Epigenetic aging and musculoskeletal outcomes in a cohort of women living with HIV

Authors: Stephanie Shiau, PhD, MPH[1]; Francesca Zumpano, BS[1], Ziyi Wang, MS[1]; Jayesh Shah[2], Phyllis C. Tien, MD[3,4], Ryan D. Ross, PhD[5]; Anjali Sharma, MD, MS[6]; Michael T. Yin, MD, MS[2]

Affiliations:

1. Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, USA
2. Department of Medicine, College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY, USA
3. Department of Medicine, Veterans Affairs Medical Center, San Francisco, CA, USA
4. University of California San Francisco, San Francisco, CA, USA
5. Department of Anatomy & Cell Biology, Rush University Medical Center, Chicago, IL, USA
6. Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA

Corresponding Author: Stephanie Shiau, PhD, MPH, Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, 08854, USA; Tel: 732-235-9104; E-mail: stephanie.shiau@rutgers.edu

Alternate Corresponding Author: Michael Yin, MD, MS, Division of Infectious Diseases, Columbia University Irving Medical Center, 630 W. 168th Street, PH 8-876, New York, NY, 10032, USA. Tel: 212-305-7185, Fax: 212-305-7290; E-mail: mty4@cumc.columbia.edu

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Abstract

Background: The relationship between accelerated epigenetic aging and musculoskeletal outcomes in women with HIV (WWH) has not been studied.

Methods: We measured DNA methylation age using the Infinium MethylationEPIC BeadChip in a cohort from the Women's Interagency HIV Study (N=190) with measures of bone mineral density (BMD) and physical function. We estimated six biomarkers of epigenetic aging: epigenetic aging acceleration (EAA), extrinsic epigenetic age acceleration (EEAA), intrinsic epigenetic age acceleration (IEAA), GrimAge, PhenoAge, and DNA methylation-estimated telomere length (DNAmTL) and evaluated associations of epigenetic aging measures with BMD and physical function. We also performed epigenome-wide association studies (EWAS) to examine associations of DNA methylation signatures with BMD and physical function.

Results: 118 WWH (mean age 49.7 years; 69% Black) and 72 without HIV (mean age 48.9 years; 69% Black) were included. WWH had higher EAA (1.44±5.36 vs. -1.88±5.07, $p<0.001$) and lower DNAmTL (7.13±0.31 vs. 7.34±0.23, $p<0.001$) than women without HIV. There were no significant associations between accelerated epigenetic aging and BMD. Rather, measures of accelerated epigenetic aging were associated with lower physical function.

Conclusions: Accelerated epigenetic aging was observed in WWH compared to women without HIV and associated with lower physical function in both groups.

Key Words: bone mineral density, osteoporosis, menopause, HIV, aging, women, EWAS, epigenetic aging, physical function

Introduction

Adults with HIV appear to have a higher prevalence of frailty, poor physical function, and osteoporosis than age- and sex-matched adults without HIV [1,2]. For women with HIV (WWH), conditions of musculoskeletal aging, including osteoporosis and fractures, occur more frequently after the menopausal transition, and the differences in prevalence between WWH and women without HIV are greater among postmenopausal women [3].

DNA methylation (DNAm), specifically methylation of 5'-cytosine in CpG-rich regions of DNA, is the most commonly studied epigenetic change due to its stability and accessibility [4,5]. DNAm age correlates with chronological age, and individuals with increased DNAm age compared to their chronological age have epigenetic age acceleration [6]. There are a number of methylation-based biomarkers of aging (i.e. epigenetic clocks) that measure age acceleration, including epigenetic age acceleration (EAA), extrinsic epigenetic age acceleration (EEAA), intrinsic epigenetic age acceleration (IEAA), GrimAge, and PhenoAge, as well as a DNA-methylation estimator of telomere length (DNAm-TL) [6–10]. Accelerated epigenetic age by DNAm has been reported among adults with HIV [11–15]; however, most of the data are in men.

Few studies have examined the relationship between epigenetic age acceleration and bone mineral density (BMD) or physical function in WWH. A study in children did not find any evidence of accelerated epigenetic aging at birth or age 7 with BMD [16]. A previous study showed that increased DNAm of *Alu*, a cluster of interspersed DNA elements, is associated with accelerated aging and lower BMD in post-menopausal women with osteoporosis [17]. In the general population, GrimAge was found to be associated with a decline in physical function, including lower performance on 6-minute and 10-meter walk tests and knee extension and ankle plantar flexion strength tests, over three years of follow up in older women [18].

In the primary analysis of the Women's Interagency HIV Study (WIHS) Musculoskeletal Substudy (MSK) of pre-, peri-, and post-menopausal women, we found that WWH had lower areal BMD (aBMD) by dual energy X-Ray absorptiometry (DXA) and lower volumetric BMD by quantitative computed tomography than age- and race/ethnicity-matched women without HIV [3]. In this secondary analysis, we compare methylation-based biomarkers of aging between WWH and women without HIV and evaluate associations of epigenetic age acceleration with BMD and physical function. We explore associations with DNAm signatures using large-scale epigenome-wide association studies (EWAS) to identify potentially modifiable mechanistic pathways.

Methods

Study participants

This analysis includes data from participants in the WIHS MSK, which enrolled 250 WIHS participants aged 40-60 years from 3 WIHS sites (San Francisco, Bronx, and Chicago) with HIV who were on ART for >1 year and had CD4>100 cells/μl, and a comparison group of women without HIV with similar age, ethnicity, and risk behaviors [3]. Exclusion criteria for the MSK included weight >264 lbs, height greater than 6'1", pregnant or breastfeeding in the past 6 months, estimated glomerular filtration rate by MDRD of <60 ml/min/1.73m2, and current hormone replacement therapy, osteoporosis treatment, or glucocorticoid use. For this epigenetic analysis, a total of 195 samples were selected, including 89 from the Bronx, 65 from San Francisco, and 41 from Chicago WIHS sites. This study was approved by the Institutional Review Boards of all participating institutions and informed consent was provided by all participants.

Measurements

***Demographics and clinical characteristics*:** Demographic and clinical information on age, race (Black vs. non-Black), weight, body mass index (BMI), menopausal stage defined using SWAN (Study of Women's Health Across the Nation) study [3,19], substance use, and co-morbidities were extracted from the WIHS database. HIV-related variables included information on CD4 count, HIV-RNA level, and use and type of antiretroviral regimens.

Bone mineral density: DXA was utilized to measure aBMD at the lumbar spine and total hip. DXA scans were performed using Lunar Prodigy densitometers (GE Medical Systems, Madison WI) at all WIHS MSK study sites and read centrally at the Image Analysis Lab (New York, NY) as described previously [3].

***Physical function*:** A battery of muscle strength, walking speed, balance and endurance measures was developed based upon the Baltimore Longitudinal Study on Aging (BLSA) and other aging cohorts [20,21]. Muscle strength was assessed by grip strength [22] and repeated chair stands. For grip strength, the participant was asked to hold a hand-held Jamar dynamometer with their dominant hand and squeeze with maximum force in kg, and the best of three attempts was utilized in analysis. For the repeated chair stand, the participant was asked to stand from a seated position without the aid of their arms. The time to completion of 10 repetitions was recorded to minimize the ceiling effect for higher functioning women [23]. Walking speed was defined by the faster of two measurements at a "normal, comfortable pace" over a 4-meter course. Endurance was assessed by a 400-meter walk, which measures time taken to complete a 400-meter walk. Static balance was assessed with the 4-stage Standing Balance Test (parallel, semi-tandem, tandem, and single-leg) [24], with the duration that subjects were asked to hold each position increased to 30 seconds to reduce possibility of a ceiling effect [25]. Only data from the single-leg stance test were included here. The functional reach test was also performed, with results reported as the mean scores of 3 reaches, where each individual would slide their hand as far forward as they could without losing their balance or taking a step [26].

***DNA extraction and genome-wide methylation profiling*:** DNA was extracted using the Qiagen QIAmp DNA Blood Midi Kit (Qiagen, Germantown, MD) and quantified using the Qubit dsDNA BR Assay Kit and Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA) at CUIMC. DNAm levels were measured using the Infinium MethylationEPIC BeadChip v1.0 (Illumina, San Diego, CA) at Roswell Park Cancer Institute (Buffalo, NY). Genomic DNA (50ng/µL) was isolated and quantified with PicoGreen (Thermo Fisher Scientific, Waltham, MA) from PBMCs of the 195 participants. The Infinium Methylation EPIC BeadChip v1.0 arrays (Illumina, Inc., San Diego, CA) which interrogate ~850,000 CpG sites, were run at the Northwestern University Genomics Core Facility according to the manufacturer's protocol. Briefly,

genomic DNA samples were bisulfite converted using the EZ DNA Methylation kit (Zymo Research Col, Irvine, CA). Samples were amplified, enzymatically fragmented, and hybridized to the BeadChips. Following hybridization, the chips were stained, washed, and scanned using the Illumina HiScan System. Raw intensity data (IDAT) files were obtained.

Bioinformatics pre-processing of DNA methylome data: Standard pre-processing pipeline procedures, including filtering, quality control and dye-bias correction were performed with the R package *ewastools* v1.5 [27,28]. Control metrics were checked for quality control. With the threshold of SNP outliers set as -4, five samples were removed, for a total of 190 remaining for analysis. A total of 843,393 CpG sites were obtained after removing the control probes, non-CpG probes, failed probes with detection p-value ≥0.05, SNP-enriched probes, probes demonstrated to cross-hybridize non-specifically in the genome, and sex chromosome probes. Estimated proportions of six different cell types (B-cells, CD4 T-cells, CD8 T-cells, natural killer cells, granulocytes, monocytes) were estimated using the Houseman method [29].

DNA methylation age: Estimated DNAm age in years was obtained from the online calculator (https://dnamage.genetics.ucla.edu/) developed by Horvath [6]. Six methylation-based biomarkers of aging were calculated: (1) epigenetic age acceleration (*EAA*), (2) extrinsic epigenetic age acceleration (*EEAA*), (3) intrinsic epigenetic age acceleration (*IEAA*), (4) *GrimAge*, (5) *PhenoAge*, and (6) DNAm-estimated telomere length (*DNAmTL*). ***EAA*** is the (raw) residual resulting from regressing the Horvath-estimated DNAm age estimate on chronological age [6]. ***EEAA*** is the residual resulting from regressing the Hannum-estimated DNAm age up-weighted for the contributions of age-related blood cell counts on chronological age [30,31]. ***IEAA*** is the residual resulting from regressing the Horvath-estimated DNAm age on chronological age + CD8.naive + CD8pCD28nCD45RAn + PlasmaBlast + CD4T + NK + Mono + Gran. ***GrimAge*** is based on 1030 CpG sites that predicts time-to-death [8]. ***PhenoAge*** is based on 513 CpG sites

that predict morbidity and mortality [9]. For these five measures, positive values indicate that the participant's biological age is older than expected based on chronological age and negative values indicate that the participant's biological age is younger than expected based on chronological age. The final measure ***DNAmTL*** estimates telomere length, where a shorter telomere length is indicative of accelerated biological aging [32].

Statistical analysis

Continuous variables were descriptively summarized by means and standard deviations and categorical variables by percentages. Comparisons between WWH and women without HIV were performed using t-tests for continuous variables and Fisher's exact tests for categorical variables. Biomarkers of epigenetic aging were compared between groups using t-tests and linear regression, unadjusted and adjusted for race and smoking status. Associations between biomarkers of epigenetic aging and continuous measures of bone and physical function were assessed using linear regression models.

For EWAS analyses, we fit a model using the empirical Bayes moderated linear regression approach implemented by *limma* [33] with DNAm as the dependent variable and HIV status as the primary independent variable. We conducted an unadjusted analysis as well as an analysis adjusted for age, race, and smoking status. CpG sites were considered to be differentially methylated if they had an p-value meeting the Holm-Bonferroni threshold ($p<5.92 \times 10^{-8}$) and $|\Delta\beta|>0.05$, where $\Delta\beta$ is the mean difference between the average DNAm of the groups. Gene annotations used in the analysis were based on the IlluminaHumanMethylationEPICanno.ilm10b2.hg19 database [34]. All analyses were performed using R statistical software (version 4.1.2).

Results

Characteristics of study population

A total of 118 WWH (mean age 49.7 years; 69% Black) and 72 women without HIV (mean age 48.9 years; 69% Black) were included, and characteristics are shown in **Table 1**. WWH had similar weight and BMI compared to women without HIV (**Table 1**). WWH were less likely to be a current smoker compared to women without HIV. Among the WWH, median (IQR) CD4 count was 567 (467-756) (cells/mm^3) and 72% had an HIV RNA <50 copies/mL. WWH had lower BMD T-scores at the lumbar spine and total hip compared to women without HIV. There was little difference in physical function measures (grip strength, repeated chair stand, walk speed, single-leg stand, and functional reach) between women with or without HIV.

HIV

WWH had a significantly higher EAA (1.44±5.36 vs -1.88±5.07, p<0.001) and a lower DNAmTL (7.13±0.31 vs 7.34±0.23, p<0.001) compared to women without HIV (**Figure 1**). EEAA, IEAA, GrimAge, and PhenoAge were not significantly different between groups. Findings were similar when adjusted for smoking status and race.

In an unadjusted model, we identified 2,286 differentially methylated CpG sites associated with HIV that met the Holm-Bonferroni threshold and had $|\Delta\beta|>0.05$. In a model adjusted for age, race, and smoking status, we identified 2,094 differentially methylated CpG sites associated with HIV. The top 50 CpG sites are shown in **Supplemental Table 1**.

Bone

There were no differences in biomarkers of aging between women with and without a lumbar spine BMD T-score <-1 or between women with and without a total hip BMD T-score <-1 for the overall cohort (**Table**

2). Among WWH, there were also no differences between groups (data not shown). Among women without HIV, EAA was significantly lower for those with a lumbar spine BMD T-score <-1 compared to those with a lumbar spine BMD T-score >-1 (-5.43±3.25 vs. -1.35±5.14). When examining continuous bone outcomes for the overall cohort, greater lumbar spine BMD (β=0.238, 95%CI: 0.010, 0.467, p=0.04) and total hip BMD (β=0.316, 95%CI: 0.029, 0.602, p=0.03) were associated with increased DNAmTL.

In an EWAS, no BMD measures (lumbar spine T-score, lumbar spine BMD, total hip T-score, or total hip BMD) were associated with DNAm. No CpG sites met criteria for association (Holm-Bonferroni significance and $|\Delta\beta|>0.05$).

Physical function

We examined associations between biomarkers of aging and various measures of physical function. Women who could not hold a single-leg stand for 30 seconds (N=112) had higher EAA, EEAA, IEAA, GrimAge, and PhenoAge, and lower DNAmTL compared to women who could hold the single-leg stand for 30 seconds (N=78) (**Table 3**). When stratified by HIV status, WWH who could not hold a single-leg stand for 30 seconds had higher EEAA (2.17±8.30 vs. -0.95±7.34, p=0.03), GrimAge (0.78±5.03 vs. -1.51±4.47, p=0.01) and PhenoAge (2.01±7.23 vs. -1.29±7.09, p=0.01) compared to WWH who could hold the single-leg stand for 30 seconds (**Supplemental Table 2**). For women without HIV, those who could not hold a single-leg stand for 30 seconds had higher EAA (-1.02±5.38 vs. -3.32±4.22, p=0.048) and lower DNAmTL (7.30±0.24 vs. 7.41±0.20, p=0.045) compared to those who could hold the single-leg stand for 30 seconds (**Supplemental Table 2**).

Longer time holding a single-leg stand was associated with lower EEAA (β=-0.141, 95%CI: -0.274, -0.009, p=0.037) and PhenoAge (β=-0.145, 95%CI: -0.278, -0.011, p=0.033) (**Table 4**). The time in seconds to

complete 10 repeated chair stands was associated with greater GrimAge acceleration (β=0.147, 95%CI: 0.031, 0.263, p=0.013) and lower DNA telomere length (β=-0.008, 95% CI: -0.015, -0.001, p=0.032). Longer time to complete a 4-meter walk in seconds was associated with higher PhenoAge (β=2.227, 95%CI: 0.918, 3.542, p=0.001).

We then performed EWAS of the association between DNAm and physical function. No CpG sites met criteria for association (Holm-Bonferroni significance and $|\Delta\beta|>0.05$).

Discussion

We report for the first time in a sample of only women (ages 40-60 years) that WWH have a higher EAA and shorter DNAmTL compared to women without HIV. Accelerated aging among adults with HIV has been demonstrated using various epigenetic aging estimates in samples with mostly men [14,15]. WWH are important to study given that globally over 50% of people living with HIV are women, and women accounted for an estimated 49% of all new infections in 2021 [35]. Our finding of accelerated epigenetic age in a cohort of older WWH (mean age 49.7 years) is an important addition to the literature on differences in aging among PWH by biological sex [36].

We found associations between epigenetic age acceleration and measures of physical function including balance and gait speed. Similar findings for some but not all the measures were observed when stratified by HIV status, possibly due to smaller sample sizes. In a study of women at ages 53 and 64 enrolled in the National Survey for Health and Development, baseline epigenetic age acceleration was not associated with balance, in contrast to our findings [37]. A study of 63-76 year-old women from the Finnish Twin Study on Aging found an association between higher GrimAge and decreased performance in 6-minute and 10-meter walk tests, but did not conduct a 4-meter walk test as used in our study [18].

Two prior studies found associations between increased epigenetic age and decreased grip strength [37,38]. Despite these previous results, we found no associations of any epigenetic aging measures in our study with grip strength. A recent longitudinal study did not find an association of any epigenetic aging measure with functional assessments covering different domains of aging (e.g. frailty, mobility, ability to perform activities of daily living) [39]. Given inconsistent findings, additional research is needed to further corroborate these findings, particularly in cohorts of WWH.

Contrary to our hypothesis, we did not find any associations between epigenetic aging measures and bone outcomes. This is consistent with a small non-HIV study of 32 individuals with osteoporosis and 16 controls which found no association between bone parameters and HorvathAge acceleration [40]. The lack of findings in that study and ours could be due to a lack of power for these outcomes and additional larger studies are needed.

Similar to other studies, we found a large number of CpG sites associated with HIV. The top differentially methylated CpG site (cg07839457) was located on the gene NLRC5, which encodes a transcription factor that regulates major histocompatibility complex (MHC) class I molecule expression. This CpG site has also been implicated in other studies of both children and adults with HIV [11,41]. In contrast to a study comparing bone samples of 27 osteoporotic and 23 osteoarthritic patients which identified 241 differentially methylated CpG sites [42], our EWAS analyses did not identify any associations between BMD and DNAm from blood samples. A recent EWAS of BMD in European individuals profiled DNAm in blood and only found one CpG site, cg23196985, located on the 5' untranslated region of *CES1*, to be significantly associated with femoral neck BMD in women [43]. These studies have yet to be replicated, and used older Illumina arrays, including the HumanMethylation27 BeadChip (~27,000 CpG sites) and the HumanMethylation450 array (~450,000 CpG sites), respectively. Genome-wide association studies (GWAS) of BMD by DXA in the general population have identified variants at 73 trait-associated genetic loci, including several associated with fracture risk [44,45]. While genetic advances may pave the way for precision medicine in osteoporosis, genetic variants explain only 5-12% of the total phenotypic variance in BMD. Beyond genetics, many other environmental, lifestyle factors, medications, and health conditions affect BMD, including hormone levels, tobacco and alcohol use, physical activity, and other comorbidities [46–48].

For physical function measures, there were also no associations at a Holm-Bonferroni threshold and a 5% methylation difference. Similarly, other EWAS studies of grip strength in the general population have not reported significant findings [38,43,49]. Taken together, there is limited evidence to support associations between epigenetic changes and physical function. Larger studies and longitudinal studies are needed to more fully assess these potential associations.

Our study is limited by its cross-sectional design. There could be reverse causation, such that a change in physical function could result in changes in DNAm, as opposed to the direction we hypothesized. We also did not adjust for inflammatory biomarkers or other factors that could be associated with both DNAm change, BMD, and physical function. Lastly, a major limitation for all DNAm studies is tissue specificity. Epigenetic changes may be tissue-specific and we do not have bone or muscle-specific DNAm data. Of note, women without HIV had higher rates of tobacco use, cocaine, and alcohol use in our study. Given evidence that these substances can be associated with increased accelerated epigenetic aging, our finding of accelerated aging in WWH may be conservative [50].

In conclusion, we found evidence of associations between certain methylation-based biomarkers of aging and measures of physical function in a cohort of WWH and women without HIV but did not find any significant associations in EWAS analyses with either BMD or functional outcomes. Future studies will need to assess whether these findings persist longitudinally, and to evaluate the directionality of these associations.

Data availability

Data are available upon request.

References

1. Hawkins KL, Brown TT, Margolick JB, Erlandson KM. Geriatric Syndromes: New Frontiers in HIV and Sarcopenia. AIDS **2017**; 31:S137–S146.

2. Jones HT, Levett T, Barber TJ. Frailty in people living with HIV: an update. Curr Opin Infect Dis **2022**; 35:21–30.

3. Sharma A, Hoover DR, Shi Q, et al. Human Immunodeficiency Virus (HIV) and Menopause Are Independently Associated With Lower Bone Mineral Density: Results From the Women's Interagency HIV Study. Clin Infect Dis **2022**; 75:65–72.

4. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nat Rev Genet **2011**; 12:529–541.

5. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. Aging Cell **2015**; 14:924–932.

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

7. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell **2013**; 49:359–367.

8. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY) **2019**; 11:303–327.

9. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) **2018**; 10:573–591.

10. Lu AT, Seeboth A, Tsai P-C, et al. DNA methylation-based estimator of telomere length. Aging (Albany NY) **2019**; 11:5895–5923.

11. Zhang X, Justice AC, Hu Y, et al. Epigenome-wide differential DNA methylation between HIV-infected and uninfected individuals. Epigenetics **2016**; 11:750–760.

12. Gross AM, Jaeger PA, Kreisberg JF, et al. Methylome-wide Analysis of Chronic HIV Infection Reveals Five-Year Increase in Biological Age and Epigenetic Targeting of HLA. Mol Cell **2016**; 62:157–168.

13. Horvath S, Levine AJ. HIV-1 Infection Accelerates Age According to the Epigenetic Clock. J Infect Dis **2015**; 212:1563–1573.

14. Breen EC, Sehl ME, Shih R, et al. Accelerated aging with HIV begins at the time of initial HIV infection. iScience **2022**; 25:104488.

15. Sehl ME, Breen EC, Shih R, et al. Increased Rate of Epigenetic Aging in Men Living With HIV Prior to Treatment. Front Genet **2021**; 12:796547.

16. Simpkin AJ, Howe LD, Tilling K, et al. The epigenetic clock and physical development during childhood and adolescence: longitudinal analysis from a UK birth cohort. Int J Epidemiol **2017**; 46:549–558.

17. Jintaridth P, Tungtrongchitr R, Preutthipan S, Mutirangura A. Hypomethylation of Alu Elements in Post-Menopausal Women with Osteoporosis. PLoS One **2013**; 8:e70386.

18. Föhr T, Törmäkangas T, Lankila H, et al. The association between epigenetic clocks and physical functioning in older women: a three-year follow-up. J Gerontol A Biol Sci Med Sci **2021**; :glab270.

19. Finkelstein JS, Brockwell SE, Mehta V, et al. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab **2008**; 93:861–868.

20. Lauretani F, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. J Appl Physiol (1985) **2003**; 95:1851–1860.

21. Bandinelli S, Milaneschi Y, Ferrucci L. Chair stands test and survival in the older population. J Am Geriatr Soc **2009**; 57:2172–2173.

22. Terzian AS, Holman S, Nathwani N, et al. Factors associated with preclinical disability and frailty among HIV-infected and HIV-uninfected women in the era of cART. J Womens Health (Larchmt) **2009**; 18:1965–1974.

23. Mansson L, Pettersson B, Rosendahl E, Skelton DA, Lundin-Olsson L, Sandlund M. Feasibility of performance-based and self-reported outcomes in self-managed falls prevention exercise interventions for independent older adults living in the community. BMC Geriatr **2022**; 22:147.

24. Rossiter-Fornoff JE, Wolf SL, Wolfson LI, Buchner DM. A cross-sectional validation study of the FICSIT common data base static balance measures. Frailty and Injuries: Cooperative Studies of Intervention Techniques. J Gerontol A Biol Sci Med Sci **1995**; 50:M291-297.

25. Hurvitz EA, Richardson JK, Werner RA, Ruhl AM, Dixon MR. Unipedal stance testing as an indicator of fall risk among older outpatients. Arch Phys Med Rehabil **2000**; 81:587–591.

26. Duncan PW, Weiner DK, Chandler J, Studenski S. Functional reach: a new clinical measure of balance. J Gerontol **1990**; 45:M192-197.

27. Heiss JA, Just AC. Identifying mislabeled and contaminated DNA methylation microarray data: an extended quality control toolset with examples from GEO. Clinical Epigenetics **2018**; 10:73.

28. Heiss JA, Just AC. Improved filtering of DNA methylation microarray data by detection p values and its impact on downstream analyses. Clinical Epigenetics **2019**; 11:15.

29. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics **2012**; 13:86.

30. Klemera P, Doubal S. A new approach to the concept and computation of biological age. Mech Ageing Dev **2006**; 127:240–248.

31. Chen BH, Marioni RE, Colicino E, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY) **2016**; 8:1844–1865.

32. Vaiserman A, Krasnienkov D. Telomere Length as a Marker of Biological Age: State-of-the-Art, Open Issues, and Future Perspectives. Front Genet **2020**; 11:630186.

33. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res **2015**; 43:e47.

34. Hansen KD. IlluminaHumanMethylationEPICanno.ilm10b2.hg19. 2016; Available at: https://bioconductor.org/packages/IlluminaHumanMethylationEPICanno.ilm10b2.hg19. Accessed 20 April 2022.

35. UNAIDS Global HIV & Aids Statistics (2021). Available at: https://www.unaids.org/en/resources/fact-sheet. Accessed 15 October 2019.

36. Brañas F, Sánchez-Conde M, Carli F, et al. Sex Differences in People Aging With HIV. J Acquir Immune Defic Syndr **2020**; 83:284–291.

37. Simpkin AJ, Cooper R, Howe LD, et al. Are objective measures of physical capability related to accelerated epigenetic age? Findings from a British birth cohort. BMJ Open **2017**; 7:e016708.

38. Marioni RE, Shah S, McRae AF, et al. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. Int J Epidemiol **2015**; 44:1388–1396.

39. Vetter VM, Kalies CH, Sommerer Y, et al. Relationship between five Epigenetic Clocks, Telomere Length and Functional Capacity assessed in Older Adults: Cross-sectional and Longitudinal Analyses. J Gerontol A Biol Sci Med Sci **2022**; :glab381.

40. Fernandez-Rebollo E, Eipel M, Seefried L, et al. Primary Osteoporosis Is Not Reflected by Disease-Specific DNA Methylation or Accelerated Epigenetic Age in Blood. J Bone Miner Res **2018**; 33:356–361.

41. Shiau S, Strehlau R, Wang S, et al. Distinct epigenetic profiles in children with perinatally-acquired HIV on antiretroviral therapy. Scientific Reports **2019**; 9:10495.

42. Delgado-Calle J, Fernández AF, Sainz J, et al. Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. Arthritis Rheum **2013**; 65:197–205.

43. Morris JA, Tsai P-C, Joehanes R, et al. Epigenome-wide Association of DNA Methylation in Whole Blood With Bone Mineral Density. J Bone Miner Res **2017**; 32:1644–1650.

44. Estrada K, Styrkarsdottir U, Evangelou E, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet **2012**; 44:491–501.

45. Zheng H-F, Forgetta V, Hsu Y-H, et al. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. Nature **2015**; 526:112–117.

46. Yin MT, Brown TT. HIV and Bone Complications: Understudied Populations and New Management Strategies. Curr HIV/AIDS Rep **2016**; 13:349–358.

47. Cohen A. Premenopausal Osteoporosis. Endocrinol Metab Clin North Am **2017**; 46:117–133.

48. Compston J. Osteoporosis: advances in risk assessment and management. Clin Med (Lond) **2016**; 16:s121–s124.

49. Soerensen M, Li W, Debrabant B, et al. Epigenome-wide exploratory study of monozygotic twins suggests differentially methylated regions to associate with hand grip strength. Biogerontology **2019**; 20:627–647.

50. Beach SRH, Dogan MV, Lei M-K, et al. Methylomic Aging as a Window onto the Influence of Lifestyle: Tobacco and Alcohol Use Alter the Rate of Biological Aging. J Am Geriatr Soc **2015**; 63:2519–2525.

Figure Legends

Figure 1: Box plots of A) EAA, B) EEAA, C) IEAA, D) GrimAge, E) PhenoAge, and F) DNAmTL in women with HIV (HIV+, N=118) and women without HIV (HIV-, N=72). Comparisons between groups performed using t-tests. P-value <0.05 is indicated by asterisks (***). Abbreviations: DNAm-TL, DNAm-based telomere length; EAA, epigenetic age acceleration; EEAA, extrinsic epigenetic age acceleration; HIV, human immunodeficiency virus; IEAA, intrinsic epigenetic age acceleration.