# 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.

**Acknowledgments**

The authors express their gratitude to the CHS participants. A full list of participating CHS investigators and institutions is at http://www.chs-nhlbi.org.

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01 HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant U01HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

Luisa Soares-Miranda is supported by the Portuguese Foundation of Science and Technology (FCT), SFRH/BPD/76947/2011, PTDC/DES/099018/2008 - FCT/FCOMP-01- 0124-FEDER-009573, and The Research Centre in Physical Activity Health and Leisure is supported by UID/DTP/00617/2013.

Dr Imamura received support from the Medical Research Council Epidemiology Unit Core Support (MC_UU_12015/5).

The funders had no role in study design or conduct; data collection, management, analysis, or interpretation; or manuscript preparation, review, or approval.

Results of the present study do not constitute endorsement by ACSM.

**Conflict of Interest Disclosures**

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

Supplemental Word Content 1. pdf

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548 **Table 1**. Baseline (1992-93) characteristics of 582 older US adults in the Cardiovascular Health Study
549 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 |

550 Values are mean ± SD (continuous variables) or percentage (categorical variables).
551 Coronary heart disease=history of myocardial infarction, angina, or coronary revascularization.
552 Congestive Heart Failure = according to the presence of following symptoms: sleep on 2 pillows to
553 breathe, awakened at night by trouble breathing, swelling of feet and ankles during the day which goes
554 down overnight. Diabetes =fasting glucose >140 mg/dl, two hour post-oral challenge glucose >200
555 mg/dl, or use of insulin or oral hypoglycemic medications.
556

**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.

$^{\delta}$ 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).

$^{\text{Ŧ}}$ 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) = ($TL_{1997\text{-}98} - TL_{1992\text{-}93}$)/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 | 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* ** | | | |
| 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; 10th percentile: -42.1 ; 90th 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; 10th percentile: -1653.8 ; 90th 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 ft$^{Ŧ}$ (mean± SD: 0.4±1.9; 10th percentile: -0.9; 90th 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 kg$^{Ŧ}$ (mean± SD: -0.6 ± 3.6; 10th percentile: -5.0 ; 90th 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 stands$^{Ŧ}$ (mean± SD: 2.2 ± 3.6; 10th percentile: -1.7 ; 90th 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 unit$^{Ŧ\ \delta}$ (≤-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) = ($TL_{1997\text{-}98} - TL_{1992\text{-}93}$)/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

$^{\delta}$ 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.