2009;1(1):81-8.
10. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA*. 2010;303(3):250-7.
11. 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.
12. 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):421-9.
13. Fried LP, Borhani NO, Enright P et al. The Cardiovascular Health Study: design and rationale. *Annals of epidemiology*. 1991;1(3):263-76.
14. Garatachea N, Pareja-Galeano H, Sanchis-Gomar F et al. Exercise attenuates the major hallmarks of aging. *Rejuvenation research*. 2015;18(1):57-89.
15. Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative medicine and cellular longevity*. 2012;2012:756132.
16. Kelly DP. Cell biology: Ageing theories unified. *Nature*. 2011;470(7334):342-3.

17. Kim JH, Ko JH, Lee DC, Lim I, Bang H. Habitual physical exercise has beneficial effects on telomere length in postmenopausal women. *Menopause*. 2012;19(10):1109-15.

18. LaRocca TJ, Seals DR, Pierce GL. Leukocyte telomere length is preserved with aging in endurance exercise-trained adults and related to maximal aerobic capacity. *Mech Ageing Dev*. 2010;131(2):165-7.

19. Longstreth WT, Jr., Bernick C, Fitzpatrick A et al. Frequency and predictors of stroke death in 5,888 participants in the Cardiovascular Health Study. *Neurology*. 2001;56(3):368-75.

20. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153(6):1194-217.

21. Ludlow AT, Zimmerman JB, Witkowski S, Hearn JW, Hatfield BD, Roth SM. Relationship between physical activity level, telomere length, and telomerase activity. *Med Sci Sports Exerc*. 2008;40(10):1764-71.

22. McArdle A, Jackson MJ. Exercise, oxidative stress and ageing. *J Anat*. 2000;197 Pt 4:539-41.

23. Mozaffarian D, Furberg CD, Psaty BM, Siscovick D. Physical activity and incidence of atrial fibrillation in older adults: the cardiovascular health study. *Circulation*. 2008;118(8):800-7.

24. Nordfjall K, Svenson U, Norrback KF, Adolfsson R, Lenner P, Roos G. The individual blood cell telomere attrition rate is telomere length dependent. *PLoS Genet*. 2009;5(2):e1000375.

25. Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, Aviv A. Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. *Atherosclerosis*. 2000;152(2):391-8.

26. Ornish D, Lin J, Daubenmier J et al. Increased telomerase activity and comprehensive lifestyle changes: a pilot study. *Lancet Oncol*. 2008;9(11):1048-57.

27. Psaty BM, Kuller LH, Bild D et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. *Annals of epidemiology*. 1995;5(4):270-7.

28. 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.

29. Ristow M, Zarse K, Oberbach A et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;106(21):8665-70.

30. Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care*. 2006;29(2):283-9.

31. Sanchis-Gomar F, Lucia A. Acute myocardial infarction: ‘telomerasing’ for cardioprotection. *Trends in Molecular Medicine* 2015 Article in Press.

32. Sanders JL, Fitzpatrick AL, Boudreau RM et al. Leukocyte telomere length is associated with noninvasively measured age-related disease: The Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2012;67(4):409-16.

33. Sattelmair JR, Pertman JH, Forman DE. Effects of physical activity on cardiovascular and noncardiovascular outcomes in older adults. *Clin Geriatr Med*. 2009;25(4):677-702, viii-ix.

34. Savela S, Saijonmaa O, Strandberg TE et al. Physical activity in midlife and telomere length measured in old age. *Exp Gerontol*. 2013;48(1):81-4.
35. Shammas MA. Telomeres, lifestyle, cancer, and aging. *Current opinion in clinical nutrition and metabolic care*. 2011;14(1):28-34.
36. Shin YA, Lee JH, Song W, Jun TW. Exercise training improves the antioxidant enzyme activity with no changes of telomere length. *Mech Ageing Dev*. 2008;129(5):254-60.
37. Siscovick DS, Fried L, Mittelmark M et al. Exercise Intensity and Subclinical Cardiovascular Disease in the Elderly: The Cardiovascular Health Study. *Am. J. Epidemiol.* 1997;145(11):977-86.
38. 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. *British journal of sports medicine*. 2014;48(19):1407-9.
39. Song Z, von Figura G, Liu Y et al. Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood. *Aging Cell*. 2010;9(4):607-15.
40. Steenstrup T, Hjelmborg JV, Kark JD, Christensen K, Aviv A. The telomere lengthening conundrum--artifact or biology? *Nucleic acids research*. 2013;41(13):e131.
41. Stewart LK, Flynn MG, Campbell WW et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. *Med Sci Sports Exerc*. 2007;39(10):1714-9.
42. Svenson U, Nordfjall K, Baird D et al. Blood cell telomere length is a dynamic feature. *PLoS One*. 2011;6(6):e21485.

43. Taylor HL, Jacobs DR, Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. *Journal of Chronic Diseases*. 1978;31(12):741-55.

44. Tell GS, Fried LP, Hermanson B, Manolio TA, Newman AB, Borhani NO. Recruitment of adults 65 years and older as participants in the Cardiovascular Health Study. *Annals of epidemiology*. 1993;3(4):358-66.

45. Terai M, Izumiyama-Shimomura N, Aida J et al. Association of telomere shortening in myocardium with heart weight gain and cause of death. *Scientific reports*. 2013;3:2401.

46. Varela E, Blasco MA. 2009 nobel prize in physiology or medicine: telomeres and telomerase. *Oncogene*. 2010;29(11):1561-5.

47. 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.

48. 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*. 2010.

Table 1. Baseline (1992-93) characteristics of 582 older US adults in the Cardiovascular Health Study with longitudinal assessment of physical activity, physical fitness and telomere length.

Characteristic	
Age, years	73±5
Gender, % male	38
Race, % white	85
Education	
< High school, %	24
High school, %	32
> High school, %	43
Annual income ≥ $25,000, %	39
Smoking habits	
Former smoker, %	44
Current smoker, %	10
Body mass index, kg/m²	27±5
Prevalent coronary heart disease, %	20
Prevalent congestive heart failure, %	5
Prevalent diabetes mellitus, %	14
Physical activity	
Walking pace, mph	
< 2, %	33
> 2, %	67
Walking blocks, blocks/week	41±65
Exercise intensity	
None, %	8
Low, %	45
Moderate, %	35
High, %	12
Leisure-time activity, kcal/week	1045±1446
Physical fitness	
Walk test, sec/15 ft	5.5±2.0
Hand grip test, kg	27.5±9.8
Chair test, sec/5 chair stands	14.8±4.9

Values are mean ± SD (continuous variables) or percentage (categorical variables).
Coronary heart disease=history of myocardial infarction, angina, or coronary revascularization.
Congestive Heart Failure = according to the presence of following symptoms: sleep on 2 pillows to breathe, awakened at night by trouble breathing, swelling of feet and ankles during the day which goes down overnight. Diabetes =fasting glucose >140 mg/dl, two hour post-oral challenge glucose >200 mg/dl, or use of insulin or oral hypoglycemic medications.

Table 2. Multivariable-adjusted cross-sectional associations in cumulatively averaged physical activity and physical fitness, between 1989-90 and 1992-93 and between 1993-94 and 1997-98, with telomere length, from1992-93 and 1997-98, among 1164 older US adults.

	Telomere Length, (95% CI), base pairs *		
	All participants	Excluding participants with poor or fair self-reported health status	Excluding participants with limitations in activities of daily living
	N=582	N=458	N=438
*Physical Activity***			
Walking pace, mph			
< then 2	reference	reference	reference
2-3	9.5 (-18.3, 37.4)	10.2 (-21.6, 42.0)	11.6 (-21.1, 44.3)
> then 3	-19.5 (-67.3, 28.3)	-14.1 (-66.6, 38.5)	-19.8 (-72.1, 32.5)
P trend	0.78	0.86	0.72
Walking distance, blocks/week			
≤ 5	reference	reference	reference
6 to 13	33.0 (-6.6, 72.6)	54.7 (8.2, 101.2)	17.3 (-32.6, 67.2)
14 to 27	36.9 (-6.9, 80.8)	62.6 (12.6, 112.4)	17.8 (-35.6, 71.2)
28 to 54	46.2 (-1.4, 91.8)	66.6 (13.9,119.3)	24.9 (-30.4, 80.1)
≥55	79.4 (27.6, 131.3)	109.6 (50.7, 168.6)	60.0 (-0.4, 120.4)
P trend	0.007	0.002	0.06
Walking Score[δ]			
I	reference	reference	reference
II	17.9 (-26.9, 62.8)	23.2 (-32.3, 78.6)	28.9 (-28.1, 85.9)
III	4.3 (-53.1, 61.7)	22.1 (-44.9, 89.0)	17.8 (-51.4, 87.0)
IV	13.3 (-34.7, 61.4)	38.6 (-19.9, 97.1)	28.0 (-30.9, 86.9)
V	18.7 (-29.4, 66.8)	37.1(-21.8,96.1)	30.2(-28.8, 89.1)
P trend	0.54	0.95	0.49
Intensity			
None	reference	reference	reference
Low	28.4 (-14.8, 71.6)	6.0 (-47.6, 59.6)	24.1 (-32.2, 80.3)
Moderate	32.9 (-12.7, 78.6)	10.6 (-44.4, 65.6)	35.6 (-23.5, 94.7)
High	58.4 (-4.1, 120.9)	35.9 (-34.7, 106.4)	79.0 (4.2, 153.9)
P trend	0.12	0.33	0.04
Leisure-time activity, kcal/week			
<104	reference	reference	reference
105 to 420	34.9 (-4.5, 74.5)	31.9 (-14.3, 78.0)	59.2 (9.9, 108.5)
431 to 875	28.9 (-15.5, 73.4)	27.4 (-24.4, 79.2)	47.6 (-6.7, 101.8)
889 to 1740	35.3 (-11.4, 82.1)	34.6 (-19.3, 88.6)	44.2 (-13.7, 102.1)
≥1761	38.8 (-11.1, 88.7)	35.6 (-20.5, 91.8)	61.8 (2.4, 121.2)
P trend	0.21	0.39	0.31
*Physical Fitness***			
Walk test , sec/15 ft [Ŧ]			
≥6.7	reference	reference	reference
6.5 to 5.7	16.2 (-11.9, 8.7)	10.5 (-37.9, 58.8)	14.5 (-33.0, 62.0)
5.5 to 5.0	37.5 (-10.3, 8.5)	41.1 (-11.9, 94.1)	50.7 (-1.6, 103.1)
4.7 to 4.3	46.1 (-6.9, 14.2)	51.7 (-4.4, 107.7)	47.2 (-9.0, 103.4)
4.0 to 3.0	31.5 (-15.1, 7.5)	33.2 (-29.3, 95.6)	25.4 (-37.6, 88.4)
P trend	0.20	0.18	0.41
Hand grip test, kg[Ŧ]			
<19.6	reference	reference	reference
19.7 to 23.6	-13.9 (-56.4, 28.6)	-24.0 (-69.1, 21.1)	-5.8 (-53.4, 41.7)
23.7 to 28.8	-1.6 (-56.4, 53.3)	-27.3 (-85.8, 31.3)	21.9 (-41.4, 85.4)
29.1 to 37.1	20.1 (-47.5, 87.6)	-3.5 (-76.7, 69.6)	42.2 (-35.8, 120.2)
≥37.3	37.9 (-52.9, 128.7)	12.2 (-85.7, 110.1)	35.9 (-66.7, 138.5)
P trend	0.47	0.95	0.36
Chair test, sec/5 chair stands [Ŧ]			
≥17.0	reference	reference	reference
16.7 to 14.0	-2.9 (-41.2, 35.3)	-16.3 (-60.3, 27.7)	-18.5 (-61.3, 24.3)
13.7 to 12.3	8.5 (-34.5, 51.5)	8.4 (-40.6, 57.3)	2.6 (-44.6, 49.8)
12.0 to 10.7	29.7 (-15.2, 74.6)	34.9 (-15.2, 84.9)	18.7 (-31.3, 68.6)
<10.6	39.8 (-7.3, 86.9)	41.2 (-11.8, 94.1)	21.9 (-29.5, 73.4)
P trend	0.04	0.02	0.18
Physical fitness score [Ŧ δ]			
I	reference	reference	reference
II	-11.6 (-50.7, 27.4)	-31.7 (-78.2, 14.8)	-22.9 (-70.0, 24.2)
III	8.8 (-35.9, 53.6)	-18.5 (-72.6, 35.7)	1.0 (-52.7, 54.8)
IV	35.1 (-14.9, 85.1)	16.6 (-42.3, 75.5)	20.0 (-38.6, 78.6)
V	31.9 (-28.5, 92.3)	9.8 (-60.3, 80.0)	13.5 (-54.42, 80.7)
P trend	0.09	0.18	0.29

* All analyses adjusted for age (years), gender (male/female), race (white/nonwhite), clinical site (4 categories), education (< high school, high school > high school), income (≤/> $ 25 000/year) and smoking status (never/former/current).

** Cross-sectional (mix-model) analysis according to physical activity and physical fitness cumulative average between 1997-98 and 1992-93.

[δ] Walking score is an ordinal score based on the combination of walking pace and walking distance. Physical fitness score is an ordinal score based on the combination of performances on the walk test, hand grip test and chair test (each in quintiles).

[Ŧ] Sample size included ~ 25-40 fewer participants in each analysis due to missing data on the exposure.

Table 3. Multivariable-adjusted longitudinal associations in cumulatively averaged physical activity and physical fitness, between 1989-93, with changes in telomere length, between 1992-93 and 1997-98, among 582 older US adults.

Telomere Length, (95% CI), base pairs per year*	All participants	Excluding participants with poor or fair self-reported health status	Excluding participants with limitations in activities of daily living
	N=582	N=458	N=438
Physical Activity [**]			
Walking pace, mph			
< then 2	reference	reference	reference
2-3	1.2 (-6.2, 8.4)	3.1 (-5.7, 12.0)	6.5 (-3.0, 16.0)
> then 3	-2.8 (-12.5, 6.8)	-0.6 (-11.8, 10.5)	2.9 (-8.6, 14.3)
P trend	0.62	0.88	0.74
Walking distance, blocks/week			
≤ 5	reference	reference	reference
6 to 13	-2.5 (-12.3, 7.2)	-3.9 (-15.3, 7.6)	-0.8 (-13.2, 11.5)
14 to 27	7.4 (-2.2, 16.9)	6.0 (-5.1, 17.1)	12.7 (0.9, 24.4)
28 to 54	-1.4 (-10.9, 8.2)	-2.8 (-12.9, 8.3)	-0.3 (-11.8, 11.2)
≥55	3.3 (-6.4, 13.0)	3.1 (-7.9, 14.3)	3.9 (-7.6, 15.3)
P trend	0.50	0.56	0.69
Walking score [δ]			
I	reference	reference	reference
II	0.6 (-12.0 13.3)	1.5 (-15.2, 18.1)	3.4 (-14.6, 21.4)
III	2.7 (-9.2, 14.5)	6.2 (-9.7, 22.1)	12.3 (-4.4, 28.9)
IV	4.0 (-6.9, 14.9)	5.4 (-9.3, 20.2)	11.4 (-4.1, 26.9)
V	3.5 (-8.2, 15.2)	6.2 (-9.1, 21.5)	9.2 (-6.9, 25.2)
P trend	0.43	0.36	0.28
Intensity			
None	reference	reference	reference
Low	-8.7 (-24.3, 6.8)	-9.5 (-28.6, 9.6)	-11.5 (-34.1, 11.2)
Moderate	-9.4 (-24.9, 6.2)	-10.4 (-29.4, 8.6)	-14.3 (-36.9, 8.3)
High	-0.3 (-17.9, 17.3)	0.8 (-20.1, 21.6)	-3.4 (-27.7, 20.9)
P trend	0.59	0.44	0.76
Leisure-time activity, kcal/week			
≤104	reference	reference	reference
105 to 420	-2.3 (-11.6, 7.1)	0.6 (-10.4, 11.6)	-1.2 (-13.0, 10.6)
431 to 875	4.2 (-5.4, 13.7)	7.5 (-3.6, 18.7)	3.2 (-8.6, 15.1)
889 to 1740	4.3 (-5.4, 14.0)	5.7 (-5.4, 16.9)	5.9 (-6.2, 18.0)
≥1761	-1.9 (-11.8, 7.9)	0.5 (-10.6, 11.6)	-2.2 (14.1, 9.7)
P trend	0.83	0.78	0.98
Physical Fitness [**]			
Walk test, sec/15 ft[Ŧ]			
≥6.7	reference	reference	reference
6.5 to 5.7	-1.6 (-11.9, 8.7)	1.6 (-10.6, 13.8)	2.4 (-11.4, 16.2)
5.5 to 5.0	-0.9 (-10.3, 8.5)	0.3 (-10.6, 11.2)	4.1 (-8.7, 16.9)
4.7 to 4.3	3.6 (-6.9, 14.2)	4.5 (-7.3, 16.3)	9.6 (-4.2, 23.4)
4.0 to 3.0	3.8 (-15.1, 7.5)	-1.7 (-14.4, 10.9)	0.7 (-13.8, 15.1)
P trend	0.94	0.99	0.62
Hand grip test, kg[Ŧ]			
≤19.6	reference	reference	reference
19.7 to 23.6	5.0 (-4.4, 14.4)	1.7 (-8.8, 12.1)	7.8 (-3.3, 18.9)
23.7 to 28.8	10.7 (1.1, 20.3)	6.3 (-4.3, 16.9)	12.8 (0.9, 24.7)
29.1 to 37.1	8.6 (-2.9, 20.2)	4.7 (-7.8, 17.2)	6.7 (-7.4, 20.8)
≥37.3	9.7 (-4.4, 23.7)	3.8 (-11.5, 19.1)	11.9 (-5.3, 29.1)
P trend	0.07	0.41	0.13
Chair test, sec/5 chair stands[Ŧ]			
≥17.0	reference	reference	reference
16.7 to 14.0	-1.4 (-10.9, 8.1)	2.9 (-7.2, 12.9)	1.6 (-8.9, 12.1)
13.7 to 12.3	-2.9 (-12.5, 6.6)	-3.9 (-14.2, 6.4)	-2.0 (-12.7, 8.6)
12.0 to 10.7	2.7 (-6.6, 12.1)	4.1 (-6.1, 14.4)	4.2 (-6.4, 14.7)
≤10.6	-3.2 (-13.4, 6.9)	0.7 (-10.5, 11.9)	-2.5 (-14.4, 9.4)
P trend	0.93	0.78	0.98
Physical fitness score [Ŧ δ]			
I	reference	reference	reference
II	3.5 (-6.4, 13.5)	1.9 (-10.2, 13.9)	4.7 (-8.7, 18.0)
III	4.1 (-5.8, 13.9)	0.2 (-11.5, 11.9)	4.7 (-8.6, 17.9)
IV	2.2 (-8.3, 12.7)	2.9 (-9.4, 15.3)	4.1 (-9.7, 17.9)
V	-0.2 (-13.1, 12.8)	-1.6 (-16.7, 13.4)	3.4 (-12.6, 19.3)
P trend	0.94	0.99	0.84

* Rate of change in TL (bp/year) = ($TL_{1997-98} - TL_{1992-93}$)/follow-up years. Positive values indicate lesser shortening in telomere length according to comparison to reference group, whereas negative values indicate greater shortening in telomere length. All analyses adjusted for age (years), gender (male/female), race (white/nonwhite), clinical site (4 categories), education (< high school, high school, > high school), income (≤/> $25 000/year) and smoking status (never/former/current).

[**] Longitudinal analysis according to physical activity and physical fitness cumulative average of 1989-90, 1990-91, 1991-92, 1992-93 (or the ones available).

[δ] Walking score is an ordinal score based on the combination of walking pace and walking distance. Physical fitness score is an ordinal score based on the combination of performances on the walk test, hand grip test and chair test (each in quintiles).

[Ŧ] Sample size included ~ 25-40 fewer participants in each analysis due to missing data on the exposure.

Table 4. Multivariable-adjusted associations of changes in cumulatively averaged physical activity and physical fitness between 1989-93 and 1993-98 with changes in telomere length between 1992-93 and 1997-98 among 582 older US adults.

	Differences in Telomere Length, (95% CI), base pairs per year*		
	All participants N=582	Excluding participants with poor or fair self-reported health status N=458	Excluding participants with limitations in activities of daily living N=438
Physical Activity **			
Change in walking pace, per each higher mph (≤-2: 3.3%; -1.5 to -1: 24.3%; 0.5: 15.0%; 0: 44.6%; 0.5: 6.9%; ≥1: 5.9%)***	-0.6 (-4.9, 3.7)	-2.0 (-6.9, 2.8)	-3.2 (-8.2,1.7)
	0.78	0.41	0.20
Change in walking distance, per higher blocks/week (mean± SD: -7.7 ± 33.5; 10^{th} percentile: -42.1 ; 90^{th} percentile: 22.1)	0.04 (-0.05, 0.13)	-0.01 (-0.10, 0.1)	0.06 (-0.03, 0.15)
	0.40	0.90	0.19
Change in walking score, per 1 higher unit$^{\delta}$ (≤-1.3: 4.1%; -1: 20%; -0.75 to -0.25: 5.7%; 0: 50.8%; 0.27to 0.74: 3.4%; ≥ 2: 16%)***	-1.6 (-5.5, 2.2)	-3.1 (-7.5, 1.3)	-0.7 (-5.1, 3.8)
	0.41	0.17	0.76
Change in leisure-time activity, per higher 1000kcal/week (mean± SD: -345.9 ± 1238.8; 10^{th} percentile: -1653.8 ; 90^{th} percentile: 735)	2.2 (-0.18, 4.6)	2.3 (-0.20, 4.8)	2.8 (0.15, 5.4)
	0.07	0.07	0.04
Physical Fitness **			
Change in walk test, per 1 higher sec/15 ftŦ (mean± SD: 0.4±1.9; 10^{th} percentile: -0.9; 90^{th} percentile: 1.8)	0.2 (-1.4, 1.8)	0.5 (-1.2, 2.3)	2.1 (-0.5,4.6)
	0.80	0.56	0.11
Change in hand grip test, per higher kgŦ (mean± SD: -0.6 ± 3.6; 10^{th} percentile: -5.0 ; 90^{th} percentile: 3.7)	0.4 (-0.5, 1.3)	0.4 (-0.6, 1.4)	0.3 (-0.7, 1.3)
	0.37	0.41	0.60
Change in chair test, per 1 higher sec/5 chair standsŦ (mean± SD: 2.2 ± 3.6; 10^{th} percentile: -1.7 ; 90^{th} percentile: 6.5)	0.9 (0.04, 1.8)	1.1 (0.5, 2.2)	1.2 (0.2, 2.2)
	0.04	0.04	0.02
Change in physical fitness score, per 1 higher unitŦ $^{\delta}$ (≤-1: 18.7%; 0: 43.9%; ≥1: 37,4%)***	-2.2 (-6.0, 1.6)	-2.7 (-6.8, 1.3)	-2.4 (-6.6, 1.7)
	0.25	0.19	0.25

* Rate of change in TL (bp/year) = ($TL_{1997-98} - TL_{1992-93}$)/follow-up years. Positive values indicate lesser shortening in telomere length, whereas negative values indicate greater shortening in telomere length. All analyses adjusted for age (years), gender (male/female), race (white/nonwhite), clinical site (4 categories), education (< high school, high school, > high school), income (≤/> $25 000/year) and smoking status (never/former/current).

**Longitudinal analysis according to physical activity and physical fitness cumulative average difference between 1997-98 and 1992-93.

*** Categories of change, and the proportion of participants in each category

δ Walking score is an ordinal score based on the combination of walking pace and walking distance. Physical fitness score is an ordinal score based on the combination of performances on the walk test, hand grip test and chair test (each in quintiles).

Ŧ Sample size included ~ 25-40 fewer participants in each analysis due to missing data on the exposure.