Leisure-Time and Occupational Physical Activity Associates Differently with Epigenetic Aging

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**Conflict of interest.** The authors declare that there are no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Abstract

**Purpose:** Greater leisure-time physical activity (LTPA) associates with healthier lives, but knowledge regarding occupational physical activity (OPA) is more inconsistent. DNA methylation (DNAm) patterns capture age-related changes in different tissues. We aimed to assess how LTPA and OPA are associated with three DNAm based epigenetic age estimates, namely DNAm Age, PhenoAge and GrimAge.

**Methods:** The participants were young adult (21–25-year-old, \( n = 285 \)) and older (55–74-year-old, \( n = 235 \)) twin pairs, including 16 pairs with documented long-term LTPA discordance. Genome-wide DNAm from blood samples was used to compute DNAm Age, PhenoAge and GrimAge Age acceleration (Acc), which describes the difference between chronological and epigenetic ages. Physical activity was assessed with sport, leisure-time and work indices based on the Baecke Questionnaire. Genetic and environmental variance components of epigenetic age Acc were estimated by quantitative genetic modelling.

**Results:** Epigenetic age Acc was highly heritable in young adult and older twin pairs (~60%). Sport index was associated with slower and OPA with faster DNAm GrimAge Acc after adjusting the model for sex. Genetic factors and non-shared environmental factors in common with sport index explained 1.5–2.7% and 1.9–3.5%, respectively, of the variation in GrimAge Acc. The corresponding proportions considering OPA were 0.4–1.8% and 0.7–1.8%, respectively. However, these proportions were minor (<0.5%) after adjusting the model for smoking status.
**Conclusions:** LTPA associates with slower and OPA with faster epigenetic aging. However, adjusting the models for smoking status, which may reflect the accumulation of unhealthy lifestyle habits, attenuated the associations.

**Keywords:** Biological aging, methylation, quantitative genetics, smoking
Introduction

The health benefits of leisure-time physical activity (LTPA) are well documented. High LTPA is associated with a low risk of several diseases, such as cardiovascular disease (CVD) and metabolic syndrome (1,2), and with low risk of premature death in a dose-dependent manner (3). The benefits of occupational physical activity (OPA) are more controversial. There is some evidence that OPA may even adversely impact health outcomes, such as risk of all-cause mortality, CVD and long-term sickness absence, while LTPA associates with low risk (1,4,5). This contrasting association of OPA with health outcomes is referred to as “the physical activity health paradox” (6).

Several possible reasons for the paradox have been proposed (7). LTPA and OPA may produce divergent physiological responses as LTPA typically differs in terms of intensity, duration and movement type from the monotonous OPA. The different physiological demands of LTPA and OPA are accompanied by other environmental and psychological factors regulating the response to physical activity. Therefore, these two forms of activities may produce an inverse impact on levels of inflammation and autonomic imbalance (6). However, there are also many selection issues and third variables (confounders such as genetic and social factors as well as other health habits) possibly explaining some of the difference in the associations (8), but these relationships are as yet poorly understood.

Epigenetics refers to DNA or chromatin modifications that regulate gene expression without altering the underlying DNA sequence itself. DNA methylation (DNAm, attachment of a methyl group to C-5 of cytosine base in the context of CpG dinucleotide in a DNA strand) is one type of
epigenetic modification. Many studies have provided evidence of age-related hypo- or hyper-methylation within specific CpG sites or islands (9). These findings have laid the ground for the development of epigenetic biomarkers of aging, also known as epigenetic clocks. Horvath’s DNAm age was the first widely used epigenetic estimate for chronological age (10). It has been argued that DNAm age may exclude CpGs whose methylation patterns may reflect a deviation of the biological age from the chronological age (11). Therefore, novel DNAm-based biomarkers for aging, that capture CpGs associated with the functional stage along with the chronological age have been developed in recent years. DNAm PhenoAge was developed using “phenotypic age measure” instead of chronological age as a reference in the biological age prediction (11). DNAm GrimAge is a combination of DNAm-based surrogate biomarkers for health-related plasma proteins and smoking pack-years as well as sex and chronological age, predicting best mortality risk (12).

Epigenetic age acceleration measures the discrepancy between chronological age and epigenetic age. According to a systematic review and meta-analysis, there was no consistent association between LTPA and epigenetic aging assessed with Horvath’s DNAm Age (13). The first results considering the novel epigenetic age estimates seem to be more promising. LTPA has been shown to be correlated negatively with both DNAm PhenoAge and GrimAge acceleration (12,14). However, there is no conclusive evidence on this topic, as the novel epigenetic clocks were published very recently. To our knowledge, no studies are investigating the association between OPA and epigenetic aging.
Here, we investigated the relative contributions of the genetic and environmental factors predicting epigenetic aging measured by DNAm Age, PhenoAge and GrimAge estimates in young adulthood and older age. We further assessed cross-sectional associations of LTPA and OPA with epigenetic aging, as well as the genetic and environmental factors explaining the association. Finally, we studied long-term effects of LTPA on epigenetic aging by comparing co-twins that differed for LTPA for over three decades at least.

Materials and methods

The participants were members of the FinnTwin12 study (born in 1983–87) and an older cohort (born before 1958) of The Finnish Twin Cohort (FTC) (15,16). Both cohorts included monozygotic (MZ) and dizygotic (DZ) twin pairs. Data on health-related behavior was collected with questionnaires and interviews. More detailed information on data collection is available in the Supplemental text appendix (see Text, Supplemental Digital Content 1, additional information on material and methods, http://links.lww.com/MSS/C106). Blood samples for DNA analyses were collected during in-person clinical studies after written informed consent was signed. A total of 1,295 twins of the FinnTwin12 study and 447 of the older cohort were examined and measured. The same-sex twin pairs in which both had information on DNA methylation in a young adult (age range 21–25, 163 MZ and 122 DZ pairs) and an older cohort (age range 55–72, 140 MZ and 78 DZ pairs) were included in this study. The FTC data collection has been approved by the ethics committees of the University of Helsinki and Helsinki University Central Hospital.
**Physical activity–discordant twin pairs (TWINACTIVE)** initiated from the older cohort of the FTC (15,17). The comprehensive selection process that included multiple measurements of physical activity since 1975 has been described in detail by Leskinen et al. (18,19). Leisure-time physical activity (LTPA) was examined with standardized repeated questions, which were quantified as metabolic equivalent (MET) [intensity × duration × frequency] and expressed as a sum score of leisure-time MET hours/day. Twin pairs whose difference in the volume of physical activity were > 3 MET hours/ day were invited to the retrospective follow-up interviews on leisure activity (covering the years from 1980 to 2005 in 5-year intervals), which were carried out during the years 2005–2007 (19). Of the 5663 originally healthy same-sex twin pairs, 16 twin pairs (age range 50–74 years, 7 MZ and 9 DZ pairs, total 5 female pairs) participated in the TWINACTIVE study. During the 30+ years before the clinical examination and DNA sample collection the mean intrapair difference in LTPA was 8.8 MET-hours/day. The participants representing pairs with the longest and highest discordance in LTPA were comprehensively selected from the FTC. The Ethics Committee of the Central Finland Health Care District has approved the TWINACTIVE study.

**Physical activity** was assessed by the Baecke Questionnaire (20). It includes four questions on sports activity and leisure-time activity excluding sports and eight questions on work-related physical activity on a five-point scale. Activities were scored as 1, 3 or 5 according to how physically demanding they are. A sport index, a non-sport leisure-time (leisure) index and a work index, respectively, were yielded from mean scores of each section as described earlier by Baecke et al. (20) and Mustelin et al. for the FinnTwin12 study (21).
Confounding variables. Body mass index was calculated as the ratio of measured body weight (kg) to height squared (m²). Sex, education years and smoking status (never, former, and current smoker [includes regular and occasional use]) and alcohol use (in grams per day) were assessed through interviews.

Epigenetic age estimates

DNA methylation. Generating and normalizing the DNA methylation (DNAm) data has been described in the Supplementary text appendix (Text, Supplemental Digital Content 1, preprocessing the DNA methylation data, http://links.lww.com/MSS/C106). Genome-wide DNAm from blood samples was determined on Illumina 450K and EPIC BeadChips, and the epigenetic age estimates DNAm age (10), DNAm PhenoAge (11), and DNAm GrimAge (12) were calculated by an online calculator (https://dnamage.genetics.ucla.edu/new) (see Text, Supplemental Digital Content 1, additional information on epigenetic age estimates, http://links.lww.com/MSS/C106). Epigenetic age acceleration (Acc), which describes the difference between chronological age and epigenetic age estimate, was calculated as the residuals from a linear regression model of epigenetic age estimate on chronological age.

The components of DNAm GrimAge (adjusted for age) were obtained using the calculator as well, including DNAm based smoking pack-years (PACYRS) and the surrogate biomarkers for plasma proteins (DNAm based plasma proteins): DNAm adrenomedullin (ADM), DNAm beta-2-microglobulin (B2M), DNAm cystatin-C, DNAm growth differentiation factor 15 (GDF15), DNAm leptin, DNAm plasminogen activator inhibitor 1 (PAI-1) and DNAm tissue inhibitor metalloproteinases 1 (TIMP-1).
Statistical methods

Descriptive statistics were calculated using IBM SPSS statistics, and further modelling was performed by using Mplus statistical package (version 8.2) (22).

Quantitative genetic modelling was conducted using a structural equation framework. First, intraclass correlation coefficients (ICCs) and correlations between epigenetic age Acc measures and LTPA (sport index and leisure index), as well as OPA (work index) were studied. Second, univariate modelling was carried out to study the magnitude of genetic and environmental factors affecting epigenetic age Acc measures and PA indices in young adult and older twin pairs as described in the Supplemental text appendix (Text, Supplemental Digital Content 1, modelling procedure of the univariate models, http://links.lww.com/MSS/C106) (23). The univariate models were adjusted for sex, education and smoking status. Third, structural equation modelling was used to study the associations of PA indices on epigenetic aging. Shared genetic and environmental effects between epigenetic age Acc and PA, as well as the genetic and environmental factors unique to epigenetic age Acc were studied. For these purposes, Cholesky’s decomposition was applied to PA indices and epigenetic age Acc measures after controlling for covariates (see Figure 1). The latent variables representing PA (CH1) and the residual of epigenetic age Acc after the impact of PA has been taken into account (CH2) were specified (24). The model was initially adjusted for sex only. The variation in both the latent variables CH1 and CH2 was partitioned in the genetic and environmental components as described in the Supplementary text appendix (Text, Supplemental Digital Content 1, modelling procedure of the bivariate models, http://links.lww.com/MSS/C106).
The potential confounding variables, including sex, education, BMI, smoking status and alcohol use, were added sequentially to the model. At each stage, the regression coefficients between PA and epigenetic age Acc ($b$) were studied. In addition, the parameters of the model were used to calculate the relative proportions in the total variation of epigenetic age Acc $[(a_1^2 + c_1^2 + e_1^2) \ast b^2 + (a_2^2 + c_2^2 + e_2^2)]$ explained by the genetic and environmental effects in common with PA ($a_1^2 \ast b^2$, $c_1^2 \ast b^2$ and $e_1^2 \ast b^2$, respectively) as well as the unique genetic and environmental factors of epigenetic age Acc ($a_2^2$, $c_2^2$ and $e_2^2$, respectively).

**Discordant twin pair analysis.** The mean within-pair differences in epigenetic age estimates between active and inactive co-twins were calculated. Standardized mean difference (SMD; within-pair difference/standard deviation of the variable among the members of the pairs) was used to evaluate effect size. The within-pair differences were regressed on zygosity to study whether there were differences in the effect of LTPA on epigenetic aging between MZ and DZ twins. The study design controls for chronological age and sex, as the twins are of the same sex and age.

**Results**

Among the young adults, DZ twins had a slightly higher BMI and leisure PA index compared with MZ twins (Table 1). The mean age of the participants was 22.4 years, while the mean of the different epigenetic age estimates ranged from 15.0 to 28.6 years, depending on the estimate utilized. In the older cohort, DZ twins were slightly older compared with MZ twins (mean 62.9
vs. 62.0), and there were also differences in the means of the epigenetic age estimates. The mean of the different epigenetic age estimates ranged from 54.7 to 62.0 years.

**Heritability**

In both cohorts, ICCs for epigenetic age Acc measures were consistently higher in MZ twins than in DZ twins, suggesting the influence of a genetic component (Table 2). According to univariate modelling, additive genetic factors accounted for 69% of the variation in DNAm age Acc, 64% of the variation in DNAm PhenoAge Acc and 62% of the variation in DNAm GrimAge Acc in young adult twin pairs (see Text, Supplemental Digital Content 1, http://links.lww.com/MSS/C106; and Table, Supplemental Digital Content 2, the estimation results of the univariate model for epigenetic aging among young adult twin pairs, http://links.lww.com/MSS/C107). Correspondingly, non-shared environmental factors accounted for the remainder (31–38%) of the variation in epigenetic aging. In older twin pairs, additive genetic factors explained 61% of the variation in DNAm age Acc, 60% of the variation in DNAm PhenoAge Acc and 58% of the variation in DNAm GrimAge Acc (see Text, Supplemental Digital Content 1, http://links.lww.com/MSS/C106; and Table, Supplemental Digital Content 3, the estimation results of the univariate model for epigenetic aging among older twin pairs, http://links.lww.com/MSS/C108), while unique environmental factors accounted for the remaining variance. The proportions of the variation in epigenetic age Acc measures explained by the genetic factors did not differ between young adult and older twin pairs (Wald test: $P = 0.076$–$0.220$). The results considering PA are presented in the Supplementary material (see Text, Supplemental Digital Content 1, http://links.lww.com/MSS/C106; and Tables, Supplemental Digital Content 4–5, the estimation
results of the univariate model for PA indices among young adult and older twin pairs, respectively, http://links.lww.com/MSS/C109 and http://links.lww.com/MSS/C110).

**Bivariate twin models**

Sport index and leisure index were associated with slower and work index with faster DNAm GrimAge Acc in both young adult and older twins as shown by correlation coefficients in Table 2. Epigenetic age Acc and PA indices were not consistently correlated, when DNAm Age and DNAm PhenoAge estimates were used to assess epigenetic age. Therefore, no further modelling was considered for these measures. Additional information on the model selection is available in the Supplemental material (see Text, Supplemental Digital Content 1, http://links.lww.com/MSS/C106, and Table, Supplemental Digital Content 6, the model fit of the bivariate models, http://links.lww.com/MSS/C111).

In young adult twin pairs, sport index was associated with slower DNAm GrimAge acceleration after all the adjustments, but in an older cohort, the association was no longer significant after controlling for smoking status (Table 3). In both cohorts, leisure index was associated with slower and work index with accelerated epigenetic aging, but these associations attenuated after controlling for covariates, especially for smoking status.

In young twin pairs, genetic and environmental factors in common with sport index explained 2.7% and 1.9%, respectively, of the variation in DNAm GrimAge Acc, after adjusting the model for sex (Table 4). The corresponding proportions for leisure index were 0.6% and 0.7% and for work index 1.8% and 1.7%, respectively. In older twin pairs, genetic and environmental factors
in common with sport index explained 1.5% and 3.5%, respectively, of the variation in DNAm GrimAge Acc (Table 5). The corresponding proportions for leisure index were 0.8% and 2.4% and for OPA 0.4% and 0.7%, respectively. In both cohorts, the proportions of the variation in DNAm GrimAge Acc explained by genetic and environmental factors in common with PA indices were minor (<0.5%) after including smoking status in the models.

The associations of the DNAm based surrogates (adjusted for age) included in DNAm GrimAge with DNAm GrimAge Acc, as well as with PA indices, were studied using correlation coefficients (see Table, Supplemental Digital Content 7, the correlations among young adult and older individual twins, http://links.lww.com/MSS/C112). Sport index in young adults and PA indices in older cohort, were associated with lower levels of several DNAm based plasma proteins, while inconsistent associations of work index were observed in both cohorts. In both cohorts, sport index was negatively associated with DNAm based smoking pack-years \((r = -0.20- -0.16)\), while the opposite association of work index was observed \((r = 0.11–0.30)\). DNAm based smoking pack-years and DNAm GrimAge Acc were highly correlated \((r > 0.80)\).

Twin pairs discordant for leisure-time physical activity for 32 years—the TWINACTIVE sample

The mean age (SD) of the participants was 60.4 (6.2) years, while the means of the epigenetic age estimates DNAm age, PhenoAge and GrimAge were 56.5 (4.8), 46.8 (5.8) and 59.7 (5.7) years, respectively. There was no difference in Horvath’s DNAm age between the active and inactive co-twins among the LTPA discordant twin pairs, as we have reported previously (25). The two newer epigenetic age estimates, however, differed between active and inactive co-twins.
Active twins were on average 3.27 (95% confidence interval CI: 1.34, 5.20; SMD = 0.56) years younger compared to their inactive co-twins in DNAm PhenoAge and 2.08 (95%CI: 0.75, 3.41; SMD = 0.37) years younger in DNAm GrimAge.

Mean within-pair difference for DNAm PhenoAge was among MZ pairs 1.80 (95%CI: -1.40, 4.96; SMD = 0.30) and among DZ pairs 4.42 (95%CI: 2.33, 6.51; SMD = 0.77) (see Figure, Supplemental Digital Content 8, the epigenetic age estimates in the LTPA discordant MZ and DZ twin pairs, http://links.lww.com/MSS/C113). In DNAm GrimAge active MZ twins were on average 1.97 (95%CI: -0.04, 4.00; SMD = 0.40) years and DZ twins 2.27 (95%CI: 0.40, 3.94; SMD = 0.36) years younger compared to their inactive co-twins. Among the small number of twin pairs, the difference in the association between LTPA and DNAm PhenoAge or DNAm GrimAge between MZ and DZ twin pairs was not significant (P = 0.091 and P = 0.887, respectively).

Discussion

In this study, we showed that the heritability estimates of different epigenetic clocks (namely DNAm age, PhenoAge and GrimAge) are very similar. Genetic factors accounted for about 60% of the variation in epigenetic age acceleration, while non-shared environmental factors explained the remainder. Models with no genetic effects showed poorer fit to the data than the models with genetic effects. Our twin models did not require the inclusion of shared environmental effects as genetic models with (ACE) or without (AE) shared environmental effects fit the data adequately. The observed heritability estimates of the epigenetic age acceleration based on the newer epigenetic age estimates were considerably higher than those reported in previous studies,
possibly due to the methodological differences in constructing epigenetic age estimates and the age differences of the target cohorts. Lu and colleagues reported low to moderate estimates of heritability for DNAm GrimAge and DNAm PhenoAge based epigenetic age acceleration (30% and 11%, respectively) (12). However, in other studies, moderate to high heritability estimates have been reported for the latter one (33–51%) (11,26). The AE model was more parsimonious and therefore used as the basis for the bivariate models to explore the common genetic and environmental factors underlying both epigenetic aging and PA.

Our findings revealed that the associations between PA and epigenetic aging depended on the utilized epigenetic age estimate and the form of PA. The results supported the existence of the PA paradox: High-intensity LTPA (sport index) was related to slower epigenetic aging and OPA (work index) to faster epigenetic aging when the newest epigenetic age estimate DNAm GrimAge was used. However, the associations mainly attenuated after controlling for smoking status. Associations between other epigenetic clocks (DNAm Age and PhenoAge acceleration) and PA were very minor or non-existent. Only a few previous studies have reported on the associations between PA and epigenetic aging with the novel epigenetic clocks. Stevenson and colleagues showed a cross-sectional negative association of DNAm PhenoAge acceleration on LTPA at the age of 70, but the analysis was controlled only for age and childhood cognitive ability (14). Zhao and colleagues did not observe significant associations between LTPA and DNAm PhenoAge or DNAm GrimAge acceleration in older African Americans (27).

In addition to the cross-sectional associations, we provided evidence for beneficial association of long-term LTPA using a discordant twin pair design. Twin pairs discordant for LTPA for 32
years differed in epigenetic aging measured with DNAm PhenoAge and DNAm GrimAge although this was not seen when measured with DNAm age. Active twin pairs were epigenetically two to three years younger on average compared with their inactive co-twins, when the genetic factors were controlled for partially (DZ pairs) or fully (MZ pairs). There were no differences in the effects between MZ and DZ twin pairs, but the mean within-pair differences were not significant in MZ pairs.

All the utilized epigenetic aging acceleration measures have been shown to predict mortality and morbidity risk, but DNAm GrimAge acceleration stands out in the prediction accuracy (12,28,29). Previous studies have shown that DNAm GrimAge may capture the stimulus of a variety of health- and lifestyle-related factors (12,27). In our study, LTPA was most consistently associated with DNAm GrimAge acceleration. DNAm GrimAge is a composite biomarker based on seven DNAm surrogates for plasma markers and smoking pack-years, which strongly predict time-to-death (12). Whereas CpGs for the other clocks were selected based on their association with a single reference, DNAm GrimAge was developed in two stages. First, CpGs for DNAm surrogates were selected based on their associations with the corresponding plasma protein levels and self-reported smoking pack-years. Second, DNAm based surrogates for DNAm GrimAge estimator were selected based on their ability to predict mortality risk. This approach may have efficiently captured the CpGs associated with diverse health-related factors.

In our study, both genetic and non-shared environmental factors common to PA and the DNAm GrimAge acceleration explained the observed associations, but these influences attenuated after controlling the model for smoking status. Therefore, the observed opposite associations of LTPA
and OPA on DNAm GrimAge acceleration may reflect an accumulation of unhealthy lifestyle habits among individuals in the lower socioeconomic class performing physically demanding work (30). Both genetic and environmental factors regulate smoking (31). Moreover, smoking has been shown to predict lower LTPA, also independently of genetic factors (32). Smoking is one of the most detrimental lifestyle factors and has not only been seen to increase the risk for multiple diseases (33) and mortality (34) but also to accelerate cellular aging (35). Interestingly, DNAm-based smoking pack-years (a component of DNAm GrimAge) has been shown to predict mortality risk better than original self-reported measure (12) and fully mediate the effects of self-reported smoking on biological aging (36). In line with this, we observed a very high correlation between DNAm smoking pack-years and DNAm GrimAge acceleration. Moreover, the opposite association of LTPA and OPA with the components of GrimAge were most evident in the case of DNAm smoking pack-years. These findings may support the recently stated arguments that the PA paradox is probably partly explained by an insufficient adjustment for smoking (8,37).

We observed that the lower values in several DNAm-based surrogate biomarkers included in the GrimAge estimator were correlated with higher levels of LTPA. LTPA promotes changes in multiple mechanistic and regulatory pathways that underlie the exercise-induced adaptations in metabolic profile, fitness, and body fat and muscle distribution. Lack of these LTPA-induced adaptations may increase the risk of cardiovascular and metabolic diseases at the population level, as different metabolic profiles have also been found among LTPA-discordant twin pairs (38) although differences in life expectancy have not been observed (39). Our study suggests that benefits of the LTPA may also be seen in epigenetic aging based on DNAm levels in blood, but its role is minor. The effect size was about half or less of the magnitude of the previously
reported effect of LTPA on certain other health-related traits such as body fat, liver fat and artery structure (38) known to be associated cardiovascular and other inactivity-related diseases. LTPA induces adaptations also directly in muscle tissue which plays an important role in age-related decline in physical functioning. Future studies utilizing recently published epigenetic clock for human skeletal muscle (40) may show, whether LTPA has a more substantial effect on epigenetic aging of muscle tissue.

**Strengths and limitations**

To our knowledge, this is the first study investigating the association of both LTPA and OPA with epigenetic aging. Our study utilizes novel epigenetic clocks that were published very recently. Twin design and the use of quantitative genetic modelling enabled us to study the genetic and environmental effects on epigenetic aging. In addition, we were able to investigate the effects of long-term LTPA on epigenetic aging after controlling for genetic factors by comparing co-twins of pairs discordant for LTPA for 30+ years.

We acknowledge that our results are based on self-reported measure of physical activity, and potential recall bias and the effect of social desirability cannot be excluded. Baecke questionnaire has been shown to be valid and reliable tool to assess high intensity LTPA but all the light intensity activities may not be properly measured (41). Activities such as gardening and household, which are increasingly important determinants of physical functioning with age (42) are not directly assessed by the questionnaire. In addition, the sample size of the LTPA discordant twins is limited and therefore, statistical power to detect small effects may be insufficient. It should be noted that recent studies have shown that biological aging may be
distinct stages rather than a continuous process and aging progression may not be linear throughout the studied age ranges (43,44).

Conclusions

We show that LTPA associates with slower epigenetic aging, whereas OPA associates with accelerated epigenetic aging. The observed associations are explained by both common genetic and environmental factors. Importantly, adjusting the models for smoking status, which may reflect the accumulation of unhealthy lifestyle habits, attenuated the negative association of LTPA and the positive association of OPA with epigenetic aging.
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Conflict of interest. The authors declare that there are no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.
References

1. Li J, Loerbroks A, Angerer P. Physical activity and risk of cardiovascular disease: What does the new epidemiological evidence show? *Curr Opin Cardiol*. 2013;28(5):575–83.

2. Myers J, Kokkinos P, Nyelin E. Physical activity, cardiorespiratory fitness, and the metabolic syndrome. *Nutrients*. 2019;11(7):1–18.

3. Löllgen H, Böckenhoff A, Knapp G. Physical activity and all-cause mortality: An updated meta-analysis with different intensity categories. *Int J Sports Med*. 2009;30(3):213–24.

4. Coenen P, Huysmans MA, Holtermann A, Krause N, Van Mechelen W, Straker LM, et al. Do highly physically active workers die early? A systematic review with meta-analysis of data from 193 696 participants. *Br J Sports Med*. 2018;52(20):1320–6.

5. Holtermann A, Hansen J V, Burr H, Søgaard K, Sjøgaard G. The health paradox of occupational and leisure-time physical activity. *Br J Sports Med*. 2011;46(4):291–5.

6. Hallman DM, Jørgensen MB, Holtermann A. On the health paradox of occupational and leisure-Time physical activity using objective measurements: Effects on autonomic imbalance. *PLoS One*. 2017;12(5):e0177042.

7. Holtermann A, Krause N, Beek AJ Van Der, Straker L. The physical activity paradox : six reasons why occupational physical activity (OPA) does not confer the cardiovascular health benefits that leisure time physical activity does. *Br J Sports Med*. 2018;52(3):149–50.

8. Kujala UM. Is physical activity a cause of longevity? It is not as straightforward as some would believe. A critical analysis. *Br J Sports Med*. 2018;52(14):914–8.

9. Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent
upon CPG island context. *PLoS Genet.* 2009;5(8):e1000602.

10. Horvath S. DNA methylation age of human tissues and cell types. * Genome Biol.* 2013;14:R115.

11. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY).* 2018;10(4):573–91.

12. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY).* 2019;11(2):303–27.

13. Ryan J, Wrigglesworth J, Loong J, Fransquet PD, Woods RL. A systematic review and meta-analysis of environmental, lifestyle, and health factors associated with DNA methylation age. * Journals Gerontol Ser A.* 2019;75(3):481–94.

14. Stevenson AJ, McCartney DL, Hillary RF, Redmond P, Taylor AM, Zhang Q, et al. Childhood intelligence attenuates the association between biological ageing and health outcomes in later life. *Tranlational Psychiatry.* 2019;9:323.

15. Kaprio J. The Finnish Twin Cohort Study: an update. *Twin Res Hum Genet.* 2013;16(1):157–62.

16. Kaprio J, Bollepalli S, Buchwald J, Iso-Markku P, Korhonen T, Kovanen V, et al. The older Finnish Twin Cohort: 45 years of follow-up. *Twin Res Hum Genet.* 2019;22(4):240–54.

17. Kaprio J, Koskenvuo M. Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort. *Twin Res.* 2002;5(5):358–65.

18. Leskinen T, Sipilä S, Kaprio J, Kainulainen H, Alen M, Kujala UM. Physically active vs. inactive lifestyle, muscle properties, and glucose homeostasis in middle-aged and older
twins. *Age (Omaha)*. 2013;35(5):1917–26.

19. Leskinen T, Waller K, Mutikainen S, Aaltonen S, Ronkainen PHA, Alén M, et al. Effects of 32-year leisure time physical activity discordance in twin pairs on health (TWINACTIVE Study): Aims, design and results for physical fitness. *Twin Res Hum Genet*. 2009;12(1):108–17.

20. Baecke JAH, Burema J, Fritjters JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr*. 1982;96(5):932–42.

21. Mustelin L, Joutsi J, Latvala A, Pietiläinen KH, Rissanen A, Kaprio J. Genetic influences on physical activity in young adults: A twin study. *Med Sci Sports Exerc*. 2012;44(7):1293–301.

22. Muthén LK, Muthén BO. Mplus User’s Guide. Eighth Ed. [Internet]. Muthén & Muthén. Los Angeles, CA; Available from: https://www.statmodel.com

23. Neale MC, Cardon LR. Methodology for Genetic Studies of Twins and Families. Dordrecht, The Netherlands: Kluver Academic Publisher; 1992.

24. de Jong PF. Hierarchical regression analysis in structural equation modeling. *Struct Equ Model*. 1999;6(2):198–211.

25. Sillanpää E, Ollikainen M, Kaprio J, Wang X, Leskinen T, Kujala UM, et al. Leisure-time physical activity and DNA methylation age - A twin study. *Clin Epigenetics*. 2019;11(1):1–8.

26. Jylhävä J, Hjelmborg J, Soerensen M, Munoz E, Tan Q, Kuja-Halkola R, et al. Longitudinal changes in the genetic and environmental influences on the epigenetic clocks across old age: Evidence from two twin cohorts. *EBioMedicine*. 2019;40:710–6.

27. Zhao W, Ammous F, Ratli S, Liu J, Yu M, Mosley TH, et al. Education and lifestyle
factors are associated with DNA methylation clocks in older African Americans. *Int J Environ Res Public Health*. 2019;16(17):3141.

28. Hillary RF, Stevenson AJ, McCartney DL, Campbell A, Walker RM, Howard DM, et al. Epigenetic clocks predict prevalence and incidence of leading causes of death and disease burden. *bioRxiv* [Internet]. 2020 [cited 2020 May 2]; Available from: https://doi.org/10.1101/2020.01.31.928648

29. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. *Elife*. 2020;9:1–20.

30. Thebault JL, Ringa V, Panjo H, Bloy G, Falcoff H, Rigal L. Accumulation of unhealthy behaviors: Marked social inequalities in men and women. *Prev Med Reports*. 2018;12:1–5.

31. Vink JM, Boomsma DI. Interplay between heritability of smoking and environmental conditions? A comparison of two birth cohorts. *BMC Public Health*. 2011;11(1):316.

32. Waller K, Vähä-Ypyä H, Törmäkangas T, Hautasaari P, Lindgren N, Iso-Markku P, et al. Long-term leisure-time physical activity and other health habits as predictors of objectively monitored late-life physical activity - A 40-year twin study. *Sci Rep*. 2018;8(1):1–10.

33. Alberg AJ, Shopland DR, Cummings KM. The 2014 Surgeon General’s report: commemorating the 50th Anniversary of the 1964 Report of the Advisory Committee to the US Surgeon General and updating the evidence on the health consequences of cigarette smoking. *Am J Epidemiol*. 2014;179(4):403–12.

34. Kujala UM, Kaprio J, Koskenvuo M. Modifiable risk factors as predictors of all-cause
mortality: The roles of genetics and childhood environment. *Am J Epidemiol*. 2002;156(11):985–93.

35. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet*. 2005;366(9486):662–4.

36. Lei M, Gibbons FX, Simons RL, Philibert RA, Beach SRH. The effect of tobacco smoking differs across indices of DNA methylation-based aging in an African American sample: DNA methylation-based indices of smoking capture these effects. *Genes (Basel)*. 2020;11(3):311.

37. Shephard RJ. Is there a ‘recent occupational paradox’ where highly active physically active workers die early? or are there failures in some study methods? *Br J Sports Med*. 2019;53(24):1557–9.

38. Leskinen T, Kujala UM. Health-related findings among twin pairs discordant for leisure-time physical activity for 32 years: The TWINACTIVE study synopsis. *Twin Res Hum Genet*. 2015;18(3):266–72.

39. Karvinen S, Waller K, Silvennoinen M, Koch LG, Britton SL, Kaprio J, et al. Physical activity in adulthood: Genes and mortality. *Sci Rep*. 2015;5:18259.

40. Voisin S, Harvey NR, Haupt LM, Griffiths LR, Ashton KJ, Coffey VG, et al. An epigenetic clock for human skeletal muscle. *J Cachexia Sarcopenia Muscle*. 2020; DOI: 10.1002/jcsm.12556.

41. Richardson MT, Ainsworth BE, Wu HC, Jacobs DR, Leon AS. Ability of the atherosclerosis risk in communities (ARIC)/baecke questionnaire to assess leisure-time physical activity. *Int J Epidemiol*. 1995;24(4):685–93.

42. Peeters G, Van Gellecum YR, Van Uffelen JGZ, Burton NW, Brown WJ. Contribution of
house and garden work to the association between physical activity and well-being in young, mid-aged and older women. *Br J Sports Med.* 2014;48(12):996–1001.

43. Holzscheck N, Söhle J, Kristof B, Grönniger E, Gallinat S, Wenck H, et al. Multi-omics network analysis reveals distinct stages in the human aging progression in epidermal tissue. *Aging (Albany NY)*. 2020;12(12):12393–409.

44. Timmons JA, Volmar CH, Crossland H, Phillips BE, Sood S, Janczura KJ, et al. Longevity-related molecular pathways are subject to midlife “switch” in humans. *Aging Cell.* 2019;18(4):1–10.
Appendices

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Figure legends

Figure 1. The path diagram of the structural equation model. Circles denote latent variables; rectangles denote observed variables. CH1 represents physical activity and CH2 represents the residual of the epigenetic age Acc after the impact of physical activity has been taken into account. A, additive genetic; C, shared environmental; E, non-shared environmental components.
Figure 1
Table 1. Descriptive statistics of the study variables by zygosity among young adult \((n = 570)\) and older \((n = 470)\) twin individuals

| Variable                        | Young adult (21–25 years) | Older (55–72 years) |
|--------------------------------|---------------------------|---------------------|
|                                | MZ twins \((n = 326)\)    | DZ twins \((n = 244)\) | MZ twins \((n = 294)\) | DZ twins \((n = 176)\) |
|                                | Mean (SD) \(n\)          | Mean (SD) \(n\)     | Mean (SD) \(n\)     | Mean (SD) \(n\)     |
| Women, \(n, \%\)               | 208, 63.8%                | 142, 58.2%          | 188, 63.9%          | 94, 53.4%           |
| Chronological age (years)      | 22.4 (0.7)                | 22.4 (0.7)          | 62.0 (4.1)          | 62.9 (4.3)          |
| DNAm Age (years)               | 25.0 (3.5)                | 24.4 (3.4)          | 60.2 (6.2)          | 62.1 (7.6)          |
| DNAm PhenoAge (years)          | 15.0 (4.6)                | 15.3 (4.8)          | 54.7 (6.5)          | 56.0 (8.1)          |
| DNAm GrimAge (years)           | 28.6 (2.9)                | 28.6 (3.4)          | 59.8 (5.3)          | 60.8 (5.5)          |
| Body mass index (kg/m\(^2\))  | 23.1 (3.8)                | 23.7 (4.2)          | 27.1 (4.7)          | 27.2 (4.8)          |
| Education (years)              | 16.7 (3.5)                | 16.6 (3.6)          | 11.9 (3.8)          | 11.4 (3.5)          |
| Smoking status                 |                           |                     |                     |                     |
| Never smokers, \(n, \%\)       | 182, 56.0%                | 116, 47.5%          | 148, 50.8%          | 88, 50.0%           |
| Former smokers, \(n, \%\)     | 37, 11.4%                 | 30, 12.3%           | 97, 33.3%           | 63, 35.8%           |
| Current smokers, \(n, \%\)    | 106, 32.6%                | 98, 40.2%           | 47, 15.9%           | 25, 14.2%           |
| Alcohol use (grams per day)    | 12.4 (16.7)               | 13.4 (16.7)         | 6.9 (11.2)          | 8.8 (11.6)          |
| Leisure-time physical activity (LTPA) |                   |                     |                     |                     |
| Sport index                    | 2.9 (0.8)                 | 3.0 (0.8)           | 3.2 (0.9)           | 3.1 (0.8)           |
| Non-sport leisure index        | 2.9 (0.6)                 | 3.1 (0.6)           | 2.9 (0.7)           | 2.8 (0.7)           |
| Occupational physical activity (OPA) |                   |                     |                     |                     |
| Work index                     | 2.7 (0.6)                 | 2.8 (0.6)           | 2.4 (1.0)           | 2.3 (1.0)           |
| Out of working life            | 322                       | 243                 | 241                 | 241                 |
| Retired or unemployed, \(n, \%\) | 49, 15.2%                | 26, 10.7%           | 105, 43.6%          | 76, 50.0%           |

\(^{a}\) \(P\) value for the difference between the groups from independent samples t-test or chi-squared test.
Table 2. The intraclass correlation coefficients (ICCs) of epigenetic age acceleration (Acc) measures and physical activity indices by zygosity, as well as the correlation coefficients between the measures among young adult ($n = 570$) and older ($n = 470$) twins individuals.

| ICCs and their 95% CI | Correlation coefficients and their 95% CI $^{a,b}$ |
|-----------------------|--------------------------------------------------|
| MZ        | DZ       | 1         | 2   | 3         | 4         | 5         |
| **Young adult twins** |                     |                     |     |           |           |           |
| 1 DNAm age Acc | 0.70, 0.79 | 0.49, 0.35, 0.64 |     |           |           |           |
| 2 DNAm PhenoAge | 0.58, 0.57 | 0.18, 0.02, 0.34 | 0.35, 0.28, 0.43 |     |           |           |
| 3 DNAm GrimAge Acc | 0.67, 0.76 | 0.42, 0.22, 0.62 | 0.08, -0.01, 0.17 | 0.34, 0.25, 0.42 |     |           |
| 4 Sport index | 0.61, 0.75 | 0.32, 0.15, 0.50 | 0.09, -0.02, 0.19 | 0.09, -0.19, 0.01 | -0.21, -0.30, -0.12 |     |
| 5 Leisure index | 0.43, 0.58 | 0.34, 0.17, 0.51 | 0.05, -0.05, 0.15 | 0.03, -0.07, 0.13 | -0.12, 0.02 | 0.28, 0.19, 0.37 |
| 6 Work index | 0.51, 0.65 | 0.26, 0.07, 0.46 | 0.01, -0.12, 0.10 | 0.00, -0.10, 0.10 | 0.26, 0.16, 0.35 | -0.06, -0.17, 0.04, -0.01, -0.11, 0.10 |
| **Older twins** |                     |                     |     |           |           |           |
| 1 DNAm age Acc | 0.62, 0.73 | 0.28, 0.15, 0.41 |     |           |           |           |
| 2 DNAm PhenoAge | 0.52, 0.54 | 0.32, 0.13, 0.52 | 0.34, 0.23, 0.45 |     |           |           |
| 3 DNAm GrimAge Acc | 0.64, 0.75 | 0.40, 0.19, 0.62 | 0.21, 0.11, 0.31 | 0.40, 0.32, 0.48 |     |           |
| 4 Sport index | 0.32, 0.50 | 0.21, -0.04, 0.46 | 0.03, -0.13, 0.07 | 0.06, -0.19, 0.06 | -0.20, -0.31, -0.08 |     |
| 5 Leisure index | 0.27, 0.44 | 0.26, 0.03, 0.48 | 0.12, 0.03 | 0.00, -0.13, 0.12 | -0.21, 0.12 | 0.45, 0.36, 0.53 |
| 6 Work index | 0.33, 0.51 | 0.34, 0.12, 0.55 | 0.09, -0.02, 0.20 | 0.07, -0.04, 0.17 | 0.13, 0.03, 0.23 | -0.13, 0.03 | -0.02, -0.13, 0.09 |

CI, confidence interval.

$^{a}$ CIs were corrected for nested sampling.  $^{b}$ Significant correlations at the level 0.05 are presented in bold.
Table 3. The estimation results of the structural equation modelling: the standardized linear regression coefficients of DNAm GrimAge acceleration on physical activity indices among young adult (MZ: \( n = 163 \), DZ: \( n = 122 \)) and older (MZ: \( n = 147 \), DZ: \( n = 88 \)) twin pairs

|                     | Sport index | Leisure index | Work index | Work index $^a$ |
|---------------------|-------------|---------------|------------|-----------------|
|                     | B           | 95% CI        | \( P \)    | B               | 95% CI        | \( P \)    |
| **Young adult twin pairs** |             |               |            |                 |               |            |
| sex                 | \(-0.212\)  | \(-0.288, -0.136\) | \(<0.001\) | \(-0.113\)     | \(-0.192, -0.033\) | \(0.006\)  | \(0.189\) | \(0.105, 0.273\) | \(<0.001\) |
| + education years   | \(-0.172\)  | \(-0.244, -0.099\) | \(<0.001\) | \(-0.089\)     | \(-0.167, -0.012\) | \(0.024\)  | \(0.103\) | \(0.027, 0.179\) | \(0.008\)  |
| + body mass index    | \(-0.161\)  | \(-0.236, -0.087\) | \(<0.001\) | \(-0.085\)     | \(-0.163, -0.007\) | \(0.108\)  | \(0.106\) | \(0.029, 0.182\) | \(0.007\)  |
| + smoking status     | \(-0.085\)  | \(-0.153, -0.017\) | \(0.015\)  | \(-0.029\)     | \(-0.104, 0.046\) | \(0.451\)  | \(0.051\) | \(-0.020, 0.122\) | \(0.157\)  |
| + alcohol use        | \(-0.082\)  | \(-0.151, -0.014\) | \(0.019\)  | \(-0.029\)     | \(-0.104, 0.046\) | \(0.451\)  | \(0.052\) | \(-0.018, 0.123\) | \(0.147\)  |
| **Older twin pairs** |             |               |            |                 |               |            |
| sex                 | \(-0.212\)  | \(-0.298, -0.126\) | \(<0.001\) | \(-0.169\)     | \(-0.248, -0.090\) | \(<0.001\) | \(0.098\) | \(0.014, 0.182\) | \(0.023\)  |
| + education years   | \(-0.211\)  | \(-0.296, -0.126\) | \(<0.001\) | \(-0.165\)     | \(-0.244, -0.086\) | \(<0.001\) | \(0.078\) | \(-0.005, 0.160\) | \(0.066\)  |
| + body mass index    | \(-0.198\)  | \(-0.283, -0.113\) | \(<0.001\) | \(-0.153\)     | \(-0.232, -0.075\) | \(<0.001\) | \(0.077\) | \(-0.006, 0.161\) | \(0.070\)  |
| + smoking status     | \(-0.053\)  | \(-0.127, 0.020\) | \(0.154\)  | \(-0.048\)     | \(-0.112, 0.017\) | \(0.145\)  | \(0.028\) | \(-0.038, 0.094\) | \(0.410\)  |
| + alcohol use        | \(-0.054\)  | \(-0.126, 0.017\) | \(0.138\)  | \(-0.053\)     | \(-0.118, 0.012\) | \(0.108\)  | \(0.029\) | \(-0.037, 0.095\) | \(0.388\)  |

B, standardized regression coefficient; CI, confidence interval.

+ The model was additionally adjusted for the following variables.

$^a$ The model was additionally adjusted for indicator of being out of working life.
Table 4. The proportion of the variation in DNAm GrimAge acceleration (Acc) explained by genetic and environmental effects in young adult twin pairs

|                | Sport index | Leisure index | Work index a |
|----------------|-------------|---------------|--------------|
|                | Estimate    | 95% CI        | P            | Estimate    | 95% CI        | P            | Estimate    | 95% CI        | P            |
| A1 PA index + sex | 0.027       | 0.006, 0.048  | 0.011        | 0.006       | -0.002, 0.014 | 0.167        | 0.018       | 0.001, 0.036 | 0.039        |
| + education     | 0.018       | 0.002, 0.034  | 0.029        | 0.004       | -0.003, 0.010 | 0.253        | 0.003       | -0.002, 0.008 | 0.232        |
| + body mass index | 0.016       | 0.000, 0.032  | 0.044        | 0.003       | -0.003, 0.010 | 0.278        | 0.003       | -0.002, 0.009 | 0.225        |
| + smoking status | 0.006       | -0.004, 0.015 | 0.234        | 0.001       | -0.002, 0.003 | 0.702        | 0.001       | -0.002, 0.004 | 0.499        |
| + alcohol use   | 0.005       | -0.004, 0.015 | 0.249        | 0.001       | -0.002, 0.003 | 0.703        | 0.002       | -0.002, 0.004 | 0.487        |
| A2 PA index + sex | 0.680       | 0.597, 0.762  | <0.001       | 0.706       | 0.629, 0.782  | <0.001       | 0.661       | 0.576, 0.747  | <0.001       |
| + education     | 0.689       | 0.606, 0.771  | <0.001       | 0.706       | 0.627, 0.784  | <0.001       | 0.689       | 0.606, 0.772  | <0.001       |
| + body mass index | 0.685       | 0.602, 0.769  | <0.001       | 0.701       | 0.622, 0.780  | <0.001       | 0.689       | 0.606, 0.771  | <0.001       |
| + smoking status | 0.617       | 0.516, 0.718  | <0.001       | 0.620       | 0.520, 0.721  | <0.001       | 0.617       | 0.515, 0.718  | <0.001       |
| + alcohol use   | 0.619       | 0.515, 0.718  | <0.001       | 0.618       | 0.518, 0.719  | <0.001       | 0.615       | 0.513, 0.717  | <0.001       |
| E1 PA index + sex | 0.019       | 0.005, 0.033  | 0.008        | 0.007       | -0.003, 0.017 | 0.178        | 0.018       | 0.002, 0.034 | 0.030        |
| + education     | 0.014       | 0.002, 0.025  | 0.023        | 0.005       | -0.004, 0.013 | 0.277        | 0.008       | -0.004, 0.021 | 0.183        |
| + body mass index | 0.012       | 0.001, 0.023  | 0.038        | 0.004       | -0.004, 0.013 | 0.301        | 0.009       | -0.004, 0.021 | 0.176        |
| + smoking status | 0.004       | -0.003, 0.011 | 0.220        | 0.001       | -0.003, 0.004 | 0.709        | 0.003       | -0.005, 0.010 | 0.478        |
| + alcohol use   | 0.004       | -0.003, 0.011 | 0.235        | 0.001       | -0.003, 0.004 | 0.710        | 0.003       | -0.005, 0.010 | 0.467        |
| E2 PA index + sex | 0.275       | 0.200, 0.349  | <0.001       | 0.281       | 0.203, 0.360  | <0.001       | 0.302       | 0.223, 0.382  | <0.001       |
| + education     | 0.280       | 0.204, 0.356  | <0.001       | 0.286       | 0.206, 0.367  | <0.001       | 0.299       | 0.220, 0.379  | <0.001       |
| + body mass index | 0.287       | 0.210, 0.364  | <0.001       | 0.291       | 0.210, 0.372  | <0.001       | 0.299       | 0.220, 0.379  | <0.001       |
| + smoking status | 0.373       | 0.274, 0.471  | <0.001       | 0.378       | 0.277, 0.480  | <0.001       | 0.380       | 0.279, 0.480  | <0.001       |
| + alcohol use   | 0.371       | 0.275, 0.473  | <0.001       | 0.381       | 0.279, 0.482  | <0.001       | 0.382       | 0.281, 0.481  | <0.001       |

A1, genetic factors of DNAm GrimAge Acc in common with physical activity index; A2, unique genetic factors of DNAm GrimAge Acc; E1, environmental factors of DNAm GrimAge Acc in common with physical activity index; E2, unique environmental factors of DNAm GrimAge Acc; CI, confidence interval; + The model was additionally adjusted for the following variables.

a The model was additionally adjusted for indicator of being out of working life.
Table 5. The proportion of the variation in DNAm GrimAge acceleration (Acc) explained by genetic and environmental effects in older twin pairs

|          | Sport index | Leisure index | Work index |
|----------|-------------|---------------|------------|
|          | estimate    | 95% CI        | P          | estimate    | 95% CI        | P          | estimate    | 95% CI        | P          |
| A1       | PA index + sex | 0.015 | 0.001, 0.03 | 0.034 | 0.008 | -0.001, 0.018 | 0.074 | 0.004 | -0.003, 0.011 | 0.256 |
|          | + education  | 0.015 | 0.001, 0.03 | 0.035 | 0.008 | -0.001, 0.017 | 0.079 | 0.002 | -0.002, 0.006 | 0.380 |
|          | + body mass index | 0.014 | 0.000, 0.027 | 0.047 | 0.007 | -0.001, 0.015 | 0.099 | 0.002 | -0.002, 0.006 | 0.381 |
|          | + smoking status | 0.002 | 0.000, 0.007 | 0.455 | 0.001 | -0.002, 0.005 | 0.471 | 0.000 | -0.002, 0.003 | 0.683 |
|          | + alcohol use | 0.002 | 0.000, 0.008 | 0.437 | 0.002 | -0.002, 0.003 | 0.426 | 0.001 | -0.002, 0.003 | 0.669 |
| A2       | PA index + sex | 0.627 | 0.516, 0.738 | <0.001 | 0.623 | 0.509, 0.738 | <0.001 | 0.618 | 0.493, 0.743 | <0.001 |
|          | + education  | 0.627 | 0.517, 0.738 | <0.001 | 0.624 | 0.509, 0.738 | <0.001 | 0.623 | 0.499, 0.748 | <0.001 |
|          | + body mass index | 0.622 | 0.508, 0.737 | <0.001 | 0.617 | 0.497, 0.737 | <0.001 | 0.612 | 0.480, 0.744 | <0.001 |
|          | + smoking status | 0.584 | 0.489, 0.680 | <0.001 | 0.583 | 0.486, 0.680 | <0.001 | 0.579 | 0.481, 0.678 | <0.001 |
|          | + alcohol use | 0.597 | 0.505, 0.690 | <0.001 | 0.598 | 0.524, 0.723 | <0.001 | 0.595 | 0.501, 0.690 | <0.001 |
| E1       | PA index + sex | 0.035 | 0.005, 0.065 | 0.023 | 0.024 | 0.001, 0.046 | 0.041 | 0.007 | -0.005, 0.018 | 0.267 |
|          | + education  | 0.035 | 0.005, 0.065 | 0.022 | 0.023 | 0.000, 0.045 | 0.045 | 0.005 | -0.006, 0.016 | 0.361 |
|          | + body mass index | 0.031 | 0.003, 0.059 | 0.032 | 0.020 | -0.001, 0.041 | 0.062 | 0.005 | -0.006, 0.016 | 0.370 |
|          | + smoking status | 0.004 | -0.007, 0.015 | 0.487 | 0.004 | -0.006, 0.013 | 0.467 | 0.001 | -0.004, 0.007 | 0.678 |
|          | + alcohol use | 0.004 | -0.007, 0.015 | 0.470 | 0.004 | -0.003, 0.004 | 0.423 | 0.001 | -0.005, 0.007 | 0.664 |
| E2       | PA index + sex | 0.323 | 0.221, 0.424 | <0.001 | 0.345 | 0.235, 0.455 | <0.001 | 0.371 | 0.246, 0.496 | <0.001 |
|          | + education  | 0.322 | 0.222, 0.422 | <0.001 | 0.346 | 0.235, 0.456 | <0.001 | 0.370 | 0.245, 0.495 | <0.001 |
|          | + body mass index | 0.333 | 0.228, 0.438 | <0.001 | 0.356 | 0.240, 0.473 | <0.001 | 0.381 | 0.248, 0.514 | <0.001 |
|          | + smoking status | 0.410 | 0.316, 0.503 | <0.001 | 0.412 | 0.316, 0.508 | <0.001 | 0.419 | 0.322, 0.516 | <0.001 |
|          | + alcohol use | 0.396 | 0.305, 0.488 | <0.001 | 0.396 | 0.275, 0.476 | <0.001 | 0.403 | 0.310, 0.496 | <0.001 |

A1, genetic factors of DNAm GrimAge Acc in common with physical activity index; A2, unique genetic factors of DNAm GrimAge Acc; E1, environmental factors of DNAm GrimAge Acc in common with physical activity index; E2, unique environmental factors of DNAm GrimAge Acc; CI, confidence interval; + The model was additionally adjusted for the following variables.

a The model was additionally adjusted for indicator of being out of working life.
METHODS

Material and methods

Wave 4 of the Finntwin12 study consisted of an invitation to attend an in-person study in Helsinki, targeting twins that had been interviewed at age 14. The response rate was 73% (n = 1,347; 21–25-year-old twin individuals). Participants filled several questionnaires about their health-related behaviour and psychosocial factors, including questionnaires about lifestyle factors. They were also interviewed with a semi-structured psychiatric interview. DNA from blood samples was collected. A total of 1,295 twins of the FinnTwin12 study provided a DNA sample and written informed consent. The sample includes opposite-sex pairs who are not analyzed here due to gender differences in physical activity behaviours and experiences.

The older cohort fourth-wave questionnaire on health and risk factors was used to invite and select participants for a detailed in-person study on the epigenetics of hypertension. A total of 447 twins were examined and measured, and blood samples were collected for DNA analyses after written informed consent was signed. A lifestyle interview including items on medications, use of alcohol, smoking, diet, occupation, and physical activity was performed (1,2).

DNA methylation. High molecular weight white blood cell DNA was bisulfite-converted using EZ-96 DNA methylation-Gold Kit (Zymo Research, Irvine, CA, USA), according to the manufacturer’s instructions, and the co-twins were always converted on the same plate to
minimize potential batch effects. Genome-wide DNAm was measured using Illumina’s Infinium HumanMethylation450 BeadChip and the Infinium MethylationEPIC BeadChip (LTPA discordant twins), according to the manufacturer’s instructions (Illumina, San Diego, CA, USA). The Illumina BeadChips measure single-CpG resolution DNAm levels across the human genome. DNAm data were preprocessed using R package *minfi* (3). Detection *P* values comparing total signal for each probe to the background signal level, were calculated to evaluate the quality of the samples (4). Samples of poor quality (mean detection *P* > 0.01) were excluded from further analysis. Because data included samples from different platforms, data were normalized by using the single-sample Noob normalization method (5). Beta values representing CpG methylation levels were calculated as a ratio of methylated intensities (M) to the overall intensities (Beta value=M/(M+U+100), where U is an unmethylated probe intensity).

**Epigenetic age estimates**

The development of all the three DNAm-based age estimators (DNAm age, DNAm PhenoAge and DNAm GrimAge) have been conducted utilizing the penalized regression model (6–8). The method automatically selects the best predictors for the model from large datasets. **Horvath’s DNAm age** is an estimate for chronological age (6). It was developed by regressing the chronological age on DNAm levels within 21,369 of CpG sites. As a result, 353 optimal CpG sites were selected for the age predictor by the algorithm. **DNAm PhenoAge** was developed by using ‘phenotypic age’ as reference (7). Phenotypic age was developed by regressing the mortality risk on 42 clinical biomarkers and chronological age. Phenotypic age is a combination (weighted sum) of chronological age and the following clinical biomarkers, that best predicted the mortality risk: albumin (with negative weighting coefficient), creatinine, glucose, C-reactive
protein, lymphocyte percent (with negative weighting coefficient), mean red cell volume, red cell distribution width, alkaline phosphatase and white blood cell count. Next, phenotypic age was regressed on DNAm levels within 20,169 CpG sites. As a result, 513 CpG sites were selected for the phenotypic age predictor by the algorithm. **DNAm GrimAge** was developed at two stages, as well (8). At the first stage, DNAm based surrogate biomarkers for 88 plasma proteins and smoking pack-years were developed. At the second stage, mortality risk was regressed on the DNAm based surrogate biomarkers, chronological age and sex. As a result, eight DNAm based surrogate biomarkers, chronological age and sex were selected for mortality risk predictor by the algorithm. DNAm GrimAge is a composite biomarker (weighted sum) of chronological age, sex, DNAm based smoking pack-years (PACYRS) and the following surrogate biomarkers for plasma proteins (DNAm based plasma proteins): DNAm adrenomedullin (ADM), DNAm beta-2-microglobulin (B2M), DNAm cystatin-C, DNAm growth differentiation factor 15 (GDF15), DNAm leptin (with negative weighting coefficient), DNAm plasminogen activator inhibitor 1 (PAI-1) and DNAm tissue inhibitor metalloproteinases 1 (TIMP-1).

In this study, the epigenetic age estimates, DNAm age, DNAm PhenoAge, and DNAm GrimAge were calculated using an online calculator (https://dnamage.genetics.ucla.edu/new). We utilized normalization method implemented in the calculator. Epigenetic age acceleration (Acc), which describes the difference between chronological age and epigenetic age estimate, was calculated for all subjects as the residuals from a linear regression model of epigenetic age estimate on chronological age.
STATISTICAL ANALYSIS

The correlations and the parameters of the models were estimated using the full information maximum likelihood with robust standard errors. The data were assumed to be missing at random (MAR).

Univariate twin models

Univariate modelling was carried out to study the magnitude of genetic and environmental factors affecting epigenetic age Acc measures, and physical activity (PA) indices separately in young adult and older twin pairs. Because the dataset includes monozygotic (MZ) and dizygotic (DZ) twin pairs raised in the same home, it was possible to decompose the variation in the outcome of interest into the latent variables representing additive genetic (A), dominant genetic (D) or shared environmental (C) and non-shared environmental (E) components (ACE model or ADE model) (9). However, C and D components cannot be estimated simultaneously using twin data alone. The sequences of the models were fitted in both cohorts (ACE, ADE, AE, CE and E). The model including D and E components (DE-model), was omitted because it is less plausible biologically (dominance in the absence of additive effects is rare). The univariate models were adjusted for sex, BMI and smoking status.

The Satorra-Bentler scaled chi-squared ($\chi^2$)-test, the comparative fit index (CFI), the Tucker–Lewis index (TLI), the root mean square error of approximation (RMSEA), and the standardized root-mean-square residual (SRMR) were used to evaluate the goodness-of-fit of the models. The model fits the data well if the $\chi^2$-test is insignificant ($P > 0.05$), CFI and TLI values are close to 0.95, the RMSEA value is below 0.06, and the SRMR value is below 0.08 (10). In addition,
Bayesian information criterion (BIC) was used to compare non-nested models. A lower BIC value indicates a better model fit. The parsimonious model with the most appropriate fit to the data was considered optimal. After that, the model was fitted simultaneously in young adult and older twin pairs by using the multiple-group analysis method. The differences in the proportions explained by genetic and environmental factors were tested for significance using Wald test of parameter constraints.

**Bivariate twin models**

First, the full ACE- or ADE-model, including all the components for both latent variables CH1 and CH2 were fit. CH1 represents the PA index, and CH2 represents the residual of epigenetic age Acc after the impact of PA index has been taken into account. After that, a more parsimonious model was considered based on the results of the univariate modeling. The model fit of the nested models was compared by using the Satorra–Bentler scaled chi-squared ($\chi^2$) difference test. The more parsimonious model was chosen if the test produced a non-significant loss of fit.

**RESULTS**

**Univariate models**

Mainly, the assumptions of genetic twin modelling were met. There were no systematic differences in the means or variances in the epigenetic age Acc measures or PA indices between MZ and DZ twins. For all the epigenetic age Acc measures, the univariate model including additive genetic and non-shared environmental component (AE-model) was considered optimal in both young adult and older twin pairs (see Tables, SDC 2–3, the estimation results of the
univariate model for epigenetic aging among young adult and older twin pairs, respectively). However, ACE and ADE generally fit the data about as well. Apart from DNAm GrimAge Acc in older twins, CE models (i.e. models with no genetic component) provided significantly worse fit.

In young adults, ICCs for PA indices were consistently higher in MZ twins than in DZ twins (Table 2). In the older cohort, the ICCs were quite similar in MZ and DZ twins. However, after adjusting for covariates, AE-model was considered optimal for all the PA indices in young adult and older twin pairs (see Tables, SDC 4–5, the estimation results of the univariate model for physical activity indices among young adult and older twin pairs, respectively). In young adult twin pairs, additive genetic factors explained 60% of the variation in the sport index, 48% of the variation in the leisure index and 49% of the variation in the work index, while the rest of the variation in each index was explained by non-shared environmental effects (40%, 52% and 51%, respectively). In older twin pairs, additive genetic factors explained 35% of the variation in the sport index, 28% of the variation in the leisure index and 34% of the variation in the work index while the rest of the variation in each index was explained by non-shared environmental effects (65%, 72% and 66%, respectively).

Bivariate models

For each PA index and DNAm Grim Acc, the model including all the ACE-model components for both latent variables CH1 and CH2 was fitted. After that, the shared environmental components were omitted based on the results of univariate modelling. In young adult and older
twin pairs, the models fitted the data equally well and thus, the more parsimonious model was chosen (see Table, SDC 6, the model fit of the bivariate models).
REFERENCES

1. Kaprio J. The Finnish Twin Cohort Study: an update. Twin Res Hum Genet. 2013;16(1):157–62.

2. Kaprio J, Bollepalli S, Buchwald J, Iso-Markku P, Korhonen T, Kovanen V, et al. The older Finnish Twin Cohort: 45 years of follow-up. Twin Res Hum Genet. 2019;22(4):240–54.

3. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: A flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014;30(10):1363–9.

4. Maksimovic J, Phipson B, Oshlack A. A cross-package Bioconductor workflow for analysing methylation array data [version 3; peer review: 4 approved]. F1000Res. 2017;5:1281.

5. Fortin JP, Triche TJ, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics. 2017;33(4):558–60.

6. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.

7. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY). 2018;10(4):573–91.

8. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY). 2019;11(2):303–27.

9. Neale MC, Cardon LR. Methodology for Genetic Studies of Twins and Families.
10. Hu L, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Struct Equ Model A Multidiscip J*. 1999;6(1):1–55.
Supplementary Table 1. The estimation results of the univariate model for epigenetic aging among young adult twin pairs (MZ n = 163, DZ n = 122)

| Model fit | Parameter estimates and their 95% confidence intervals |
|-----------|---------------------------------------------------------|
| X² | df | SC | P | CFI | RMS | SR | BI | C | a² or d² / total | c² / total | total |
| DNAm Age acceleration | DNAm PhenoAge acceleration | DNAm GrimAge acceleration |
| AC | 38. | 0.9 | 0.06 | 0.9 | 0. | 0.04 | 75 | 0.4 | 0.07, 0. | -0.02, 0.3 | 0.23, 10. | 9.42, |
| E | 9 | 27 | 0 | 4 | 3 | 92 | 0.056 | 4 | 28 | 0 | 0.74 | 28 | 0.58 | 2 | 0.41 | 94 | 12.46 |
| AD | 40. | 0.9 | 0.04 | 0.9 | 0. | 0.04 | 75 | 0.6 | 0.61, 0. | 0.00, 0.3 | 0.23, 10. | 9.32, |
| E | 6 | 27 | 4 | 5 | 2 | 91 | 0.059 | 5 | 31 | 9 | 0.77 | 00 | 0.00 | 1 | 0.39 | 74 | 12.16 |
| 42. | 0.9 | 0.04 | 0.9 | 0. | 0.04 | 75 | 0.6 | 0.61, 0. | 0.3 | 0.23, 10. | 9.32, |
| AE | 1 | 28 | 1 | 3 | 1 | 91 | 0.059 | 5 | 25 | 9 | 0.77 | - | 1 | 0.39 | 74 | 12.16 |
| 48. | 0.9 | 0.01 | 0.8 | 0. | 0.04 | 75 | 0.69 | 0 | 0.52, 0.4 | 0.31, 11. | 9.51, |
| CE | 1 | 28 | 1 | 1 | 8 | 87 | 0.071 | 6 | 31 | - | 61 | 0.69 | 0 | 0.48 | 02 | 12.54 |
| 0.8 | <0.0 | 0.0 | 0. | 0.09 | 76 | - | 1.0 | - | 10. | 9.49, |
| E | 198 | 29 | 7 | 0 | 1 | 3 | 00 | 0.202 | 1 | 53 | - | 0 | 99 | 12.49 |

Note. The model was adjusted for sex, body mass index and smoking status; SC, scaling correction
Supplementary Table 2. The estimation results of the univariate model for epigenetic aging among older twin pairs (MZ \( n = 147 \), DZ \( n = 88 \))

| Model fit | Parameter estimates and their 95% confidence intervals |
|-----------|---------------------------------------------------------|
| **DNAm Age acceleration** |
| AC 36. 2 1.1 0.11 0.9 0.8 | X \( ^2 \) 70 0.6 0.50, 0.0 0.00, 0.3 0.28, 24. 18.29, 1.2 1.0 |
| E 3 7 2 0 0 9 0.054 9 | d f SC P I I EA MR C a \( ^2 \) / total c \( ^2 \) or d \( ^2 \) / total c \( ^2 \) / total 1 0.00 |
| AD 36. 2 1.0 0.10 0.9 0.8 | 0.06 70 0.2 -0.41, 0.3 -0.34, 0.3 0.27, 24. 18.27, |
| E 4 7 9 6 0 9 0.055 8 | 43 6 0.93 6 1.06 8 0.50 07 29.87 |
| AE 6 8 8 6 0 9 0.054 9 | 39 1 0.73 - 9 0.50 0 20 30.11 |
| **DNAm PhenoAge acceleration** |
| AC 36. 2 1.1 0.11 0.8 0.8 | 0.05 71 0.5 0.43, 0.0 0.00, 0.4 0.34, 27. 23.03, |
| E 0 7 0 6 7 0.055 3 | 52 5 0.66 0 0.00 5 0.57 27 31.51 |
| AD 38. 2 1.0 0.07 0.8 0.8 | 0.05 71 0.6 0.49, 0.0 0.00, 0.4 0.30, 30. 25.57, |
| E 5 7 9 0 8 7 0.060 5 | 52 0 0.70 0 0.00 0 0.51 23 34.88 |
| AE 9 8 5 7 8 7 0.060 5 | 46 0 0.70 - 0 0.51 22 34.88 |
| **DNAm GrimAge acceleration** |
| AC 23. 2 1.2 0.64 1.0 1.0 | 0.03 66 0.4 0.07, 0.1 -0.26, 0.4 0.32, 10. 8.25, |
| E 7 7 6 0 1 0.000 6 | 71 7 0.87 0 0.46 3 0.53 00 11.76 |
| AD 23. 2 1.2 0.66 1.0 1.0 | 0.03 66 0.5 0.48, 0.0 0.00, 0.4 0.32, 9.9 8.24, |
| E 3 7 9 9 0 1 0.000 6 | 71 8 0.68 0 0.00 2 0.52 8 11.72 |
| AE 2 8 5 3 0 1 0.000 6 | 66 8 0.68 - 2 0.52 8 11.72 |
| **Note.** The model was adjusted for sex, body mass index and smoking status; SC, scaling correction
Supplementary Table 3. The estimation results of the univariate model for physical activity indices among young adult twin pairs (MZ, n = 163, DZ, n = 122)

| Model fit | Parameter estimates and their 95% confidence intervals |
|-----------|--------------------------------------------------------|
|           | dX² SC P I TL RMS SRM BI a² / total | c² or d² / total | total |
| Sport index |                                        |                  |       |
| ACE 27.2 | 0.9 0.43 0.9 0.9 | 0.06 572 0.5 0.12 | 0.0 -0.39 | 0.4 0.28 | 0.5 0.51 |
| ADE 26.2 | 0.9 0.50 1.0 1.0 | 0.06 572 0.6 0.47 | 0.0 0.00 | 0.4 0.28 | 0.5 0.51 |
| AE 27.2 | 0.9 0.50 1.0 1.0 | 0.06 572 0.6 0.47 | 0.4 0.28 | 0.5 0.51 |
| Leisure index |                                        |                  |       |
| ACE 25.2 | 0.9 0.55 1.0 1.0 | 0.05 553 0.3 -0.11 | 0.1 -0.18 | 0.5 0.38 | 0.3 0.32 |
| ADE 25.2 | 0.9 0.56 1.0 1.0 | 0.05 553 0.4 0.34 | 0.0 0.00 | 0.5 0.38 | 0.3 0.32 |
| AE 26.2 | 0.9 0.56 1.0 1.0 | 0.05 552 0.4 0.34 | 0.5 0.38 | 0.3 0.32 |
| Work index |                                        |                  |       |
| ACE 36.2 | 0.9 0.09 0.8 0.8 | 0.05 554 0.4 0.35 | 0.0 0.00 | 0.5 0.38 | 0.3 0.33 |
| ADE 38.2 | 0.9 0.07 0.8 0.8 | 0.05 554 0.4 -0.36 | 0.0 -0.72 | 0.5 0.37 | 0.3 0.33 |
| AE 38.2 | 0.9 0.09 0.8 0.8 | 0.05 554 0.4 0.35 | 0.5 0.38 | 0.3 0.33 |

Note. The model was adjusted for sex, body mass index and smoking status; SC, scaling correction.
Supplementary Table 4. The estimation results of the univariate model for physical activity indices among older twin pairs (MZ \( n = 147 \), DZ \( n = 87 \))

| Model fit | Parameter estimates and their 95% confidence intervals |
|-----------|---------------------------------------------------------|
|           | \( \chi^2 \) | df | SC | \( P \) | CFI | TLI | RMSEA | SRMR | BIC | \( a^2 / \text{total} \) | \( c^2 \text{ or } d^2 / \text{total} \) | \( e^2 / \text{total} \) | total |
| Sport index | | | | | | | | | | | | | | |
| ACE | 31.1 | 27 | 1.01 | 0.266 | 0.90 | 0.89 | 0.036 | 0.065 | 5249 | 0.35 | 0.17 | 0.52 | 0.00 | 0.00 | 0.00 | 0.65 | 0.48 | 0.83 | 0.63 | 0.54 | 0.72 |
| ADE | 32.6 | 27 | 0.97 | 0.212 | 0.87 | 0.86 | 0.042 | 0.064 | 5249 | 0.30 | -0.85 | 1.44 | 0.05 | -1.13 | 1.23 | 0.65 | 0.47 | 0.83 | 0.63 | 0.54 | 0.72 |
| AE | **32.3** | **28** | **0.97** | **0.263** | **0.90** | **0.89** | **0.036** | **0.065** | **5243** | **0.35** | **0.17** | **0.52** | **-** | **0.65** | **0.48** | **0.83** | **0.63** | **0.54** | **0.72** |
| CE | 33.9 | 28 | 0.98 | 0.204 | 0.87 | 0.86 | 0.042 | 0.066 | 5245 | - | 0.30 | 0.14 | 0.45 | 0.71 | 0.55 | 0.86 | 0.63 | 0.54 | 0.73 |
| E | 51.6 | 29 | 0.97 | 0.006 | 0.50 | 0.48 | 0.081 | 0.070 | 5257 | - | - | 1.00 | 0.63 | 0.54 | 0.73 |
| Leisure index | | | | | | | | | | | | | | | | |
| ACE | 25.2 | 27 | 0.93 | 0.562 | 1.00 | 1.05 | 0.000 | 0.068 | 5071 | 0.21 | -0.37 | 0.80 | 0.05 | -0.45 | 0.55 | 0.73 | 0.56 | 0.9 | 0.40 | 0.34 | 0.45 |
| ADE | 24.2 | 27 | 0.97 | 0.620 | 1.00 | 1.08 | 0.000 | 0.068 | 5071 | 0.28 | 0.12 | 0.43 | 0.00 | 0.00 | 0.00 | 0.73 | 0.57 | 0.89 | 0.40 | 0.34 | 0.45 |
| AE | **25.1** | **28** | **0.93** | **0.623** | **1.00** | **1.08** | **0.000** | **0.068** | **5066** | **0.28** | **0.12** | **0.43** | **-** | **0.73** | **0.57** | **0.89** | **0.40** | **0.34** | **0.45** |
| CE | 25.7 | 28 | 0.93 | 0.587 | 1.00 | 1.06 | 0.000 | 0.067 | 5066 | - | 0.23 | 0.09 | 0.37 | 0.77 | 0.64 | 0.91 | 0.40 | 0.34 | 0.45 |
| E | 37.5 | 29 | 0.91 | 0.135 | 0.76 | 0.75 | 0.050 | 0.072 | 5071 | - | - | 1.00 | 0.40 | 0.34 | 0.45 |
| Work index | | | | | | | | | | | | | | | | |
| ACE | 37.2 | 27 | 0.91 | 0.091 | 0.68 | 0.65 | 0.057 | 0.061 | 5378 | 0.29 | -0.32 | 0.89 | 0.05 | -0.45 | 0.55 | 0.66 | 0.47 | 0.85 | 0.95 | 0.84 | 1.07 |
| ADE | 35.5 | 27 | 0.96 | 0.126 | 0.73 | 0.70 | 0.052 | 0.061 | 5378 | 0.34 | 0.17 | 0.52 | 0.00 | 0.00 | 0.00 | 0.66 | 0.48 | 0.83 | 0.95 | 0.84 | 1.07 |
| AE | **36.9** | **28** | **0.92** | **0.122** | **0.72** | **0.70** | **0.052** | **0.061** | **5373** | **0.34** | **0.17** | **0.52** | **-** | **0.66** | **0.48** | **0.83** | **0.95** | **0.84** | **1.07** |
| CE | 38.1 | 28 | 0.92 | 0.097 | 0.69 | 0.66 | 0.055 | 0.061 | 5374 | - | 0.28 | 0.13 | 0.43 | 0.72 | 0.58 | 0.87 | 0.95 | 0.84 | 1.06 |
| E | 53.8 | 29 | 0.92 | 0.003 | 0.23 | 0.21 | 0.085 | 0.071 | 5383 | - | - | 1.00 | 0.95 | 0.84 | 1.06 |

Note. The model was adjusted for sex, body mass index and smoking status; SC, scaling correction
Supplementary Table 5. The model fit of the bivariate models and chi-squared difference tests for comparison of the nested models among young adult (MZ n = 163, DZ n = 122) and older twin pairs (MZ n = 147, DZ n = 88)

|                     | X²  | df | SC  | P   | CFI | TLI | RMSEA | SRMR | BIC |
|---------------------|-----|----|-----|-----|-----|-----|-------|------|-----|
| **Young adult twin pairs** |     |    |     |     |     |     |       |      |     |
| Sport index         |     |    |     |     |     |     |       |      |     |
| ACE                 | 21.9| 19 | 0.97| 0.291| 0.98| 0.98| 0.032 | 0.110| 3902|
| AE                  | 24.8| 21 | 0.99| 0.255| 0.98| 0.98| 0.036 | 0.119| 3894|
| ACE vs. AE          | 2.8 | 2  | 1.12| 0.246| 0.98| 0.98|       |      |     |
| Leisure index       |     |    |     |     |     |     |       |      |     |
| ACE                 | 22.8| 19 | 0.97| 0.246| 0.97| 0.97| 0.037 | 0.105| 3709|
| AE                  | 23.0| 21 | 1.02| 0.344| 0.98| 0.98| 0.026 | 0.106| 3699|
| ACE vs. AE          | 0.9 | 2  | 1.50| 0.638| 0.98| 0.98|       |      |     |
| Work index          |     |    |     |     |     |     |       |      |     |
| ACE                 | 31.9| 19 | 1.01| 0.032| 0.92| 0.92| 0.069 | 0.102| 3736|
| AE                  | 29.8| 21 | 1.08| 0.096| 0.94| 0.95| 0.054 | 0.102| 3725|
| ACE vs. AE          | 0.01| 2  | 0.99| 0.993| 0.98| 0.98|       |      |     |

| **Older twin pairs** |     |    |     |     |     |     |       |      |     |
| Sport index          |     |    |     |     |     |     |       |      |     |
| ACE                 | 11.7| 19 | 1.25| 0.899| 1.00| 1.05| 0.000 | 0.101| 3683|
| AE                  | 12.9| 21 | 1.14| 0.912| 1.00| 1.05| 0.000 | 0.101| 3672|
| ACE vs. AE          | 0.9 | 2  | 0.1 | 0.653| 0.98| 0.98|       |      |     |
| Leisure index        |     |    |     |     |     |     |       |      |     |
| ACE                 | 8.7 | 19 | 1.12| 0.978| 1.00| 1.08| 0.000 | 0.113| 3505|
| AE                  | 9.5 | 21 | 1.12| 0.985| 1.00| 1.08| 0.000 | 0.114| 3494|
| ACE vs. AE          | 0.8 | 2  | 1.12| 0.670| 0.98| 0.98|       |      |     |
| Work index          |     |    |     |     |     |     |       |      |     |
| ACE                 | 23.5| 19 | 1.08| 0.218| 0.96| 0.96| 0.045 | 0.093| 3811|
| AE                  | 24.4| 21 | 1.05| 0.274| 0.97| 0.97| 0.037 | 0.094| 3800|
| ACE vs. AE          | 0.3 | 2  | 0.77| 0.855| 0.98| 0.98|       |      |     |

The models were adjusted for sex. SC, scaling correction.
Supplementary Table 6. The correlations between the components of DNAm GrimAge estimator (adjusted for age) and DNAm GrimAge acceleration (Acc) as well as physical activity indices among young adult (n = 570) and older individual twins (n = 470)

|                      | Correlation coefficients and their 95% CI |             |             |             |
|----------------------|------------------------------------------|-------------|-------------|-------------|
|                      | DNAm GrimAge Acc                         | Sport index | Leisure index | Work index  |
| Young adult twins    |                                          |             |             |             |
| DNAm ADM             | 0.24                                     | 0.14, 0.33  | -0.11       | 0.01        | -0.11, 0.10 | 0.02 | -0.08, 0.12 |
| DNAm B2M             | 0.33                                     | 0.24, 0.42  | -0.09       | -0.12       | -0.22, -0.02 | 0.08 | 0.00, 0.16  |
| DNAm cystatin-C      | 0.49                                     | 0.41, 0.56  | -0.04       | 0.15        | 0.05, 0.24  | 0.05 | -0.04, 0.14 |
| DNAm GDF15           | 0.46                                     | 0.38, 0.55  | -0.12       | 0.03        | -0.07, 0.13 | 0.08 | -0.03, 0.19 |
| DNAm PAI-1           | 0.34                                     | 0.25, 0.42  | -0.12       | 0.02        | -0.07, 0.11 | 0.06 | -0.03, 0.16 |
| DNAm TIMP-1          | 0.46                                     | 0.40, 0.53  | -0.13       | 0.01        | -0.10, 0.09 | -0.03 | -0.14, 0.09 |
| DNAm leptin          | -0.09                                   | -0.19, 0.01 | -0.14       | -0.09       | -0.18, 0.00 | -0.03 | -0.13, 0.08 |
| DNAm PKCYSR          | 0.83                                     | 0.80, 0.87  | -0.20       | 0.00        | -0.10, 0.11 | 0.30 | 0.21, 0.39  |

| Older twins          |                                          |             |             |             |
|                      | DNAm ADM                                 | 0.23        | 0.14, 0.32  | -0.03       | -0.14, 0.08 | 0.04 | -0.07, 0.15 | -0.04 | -0.16, 0.08 |
|                      | DNAm B2M                                 | 0.39        | 0.30, 0.49  | -0.09       | -0.20, 0.01 | 0.11 | -0.20, -0.03 | 0.01 | -0.09, 0.11 |
|                      | DNAm cystatin-C                         | 0.50        | 0.42, 0.58  | -0.14       | -0.25, -0.02 | -0.12 | -0.24, -0.01 | 0.04 | -0.07, 0.15 |
|                      | DNAm GDF15                               | 0.51        | 0.38, 0.64  | -0.03       | -0.15, 0.09 | -0.08 | -0.20, 0.05  | 0.11 | -0.02, 0.24 |
|                      | DNAm PAI-1                               | 0.52        | 0.46, 0.59  | -0.19       | -0.30, -0.07 | -0.13 | -0.26, -0.01 | 0.02 | -0.11, 0.16 |
|                      | DNAm TIMP-1                              | 0.56        | 0.49, 0.64  | -0.15       | -0.27, -0.02 | -0.10 | -0.23, 0.03  | 0.05 | -0.06, 0.17 |
|                      | DNAm leptin                             | -0.21       | -0.32, -0.10 | 0.03       | -0.08, 0.15 | 0.18 | 0.07, 0.29  | -0.11 | -0.23, 0.01 |
|                      | DNAm PKCYSR                              | 0.89        | 0.86, 0.92  | -0.16       | -0.28, -0.04 | -0.17 | -0.26, -0.08 | 0.11 | 0.01, 0.21  |

CI, confidence interval; ADM, adrenomedullin; B2M, beta-2-microglobulin; GDF15, growth differentiation factor 15; PAI-1, plasminogen activator inhibitor 1; TIMP-1, tissue inhibitor metalloproteinases 1; PACKYRS, smoking pack-years.

*a CIs were corrected for nested sampling.*
Supplementary Figure 1. Epigenetic aging in twin pairs discordant for leisure-time physical activity: DNAm PhenoAge (A and B) and DNAm GrimAge (C and D) in MZ and DZ twin pairs, respectively. Dashed line denotes the mean within-pair difference and other lines denote individual pairs.