

**Grip strength is inversely associated with DNA methylation age acceleration.**

Mark D. Peterson, Ph.D., M.S.,<sup>1-3</sup> Stacey Collins, M.A.,<sup>4</sup> Helen C.S. Meier, Ph.D., M.P.H.,<sup>3,4</sup>

Alexander Brahmsteadt, M.D.,<sup>1</sup> Jessica D.Faul, Ph.D., M.P.H.<sup>3,4</sup>

<sup>1</sup>Department of Physical Medicine & Rehabilitation, University of Michigan

<sup>2</sup>Michigan Institute for Health Policy and Innovation (IHPI)

<sup>3</sup>Michigan Center on the Demography of Aging (MiCDA)

<sup>4</sup>Survey Research Center, Institute for Social Research, University of Michigan

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Corresponding Author:

Mark D. Peterson, Ph.D., M.S., FACSM

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Associate Professor  
Department of Physical Medicine and Rehabilitation  
University of Michigan-Medicine  
325 E. Eisenhower Parkway, Suite 2  
Ann Arbor, Michigan 48108  
Email: [mdpeterz@med.umich.edu](mailto:mdpeterz@med.umich.edu)

## Abstract

**Background:** There is a large body of evidence linking muscular weakness, as determined by low grip strength, to a host of negative aging-related health outcomes. Given these links, grip strength has been labeled a “biomarker of aging”; and yet, the pathways connecting grip strength to negative health consequences is unclear. The objective of this study was to determine whether grip strength was associated with measures of DNA methylation (DNAm) age acceleration.

**Methods:** Middle age and older adults from the 2006-2008 waves of the Health and Retirement Study with 8-10 years of follow-up were included. Cross sectional and longitudinal regression modeling was performed to examine the association between normalized grip strength (NGS) and three measures of DNAm age acceleration, adjusting for cell composition, sociodemographic variables, and smoking. Longitudinal modeling was also completed to examine the association between change in absolute grip strength and DNAm age acceleration. The three DNAm clocks used for estimating age acceleration including the established DunedinPoAm, PhenoAge, and GrimAge clocks.

**Results:** There was a robust and independent cross-sectional association between NGS and DNAm age acceleration for men using the DunedinPoAm ( $\beta$ : -0.36;  $p < 0.001$ ) PhenoAge ( $\beta$ : -8.27;  $p = 0.01$ ) and GrimAge ( $\beta$ : -4.56;  $p = 0.01$ ) clocks, and for women using the DunedinPoAm ( $\beta$ : -0.36;  $p < 0.001$ ) and GrimAge ( $\beta$ : -4.46;  $p = 0.01$ ) clocks. There was also an independent longitudinal association between baseline NGS and DNAm age acceleration for men ( $\beta$ : -0.26;  $p < 0.001$ ) and women ( $\beta$ : -0.36;  $p < 0.001$ ) using the DunedinPoAm clock, and for women only using the PhenoAge ( $\beta$ : -8.20;  $p < 0.001$ ) and GrimAge ( $\beta$ : -5.91;  $p < 0.001$ ) clocks. Longitudinal modeling revealed a robust association between change in grip strength from wave 1 to wave 3 was independently associated with PhenoAgeAA ( $\beta$ : -0.13; 95% CI: -0.23, -0.03) and GrimAgeAA ( $\beta$ : -0.07; 95% CI: -0.14, -0.01) in men only (both  $p < 0.05$ ).

**Conclusions:** Our findings provide some initial evidence of age acceleration among men and women with lower NGS and loss of strength over time. Future research is needed to understand the extent to which DNAm age mediates the association between grip strength and chronic disease, disability, and mortality.

**Key words:** grip strength; DNA methylation; strength training; aging

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## Introduction

There is a large body of evidence linking muscular weakness, as determined by low grip strength, to a host of negative aging-related health outcomes including diabetes,<sup>1,2</sup> physical disability,<sup>3-5</sup> cognitive decline (including Alzheimer's disease<sup>6</sup>),<sup>7,8</sup> and early all-cause mortality.<sup>9-12</sup> Given these findings, grip strength has been labeled a “biomarker of aging”<sup>13</sup>; and yet, this crude metric provides no direct biological plausibility or mechanism linking weakness with chronic disease, physical and cognitive impairments, or specific causes of mortality.

Aging is indeed a complex, multidimensional phenomenon that manifests differently between individuals throughout the lifespan, and is highly conditional on interactions between genetic, environmental, behavioral, and demographic characteristics.<sup>14</sup> The prevailing context for aging research has been backward-looking, taking into consideration only the chronological age in years since birth.<sup>15</sup> However, there is a well-established literature demonstrating that certain subgroups of the population age at different rates,<sup>16,17</sup> and thus better understanding these subgroups, and the underlying mechanisms contributing to accelerated aging phenotypes could lead to improved early detection systems, development of targeted interventions, and ultimately a decrease in population disability-adjusted life years. A growth in research evidence documents that epigenetic phenomena, such as DNA methylation (DNAm), are highly implicated in the development of disease<sup>18</sup> and rate of biological aging. Given that methylation profiles are thought to be modifiable by lifestyle and other environmental factors, it has been proposed that DNAm age is a robust biological aging clock-providing a superior estimate of true biological age

over chronological age.<sup>19, 20</sup> A number of epigenetic clock measures have been generated for measuring an individual's epigenetic age, and in particular, to indicate accelerated or decelerated biological aging (i.e., as compared to chronological age).<sup>21, 22</sup> The objective of this study was to determine the association between grip strength and DNAm age acceleration derived from three epigenetic clocks trained on phenotypic aging (i.e., age-related health conditions/profiles) and mortality.

## **Methods**

### Cohort

We used a longitudinal panel study (Health and Retirement Study [HRS]) to determine the cross-sectional and longitudinal association between muscle strength DNAm age acceleration in middle age and older adults. The HRS is a prospective epidemiological resource gathering extensive questionnaires, physical and cognitive measures, and biological samples in a cohort of more than 43,000 participants. HRS is a multistage area probability survey of non-institutionalized, community dwelling Americans aged 51 years and older. Study details have been previously described, and all questionnaires are available on the HRS website (<http://hrsonline.isr.umich.edu>). Briefly, the HRS is the longest running longitudinal study of older adults in the United States, with consistently high response rates. The HRS follows respondents longitudinally until death, and new cohorts have been enrolled since the 1992 baseline interviews, in order to maintain the population representativeness of the study sample. Starting in 2006, a random one-half sample of HRS participants was selected for an enhanced

face-to-face interview that included physical and biological measurements (e.g., hand grip strength, blood, and saliva collection), and the other half completed only the core interview. The other random one-half completed the enhanced face-to-face interview that included physical and biological measurements in 2008. These half-sample alternate waves are done so that longitudinal information from the enhanced face-to-face interview is available every 4 years at the individual level, and the expanded content is available every wave on a nationally representative half-sample of the longitudinal panel. This rotating design has continued in all biennial HRS waves since 2006, resulting in follow-up data on the physical, biomarker, and cognitive health measures every 4 years for the subsamples. Our initial study sample of  $n=14,781$  included all HRS participants aged 51 or older (70.0 years) interviewed in 2006/2008, when physical measure and biomarker collection began in the HRS, and with an 8-year follow-up assessment in 2014/2016. The final cohort of  $n=1,275$  reflect only participants with full data on all exposures, covariates, and the three dependent DNAm age acceleration clocks, only available on a subsample of the 2016 respondents. The age, sex and racial breakdown of our sample is consistent with that of Americans aged 50 years and older in the United States, based on U.S. census data.<sup>23</sup>

Exposures:

*Grip strength:* In HRS, hand grip strength was assessed using a Smedley spring-type hand dynamometer (Scandidact, Odder, Denmark). Participants were instructed to squeeze the device with maximal effort and then let go. Grip strength assessments were administered while

participants were standing with their arm at their side, and with the elbow flexed at a 90-degree angle. After one practice trial, two measurements were taken with each hand, alternating hands. The maximum measurement from the four trials were considered maximal grip strength capacity. Normalized grip strength (NGS) as grip strength per body mass (i.e.,  $\left(\frac{\text{Grip Strength (kg)}}{\text{Body Mass (kg)}}\right)$ ) was examined as a continuous predictor.

### *The HRS Methylation Sample*

DNA methylation assays were done on a nonrandom subsample (N = 4018) of people who participated in the 2016 Health and Retirement Venous Blood Study (VBS),<sup>24</sup> as described thoroughly in HRS documentation (<https://hrsonline.isr.umich.edu/modules/meta/vbs/2016/desc/HRS2016VBSDD.pdf>).<sup>21</sup>

### *Methods for DNAm*

DNA methylation data are based on assays using the Infinium Methylation EPIC BeadChip completed at the Advanced Research and Diagnostics Laboratory at the University of Minnesota. Samples were randomized across plates by key demographic variables (i.e., age, cohort, sex, education, race/ethnicity) with 39 pairs of blinded duplicates, as previously described thoroughly in HRS documentation ([https://hrsdata.isr.umich.edu/sites/default/files/documentation/data-descriptions/EPICLOCKS\\_DD.pdf](https://hrsdata.isr.umich.edu/sites/default/files/documentation/data-descriptions/EPICLOCKS_DD.pdf)).<sup>25</sup>

### *Subsample Weights*

Respondents with at least one valid venous blood result were assigned a VBS weight. The weights were adjusted for the differential probabilities of participation by dividing the HRS 2016 sample weight by the predicted probability of having a valid venous blood result among community-dwelling 2016 HRS respondents born prior to 1960. A separate respondent level weight was created for the subsample of respondents in the DNAm sample to adjust for selection and was used for analyses of data from that sample (VBSI16WGTRA).<sup>25</sup> The weighted sample represents community-dwelling adults 56 years and over in the US.

### DNAm Measures

Thirteen epigenetic clocks have been estimated from the HRS data. Nine of these clocks are “first generation” clocks trained on chronological age, and four are “second generation” clocks trained on health-related outcomes (Zhang, PhenoAge, GrimAge, DunedinPoAm). Of these four, we chose three (PhenoAge, GrimAge, DunedinPoAm). PhenoAge and GrimAge were regressed on age to obtain DNAm “accelerated age” estimates, whereas DunedinPoAm was originally created as a pace-of-aging clock.

*PhenoAge* was developed by Morgen Levine using composite clinical biomarkers combined into a multi-system measure of biological age, called phenotypic age, which was developed to estimate an individual’s mortality risk using 9 markers of tissue and immune function (albumin, creatinine, serum glucose, CRP, lymphocyte percent, mean (red) cell volume, red cell distribution width, alkaline phosphatase, white blood cell count) and age.<sup>25</sup> Phenotypic age was predicted by PhenoAge based on 513 CpGs in whole blood from the same sample.



Although this clock was developed using whole blood data, values from all tested tissues and cells are correlated with age and predict mortality better than chronological age-based clocks.<sup>26</sup> PhenoAge has been shown to predict multiple aging outcomes such as mortality, cancer, lifespan, physical function and Alzheimer's disease. Moreover, PhenoAge acceleration (PhenoAgeAA) is related to biomarkers such as high CRP, glucose, triglycerides waist-to-hip ratio and low HDL cholesterol.<sup>26</sup>

*GrimAge* was constructed by HRS staff member Jonah Fisher with assistance of Steve Horvath, and was developed based on the 7 DNAm surrogates of plasma proteins and smoking pack years in a two-stage procedure.<sup>25,27</sup> First, surrogate DNAm biomarkers of physiological risk and stress factors were defined with plasma proteins (adrenomedullin, CRP, plasminogen activation inhibitor 1 (PAI-1) and growth differentiation factor 15 (GDF15)) and DNAm-based estimator of smoking pack-years. Then, time-to-death was regressed on these biomarkers and an estimator of smoking years to estimate a composite biomarker of lifespan-GrimAge. High values of this measure (GrimAgeAA) are associated with morbidity and mortality risk.<sup>27</sup>

*DunedinPoAm* was developed to represent individual variation in the pace of biological aging. Based on data from the Illumina 450k Array run on samples from the Dunedin cohort, estimates were derived by using elastic-net regression models to calculate a methylation Pace of Aging (mPoA) score.<sup>28</sup> The pace of aging was calculated with composited slopes across the 18 biomarkers that measure the rate of aging in the cardiovascular, metabolic, renal, hepatic, pulmonary, periodontal, and immune systems. Then, the pace of aging composite was scaled to

represent the mean trend in the cohort among Dunedin Study members with methylation data at age 38. The mPoA algorithm was trained on 3 waves of biomarker data from participants, including data collected at ages 26, 32, and 38. DunedinPoAm is estimated in years per chronological year (years/chronological year).<sup>25</sup>

Covariates:

The following sociodemographic measures were included in the primary analytic approach as independent covariates: age, self-reported race/ethnicity (White, Black, Hispanic, other), marital status, education (<high school graduate, high school graduate, some college, college graduate, and  $\geq$ college graduate), household wealth, history of smoking (never vs. ever smoked), and cell composition as described.<sup>29</sup>

Analyses

Descriptive statistics were used to explore the distribution, central tendency, and variation of each baseline NGS measurement, with an emphasis on graphical methods such as histograms, scatterplots, and boxplots. Weighted multivariate linear regression analyses were conducted, using the SAS surveyreg procedure, to determine the unadjusted and adjusted association between NGS and DNAm age acceleration for each clock. First, a cross-sectional analysis was performed to examine the association between NGS and DNAm age acceleration across all clocks in the same discrete time period (2016). Thereafter, we explored a retrospective longitudinal association between baseline (“wave 1”) NGS (2006/2008) and DNAm age acceleration across all clocks at 8-years follow-up (“wave 3”). Finally, to examine if change in

strength was associated with DNAm age acceleration, longitudinal modeling was completed to examine the association between changes in absolute grip strength from wave 1 to wave 3, and DNAm age acceleration for each clock at wave 3. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC), and for all analyses,  $p < 0.05$  was considered statistically significant.

## Results

**Descriptive** and health data are presented as weighted means, standard errors, and percentages for men and women in **Tables 1 and 2 for the wave 3 full study population and final sample subset, respectively**. The average age of study participants was 70 years. Approximately 45.1% of men and 44.3% of women were classified as obese (body mass index  $>30\text{kg}/\text{m}^2$ ). On average men had a stronger grip strength relative to body mass than women (0.44kg v. 0.33kg, respectively). The majority of participants were Non-Hispanic White and reported being married and having some college or greater educational attainment. The distribution of household wealth was similar in men and women.

### Cross-Sectional Analyses

For the unadjusted cross-sectional analyses, greater grip strength was inversely associated with DNAm age acceleration. Specifically, for men there was a statistically significant association between NGS and DunedinPoAm, PhenoAgeAA, and GrimAgeAA (all  $p < 0.01$ ). For women, there was a statistically significant association between NGS and DunedinPoAm only. Moreover, in models adjusted for age, race, education, marital status, income, cell composition

and smoking, there was an independent cross-sectional association between NGS and DNAm age acceleration for men and women using the DunedinPoAm clock, for men only using the PhenoAgeAA and for men and women using the GrimAgeAA clock (**Table 3**).

### Longitudinal Analyses

Greater grip strength was inversely associated with DNAm age acceleration in longitudinal models. Specifically, for men there was a statistically significant longitudinal association between wave 1 NGS and wave 3 DunedinPoAm and GrimAgeAA (all  $p < 0.01$ ). For women, there was a statistically significant association between wave 1 NGS and wave 3 DunedinPoAm, PhenoAgeAA, and GrimAgeAA. Moreover, in models adjusted for age, race, education, marital status, income, cell composition and smoking, there was an independent longitudinal association between wave 1 NGS and wave 3 DNAm age acceleration for men and women using the DunedinPoAm clock, and for women only using the PhenoAgeAA and GrimAgeAA clocks (**Table 4**).

Finally, change in grip strength from wave 1 to wave 3 was independently associated with PhenoAgeAA ( $\beta$ : -0.13; 95% CI: -0.23, -0.03) and GrimAgeAA ( $\beta$ : -0.07; 95% CI: -0.14, -0.01) in men only (both  $p < 0.05$ ) (**Model 5: Supplemental Table e1**).

### **Discussion**

The principal finding of the current study are that among middle aged and older men and women, lower NGS was associated with DNAm age acceleration across several clocks. These results suggest the DNAm clocks examined in this study represent clinically relevant biomarkers

of age acceleration. Perhaps more importantly, our findings represent the first evidence and largest study to date demonstrating that greater NGS may be protective against DNAm age acceleration (see: conceptual model **Figure 1**). The limited literature on DNAm age acceleration and functional assessments of aging is mixed.<sup>30, 31</sup> One study in a German cohort found that accumulating deficits (comprehensive frailty) was associated with increasing age acceleration derived from the Horvath DNAm clock,<sup>31</sup> which used DNA from multiple cell and tissue types and trained on chronological age.<sup>32</sup> However, another study in an older German cohort did not find an association between measures of intrinsic and extrinsic<sup>33</sup> epigenetic age acceleration and frailty or functional assessments.<sup>34</sup> Most recently, phenotypic and mortality trained epigenetic clocks were found to be associated with cross-sectional and 3-year change in frailty outcomes in the Canadian Longitudinal Study on Aging (CLSA).<sup>31</sup> The heterogeneity of findings observed in these studies is likely due to employing DNAm age acceleration measures derived from different clocks. Our results, which are consistent with the CLSA, suggest that thoughtful selection of DNAm clocks to be included in future analyses of age-related functional decline or frailty is warranted. DNAm clocks designed to represent phenotypic aging and mortality are better suited for understanding the association between grip strength and health-related outcomes.

Our findings further revealed that associations between NGS and DNAm age acceleration clocks were not consistent across sex categories in the fully adjusted models. In the cross-sectional analysis, stronger NGS was inversely associated with GrimAgeAA and DunedinPoAmAA in both men and women, but only PhenoAA in men. In our longitudinal

models, only DunedinPoAmAA was statistically significantly associated with NGS in both men and women, whereas GrimAgeAA and PhenoAA were both statistically significant in women. This suggests that future studies should include sex-stratified models to inform potential differences in aging indicators by sex.

As a biological marker of aging, the specific mechanisms underlying observed correlations between lower grip strength and negative health outcomes remain unclear. DNAm patterns may be one pathophysiological pathway by which grip strength and morbidity are related. There is also a growing consensus that the low-grade chronic inflammation in aging (“inflammaging”) is a highly significant risk factor for both morbidity and mortality in elderly people,<sup>35</sup> and may represent a strong mechanism linking age-related increases in adiposity and metabolic dysregulation with sarcopenia and muscle weakness.<sup>36</sup> Indeed, we have previously shown that chronic inflammation was associated with *lower* NGS in both men and women, and also emerged as a significant causal mediator between lower NGS and both incident disability and chronic disease multimorbidity.<sup>5</sup> We have also demonstrated that muscle weakness and testosterone deficiency were highly correlated, and independently associated with multimorbidity in young and older men.<sup>37</sup> Moreover, the largest study to date to examine the genomewide association studies of grip strength revealed that a genetic variant in a chromosomal region that regulates myotube differentiation and muscle repair may contribute to variability in grip strength in the elderly.<sup>38</sup> The extent to which immune, endocrine, and genetic mechanisms combine to

contribute to age-related weakness, DNAm age acceleration, chronic disease, and cause-specific mortality is an important future research directive.

However, muscle weakness and DNAm age acceleration may also just simply represent proxies of aging, resulting in their correlation. Conventional aging research ignores changes in life-expectancy at the population level, actual life expectancy at the individual level, and characteristics of people throughout time, and has led to an incomplete understanding of the factors that influence altered aging trajectories. For example, why is someone aged 50 years considered to be “middle-aged” in today’s society, whereas 150 years ago a person of the same age would be considered elderly? It has been conjectured that certain lifestyles (e.g., regular physical activity, healthy dietary habits) may produce these effects by altering typical aging trajectories of DNAm that underwrite health and functional status into older adulthood. Recently, a conceptual model was proposed for the sequence of events and outcomes that surround the role of muscle weakness in the disease and disabling processes.<sup>37</sup> However, future studies are certainly needed that provide specific evidence for how muscle strength and clinically relevant health outcomes are mediated or moderated by other factors, as well as specific hypothesized connections between grip strength and DNAm age acceleration to elucidate potential biological mechanisms. Future research is also needed to examine a potential causal mediation pathway between grip strength (exposure), physical activity/nutritional status (mediators), and accelerated/decelerated DNAm age.

Regardless, grip strength is a clinically relevant biomarker of aging and global DNAm is relatively stable over time.<sup>39</sup> Thus, the potential exists for combining measures of grip strength and DNAm age acceleration to screen individuals for future risk of chronic disease and functional decline before it occurs. Doing so would allow for the design of interventions to delay or prevent their onset and progression. While this is an exciting possibility, extensive research of longitudinal DNAm age acceleration measurements must be undertaken, as well as additional replication of our findings in other US and international cohorts.

We acknowledge that our study has several limitations. First, we had DNAm at only a single time point (wave 3). Future studies are needed to examine earlier onset deviations in DNAm acceleration/deceleration and factors that contribute to these alterations. Secondly, we cannot rule out time-varying confounding since baseline measurements of all covariates were included in our final models. Thus, whether declines in NGS “cause” a risk for DNAm age acceleration, or if incident disease processes (e.g., diabetes) themselves, are a cause of diminished muscle function and altered biological age is an interesting topic. We were also unable to determine if other competing risks or unmeasured confounding (i.e., other risk factors [e.g., lack of physical activity] or existing diseases [e.g., cancer]) may have influenced the observed findings. We further recognize the potential bias introduced by losing a substantial number of the participants when modeling for the independent association between NGS and prevalent/longitudinal DNAm age acceleration. Future efforts are needed to validate and extend these findings in other/larger cohorts to improve our understanding of the underlying



mechanisms linking weakness and accelerated biological aging. Lastly, a limitation in this study was not using any biomarkers of pathophysiology. For example, there are multiple markers of chronic inflammation and oxidative stress, including TNF- $\alpha$ , IL-6, IL-12, IL-1 $\beta$ , and reactive oxygen species (ROS). Future studies could examine multiple markers simultaneously, as chronic inflammation and oxidative stress are both highly dependent on complex signaling and lead to age-related diseases.

Despite these limitations, this study has various strengths. Most notably, we used grip strength as our primary exposure, which is a cost-effective, reliable proxy for total body muscle strength that can be easily administered in the clinical or community settings, and across populations of children, adolescents, and adults,<sup>11, 40-42</sup> Moreover, the results of this study are generalizable to community-dwelling older Americans since the sample used in this study is from the longest running, longitudinal nationally- representative cohort of older adults in the United States. Our analysis is superior to the results from the recently published findings from the CLSA, because we stratified by sex and examined findings at a longer time horizon (i.e., 8 years instead of 3 years follow-up). Lastly and most importantly, our findings demonstrating that lower NGS was associated with DNAm age acceleration across several clocks, were independent of smoking status-a known risk factor for negative health outcomes and strong predictor of DNAm patterns.

**Conclusion:**

Our findings provide some initial evidence of age acceleration among men and women with lower NGS and loss of strength over time. Preservation of muscle strength may positively influence healthy aging by protecting against DNAm age acceleration. Future research is needed to understand the extent to which DNAm age mediates the association between grip strength and chronic disease, disability, and early mortality, as well as the extent to which lifestyle/behavioral factors (e.g., physical activity and dietary status) can mediate the association between grip strength and accelerated/decelerated DNAm age.

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**Table 1. Demographic and health characteristics of the weighted, cross-sectional full study population by sex at wave 3 (2014/2016)**

	<b>Men</b>	<b>Women</b>
	<i>n</i> =5,888	<i>n</i> =8,893
<b>Age, years</b>	<b>70.3 (0.23)</b>	<b>69.6 (0.22)</b>
<b>Body Mass Index (BMI), kg/m<sup>2</sup></b>	<b>28.9 (0.16)</b>	<b>28.6 (0.16)</b>
<b>Obesity (BMI &gt;30), %</b>	<b>45.1</b>	<b>42.3</b>
<b>Level of Education</b>		
<b>Less than H.S., %</b>	<b>12.5</b>	<b>14.6</b>
<b>High School Graduate, %</b>	<b>30.8</b>	<b>32.8</b>
<b>Some College, %</b>	<b>21.7</b>	<b>26.8</b>
<b>College Graduate, %</b>	<b>35.0</b>	<b>25.7</b>
<b>Married, %</b>	<b>70.3</b>	<b>55.7</b>
<b>Race</b>		
<b>White, %</b>	<b>89.3</b>	<b>85.8</b>
<b>Black, %</b>	<b>6.9</b>	<b>7.9</b>
<b>Unknown/Other, %</b>	<b>3.8</b>	<b>6.4</b>
<b>Ethnicity</b>		
<b>Hispanic, %</b>	<b>6.7</b>	<b>8.7</b>
<b>Net Worth</b>		
<b>Quartile 1, %</b>	<b>16.4</b>	<b>20.1</b>
<b>Quartile 2, %</b>	<b>21.0</b>	<b>21.0</b>
<b>Quartile 3, %</b>	<b>25.7</b>	<b>22.6</b>
<b>Quartile 4, %</b>	<b>37.0</b>	<b>36.4</b>
<b>NGS (relative to body mass)</b>	<b>0.44 (0.003)</b>	<b>0.33 (0.002)</b>



**Table 2. Demographic and health characteristics of the weighted, cross-sectional final subset population by sex at wave 3**

	Men	Women
	<i>n</i> =528	<i>n</i> =746
<b>Age, years</b>	<b>69.0 (0.32)</b>	<b>69.6 (0.33)</b>
<b>Body Mass Index (BMI), kg/m<sup>2</sup></b>	<b>29.0 (0.24)</b>	<b>28.6 (0.24)</b>
<b>Obesity (BMI &gt;30), %</b>	<b>45.9</b>	<b>43.7</b>
<b>Level of Education</b>		
<b>Less than H.S., %</b>	<b>11.0</b>	<b>13.8</b>
<b>High School Graduate, %</b>	<b>27.9</b>	<b>33.9</b>
<b>Some College, %</b>	<b>23.0</b>	<b>27.1</b>
<b>College Graduate, %</b>	<b>38.2</b>	<b>25.2</b>
<b>Married, %</b>		
<b>Race</b>		
<b>White, %</b>	<b>88.9</b>	<b>84.1</b>
<b>Black, %</b>	<b>8.7</b>	<b>9.1</b>
<b>Unknown/Other, %</b>	<b>2.4</b>	<b>6.8</b>
<b>Ethnicity</b>		
<b>Hispanic, %</b>	<b>7.4</b>	<b>8.6</b>
<b>Net Worth</b>		
<b>Quartile 1, %</b>	<b>19.8</b>	<b>23.8</b>
<b>Quartile 2, %</b>	<b>24.5</b>	<b>24.0</b>
<b>Quartile 3, %</b>	<b>26.7</b>	<b>24.0</b>
<b>Quartile 4, %</b>	<b>29.1</b>	<b>28.2</b>
<b>NGS (relative to body mass)</b>	<b>0.44 (0.005)</b>	<b>0.34 (0.003)</b>





**Table 3. Cross-sectional regression models were completed stepwise for each DNAm age acceleration clock to examine the effects of incremental adjustment on the exposure variable (NGS).**

	<i>Model 1</i> $\beta$ (95% CI)	<i>Model 2</i> $\beta$ (95% CI)	<i>Model 3</i> $\beta$ (95% CI)	<i>Model 4</i> $\beta$ (95% CI)
<b>Men</b>				
<i>PhenoAgeAA</i>	-7.95 (-13.92, -1.98)*	-9.71 (-15.84, -3.57)*	-9.16 (-15.26, -3.05)*	-8.27 (-14.25, -2.29)*
<i>GrimAgeAA</i>	-5.52 (-9.56, -1.48)*	-2.89 (-6.93, 1.15)	-2.69 (-6.79, 1.41)	-4.56 (-8.45, -0.68)*
<i>DunedinPoAm</i>	-0.41 (-0.55, -0.28)*	-0.34 (-0.49, -0.19)*	-0.34 (-0.49, -0.19)*	-0.36 (-0.51, -0.21)*
<b>Women</b>				
<i>PhenoAgeAA</i>	-6.50 (-13.39, 0.39)	-4.55 (-11.86, 2.75)	-4.26 (-11.56, 3.05)	-4.96 (-12.45, 2.53)
<i>GrimAgeAA</i>	-5.98 (-9.99, 1.57)	-2.54 (-6.43, 1.35)	-2.62 (-6.43, 1.18)	-4.46 (-7.81, -1.11)*
<i>DunedinPoAm</i>	-0.45 (-0.60, -0.29)*	-0.34 (-0.48, -0.19)*	-0.33 (-0.48, -0.19)*	-0.36 (-0.50, -0.22)*

Model 1: Unadjusted

Model 2: Model 1 + Demographic variables (age, race, education, marital status, income).

Model 3: Model 1 + Model 2 + Cell Composition

Model 4: Model 1 + Model 2 + Model 3 + Smoking

\*P-value < 0.05

**Table 4. Longitudinal regression models were completed stepwise to examine the effects of incremental adjustment on the exposure variable (NGS) at baseline, to 8-year DNAm age acceleration.**

	<i>Model 1</i> $\beta$ (95% CI)	<i>Model 2</i> $\beta$ (95% CI)	<i>Model 3</i> $\beta$ (95% CI)	<i>Model 4</i> $\beta$ (95% CI)
<b>Men</b>				
<i>PhenoAgeAA</i>	-3.59 (-8.14, 0.96)	-2.94 (-7.99, 2.10)	-3.15 (-8.22, 1.92)	-3.17 (-8.24, 1.90)
<i>GrimAgeAA</i>	-4.48 (-7.64, -1.32)*	-1.81 (-4.92, 1.30)	-1.68 (-4.84, 1.47)	-2.28 (-5.20, 0.63)
<i>DunedinPoAm</i>	-0.32 (-0.42, -0.22)*	-0.26 (-0.37, -0.15)*	-0.26 (-0.36, -0.15)*	-0.26 (-0.37, -0.16)*
<b>Women</b>				
<i>PhenoAgeAA</i>	-9.00 (-13.76, -4.24)*	-7.92 (-12.63, -3.21)*	-7.73 (-12.39, -3.08)*	-8.20 (-12.73, -3.31)*
<i>GrimAgeAA</i>	-7.29 (-10.13, -4.46)*	-5.10 (-8.07, -2.14)*	-4.91 (-7.84, -1.98)*	-5.91 (-8.55, -3.26)*
<i>DunedinPoAm</i>	-0.43 (-0.55, -0.31)*	-0.36 (-0.48, -0.24)*	-0.35 (-0.47, -0.23)*	-0.36 (-0.48, -0.24)*

Model 1: Unadjusted

Model 2: Model 1 + Demographic variables (age, race, education, marital status, income).

Model 3: Model 1 + Model 2 + Cell Composition

Model 4: Model 1 + Model 2 + Model 3 + Smoking

\*P-value < 0.05

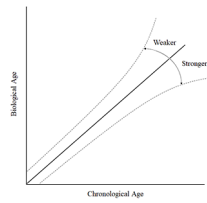
## Figure Legend

**Figure 1: Conceptual model demonstrating the deviations in DNAm age (“Biological Age”) from chronological age associated with weaker or stronger NGS.**

**Supplemental Files**

**Supplemental Table e1. Model 5: Association between changes in absolute grip strength from wave 1 to wave 3, and DNAm age acceleration for each clock at wave 3.**

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