Quantification of the pace of biological aging in humans through a blood test: a DNA methylation algorithm

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Abstract (199 of 200 words)

Biological aging is the gradual and progressive decline in system integrity that occurs with advancing chronological age, causing morbidity and disability. Measurements of the rate of biological aging are needed to serve as surrogate endpoints in trials of therapies designed to prevent disease by slowing biological aging to extend healthspan. We report a blood DNA-methylation measure that is sensitive to variation in the pace of biological aging among individuals born in the same year. We first modeled longitudinal change in a panel of 18 biomarkers tracking organ-system integrity across 12 years of follow-up in the Dunedin birth cohort. Rates of change across these biomarkers were composited to form a measure of aging-related decline in system integrity, termed Pace of Aging. We then used elastic-net regression to develop a DNA-methylation predictor of Pace of Aging, called mPoA for (m)ethylation (P)ace (o)f (A)ging. Validation analyses showed mPoA was associated with functional decline in the Dunedin Study, complemented information from other methylation clocks in the Understanding Society Study, was accelerated by early-life adversity in the E-risk Study, and was disrupted by caloric restriction in the CALERIE trial. Findings provide proof-of-principle for mPoA as a single-time-point measure of a person's pace of biological aging.

INTRODUCTION

Aging of the global population is producing forecasts of rising burden of disease and disability (Harper, 2014). Because this burden arises from multiple age-related diseases, treatments for single diseases will not address the burden challenge (Goldman et al., 2013). Geroscience research suggests an appealing alternative: treatments to slow aging itself could prevent or delay the multiple diseases that increase with advancing age, perhaps with a single therapeutic approach (Gladyshev, 2016; Kaeberlein, 2013). Aging can be understood as a gradual and progressive deterioration in biological system integrity (Kirkwood, 2005). This deterioration is thought to arise from an accumulation of cellular-level changes. These changes, in turn, increase vulnerability to diseases affecting many different organ systems (Kennedy et al., 2014; López-Otín et al., 2013). Animal studies suggest treatments that slow the accumulation of cellular-level changes can extend healthy lifespan (Campisi et al., 2019; Kaeberlein et al., 2015). However, human trials of these treatments are challenging because humans live much longer than model animals, making it time-consuming and costly to follow up human trial participants to test treatment effects on healthy lifespan. This challenge will be exacerbated in trials that will give treatments to young or middle-aged adults, with the aim to prevent the decline in system integrity that antedates disease onset by years. Involving young and midlife adults in healthspan-extension trials has been approved for development by the National Advisory Council on Aging (2019 CTAP report to NACA). In midlife trials of anti-aging treatments, traditional endpoints such as disease diagnosis or death are too far in the future to serve as outcomes. Translation of anti-aging treatments to humans could be aided by measures that quantify the pace of deterioration in biological system integrity in human aging. Such measures could be used as surrogate endpoints for healthy lifespan extension (Justice et al., 2016, 2018; Moskalev et al., 2016), even with young-to-midlife adult trial participants. A useful measure should be non-invasive, inexpensive, reliable, and highly sensitive to biological change.

Recent efforts to develop such measures have focused on blood DNA methylation as a biological substrate highly sensitive to changes in chronological age (Horvath and Raj, 2018). Methylation-clock algorithms have been developed to identify methylation patterns that

characterize individuals of different chronological ages. However, a limitation is that individuals born in different years have grown up under different historical conditions (Schaie, 1967). For example, people born 70 years ago experienced more exposure to childhood diseases, tobacco smoke, airborne lead, and less exposure to antibiotics and other medications, and lower quality nutrition, all of which leave signatures on DNA methylation (Bell et al., 2019). As a result, the clocks confound methylation patterns arising from early-life exposures to methylation-altering factors with methylation patterns related to biological aging during adulthood. An alternative approach is to study individuals who were all born the same year, and find methylation patterns that differentiate those who have been aging biologically faster or slower than their same-age peers. The current article reports four steps in our work toward developing a blood DNA methylation measure to represent individual variation in the pace of biological aging.

In Step 1, which we previously reported (Belsky et al., 2015), we collected a panel of 18 blood chemistry and organ-system function biomarkers at three successive waves of the Dunedin Longitudinal Study of a 1972-73 population-representative one-year birth cohort (N=1037). We used repeated-measures data collected when Study members were aged 26, 32, and 38 years old to quantify rates of biological change. We modelled the rate of change in each biomarker and calculated how each Study member's personal rate-of-change on that biomarker differed from the cohort norm. We then combined the 18 personal rates of change across the panel of biomarkers to compute a composite for each Study member that we called the Pace of Aging. Pace of Aging represents a personal rate of multi-system decline over a dozen years. Pace of Aging was normally distributed, and showed marked variation among Study members who were all the same chronological age, confirming that individual differences in biological aging do emerge already by age 38, years before chronic disease onset.

In Step 2, which we previously reported, we validated the Pace of Aging against known criteria. As compared to other Study members who were the same chronological age but had slower Pace of Aging, Study members with faster Pace of Aging performed more poorly on tests of physical function; showed signs of cognitive decline on a panel of dementia-relevant neuropsychological tests from an early-life baseline; were rated as looking older based on facial photographs; and reported themselves to be in worse health (Belsky et al., 2015).

Subsequently, we reported that faster Pace of Aging is associated with early-life factors important for aging: familial longevity, low childhood social class, and adverse childhood experiences (Belsky et al., 2017a), and that faster Pace of Aging is associated with older scores on Brain Age, a machine-learning-derived measure of structural MRI differences characteristic of different age groups (Elliott et al., 2019). Notably, Pace of Aging was not well-correlated with published epigenetic age clocks, which were designed to measure how old a person is biologically rather than how fast they are aging (Belsky et al., 2018).

In Step 3, which we report here, we distill the Pace of Aging into a measurement that can be obtained from a single blood sample. Here we focused on blood DNA methylation as an accessible molecular measurement that is sensitive to changes in physiology occurring in multiple organ systems (Birney et al., 2016; Bolund et al., 2017; Chambers et al., 2015; Chu et al., 2017; Hedman Åsa K. et al., 2017; Ma et al., 2019; Mill and Heijmans, 2013; Morris et al., 2017; Wahl et al., 2017). We used data about the Pace of Aging from age 26 to 38 years in the Dunedin Study along with whole-genome methylation data at age 38 years. Elastic net regression was applied to derive an algorithm that captured DNA methylation patterns linked with variation among individuals in their Pace of Aging. The algorithm is hereafter termed "mPoA".

In Step 4, which we report here, we validate the mPoA in 4 ways. First, using the Dunedin Study, we tested if Study member's mPoA measured when they were aged 38 years could predict deficits in physical and cognitive functioning seven years later, when the cohort was aged 45 years. Second, we applied the mPoA algorithm to methylation data from a second, cross-sectional, study of adults to evaluate patterning of mPoA by chronological age and sex and to test correlations of mPoA with self-reported health and proposed measures of biological age, including three epigenetic clocks. Third, we applied the mPoA algorithm to DNA methylation data from a third, longitudinal, study of young people to test if mPoA was accelerated by exposure to poverty and victimization, factors which are known to shorten healthy lifespan. Finally, to ascertain the potential usefulness of mPoA as a measure for trials of anti-aging treatments, we applied the algorithm to DNA methylation data from a randomized trial of caloric restriction, CALERIE (Ravussin et al., 2015). Earlier we reported from this trial that

the intervention (two years of prescribed 25% caloric restriction) slowed the rate of biological aging as measured by a blood-chemistry biological-age composite measure (Belsky et al., 2017b). Here, using newly generated methylation data from blood drawn at the CALERIE baseline assessment, we tested if (a) mPoA from blood drawn before caloric restriction could predict the future rate of biological aging of participants during the two-year trial, and (b) if this prediction was modified in participants who underwent caloric restriction, but not among control participants. We report promising results from this four-step research program, while appreciating that additional measurement development will be needed to support applied use of mPoA.

METHODS

Data Sources

Data were used from four studies: Dunedin Study, Understanding Society Study, Environmental Risk (E-Risk) Longitudinal Twin Study, and CALERIE Trial. The four datasets and measures analyzed within each of them are described in **Supplemental Materials** Section 1.

DNA Methylation Data

DNA methylation was measured from Illumina 450k Arrays in the Dunedin and E-Risk studies and from Illumina EPIC 850k Arrays in the Understanding Society study and the CALERIE Trial. DNA was derived from whole blood samples in all studies. Dunedin Study blood draws were conducted at the cohort's age-38 assessment during 2010-12. Understanding Society blood draws were conducted in 2012. E-Risk blood draws were conducted at the cohort's age-18 assessment during 2012-13. CALERIE blood draws were conducted at the trial baseline assessment in 2007. Dunedin and CALERIE methylation assays were run by the Molecular Genomics Shared Resource at Duke Molecular Physiology Institute, Duke University (USA). E-

Risk and Understanding Society assays were run by the Complex Disease Epigenetics Group at the University of Exeter Medical School (UK) (www.epigenomicslab.com). Processing protocols for the methylation data from all studies have been described previously (Hannon et al., 2018; Marzi et al., 2018). (CALERIE data were processed according to the same protocols used for the Dunedin Study.)

Methylation Pace of Aging (mPoA) was developed using elastic-net regression analysis carried out in the Dunedin Study, as described in detail in the Results. The criterion variable was Pace of Aging. Development of the Pace of Aging is described in detail elsewhere (Belsky et al., 2015). Briefly, we conducted mixed-effects growth modeling of longitudinal change in 18 biomarkers measuring integrity of the cardiovascular, metabolic, renal, hepatic, pulmonary, periodontal, and immune systems (biomarkers are listed in the **Supplemental Materials** Section 1, description of the Dunedin Study data). For each biomarker, we estimated random slopes quantifying each participant's own rate of change in that biomarker. We then composited slopes across the 18 biomarkers to calculate a participant's Pace of Aging. Pace of Aging was scaled in units representing the mean trend in the cohort, i.e. the average physiological change occurring during one calendar year (N=954, M=1, SD=0.38). Of the N=819 Dunedin Study members with methylation data at age 38, N=810 had measured Pace of Aging (M=0.98, SD=0.09). This group formed the analysis sample to develop mPoA.

To compute mPoA in the Understanding Society, E-Risk, and CALERIE Trial datasets, we applied the scoring algorithm estimated from elastic net regression in the Dunedin Study. CpG weights for the scoring algorithm are provided in **Table 1**.

Statistical Analysis

We conducted analysis of Dunedin, Understanding Society, E-Risk, and CALERIE data using regression models. For E-Risk analysis, which include data on twin siblings, we clustered standard errors at the family level to account for non-independence of data. CALERIE analysis used mixed-effects growth models (Singer and Willett, 2003) following the method in our original analysis of those data (Belsky et al., 2017b). All analyses included covariate adjustment for sex. In the Understanding Society and CALERIE studies, we also included covariate adjustment for chronological age. (Dunedin and E-Risk are birth-cohort studies and participants are all the same chronological age.) For regression analysis, methylation measures were adjusted for batch effects by regressing the measure on batch controls and predicting residual values.

RESULTS

Capturing Pace of Aging in a single blood test

We conducted analysis to develop the mPoA algorithm using data from Dunedin Study for whom age-38 DNA methylation data were available (N=810). We applied elastic-net regression (Zou and Hastie, 2005) using Pace of Aging between ages 26 to 38 years as the criterion. We included all methylation probes that appear on both the Illumina 450k and EPIC arrays as potential predictor variables. We selected this overlapping set of probes for our analysis to facilitate application of the derived algorithm by other research teams using either chip. We fixed the alpha parameter to 0.5, following the approach reported by Horvath (Horvath, 2013). This analysis selected a set of 46 CpG sites (**Table 1**, **Supplemental Materials** Section 2). The 46-CpG elastic-net-derived mPoA algorithm, applied in the age-38 Dunedin DNA methylation data, was associated with the longitudinal 26-38 Pace of Aging measure (Pearson r=0.56, **Supplemental Figure 1**). This is likely an overestimate of the true out-of-sample correlation because the analysis is based on the same data used to develop the mPoA algorithm; bootstrap-cross-validation analysis estimated the out-of-sample correlation to be r=0.33; **Supplemental Materials** Section 3).

mPoA in midlife predicted future functional limitations

Physical Functioning. As a primary criterion validity analysis of mPoA, we tested prospective associations of Dunedin Study members' age-38 mPoA values with their performance seven years later, when they were aged 45 years, on tests of balance, walking speed, chair stands, grip strength, and Study-member reports about physical limitations. Performance scores were reversed so that positive correlations indicated an association between faster mPoA and worse physical performance. Study members with faster mPoA at age 38 performed more poorly at age 45 on all physical performance tests, with the exception of grip strength, and reported more functional limitations (standardized effect-sizes for tests of balance, walking speed, chair stands, and physical limitations r=0.19-0.29, p<0.001 for all; grip strength r=0.05, 95% CI [-0.02-0.12], p=0.200). Effect-sizes are graphed in the dark blue bars in **Figure 1, Panel A**.

Physical Decline. For balance, grip strength, and functional limitations, the Dunedin study administered the same assessments at the age-38 and age-45 assessments. We used these data to measure change in physical function across the 7-year interval. We computed change scores by subtracting the age-45 score from the age-38 score. Change scores for balance and physical limitations indicated worsening of physical functioning across the 7-year interval (in terms of age-38 standard-deviation units (SDs): balance declined by 0.26 SDs 95% CI [0.19-0.33] and physical limitations increased by 0.26 SDs [0.18-0.33]). In contrast, grip strength increased slightly (0.05 SDs [0.00-0.11]). Study members with faster age-38 mPoA experienced greater decline in balance at age 45 (r=0.11 [0.04-0.19]) and a greater increase in physical limitations (r=0.10 [0.02-0.18]). There was no association between mPoA and change in grip strength (r=0.00 [-0.07-0.08]). Effect-sizes are graphed in the top row of **Figure 1, Panel B**.

Cognitive Functioning. We evaluated cognitive functioning from tests of perceptual reasoning, working memory, and processing speed, which are known to show aging related declines already by the fifth decade of life (Hartshorne and Germine, 2015; Park et al., 2002). Study members with faster age-38 mPoA performed more poorly on all age-45 cognitive tests (r=0.14-0.28, p<0.001 for all). Effect-sizes are graphed in the light-blue bars of **Figure 1, Panel A**.

Cognitive Decline. Cognitive functioning in early life is a potent risk factor for chronic disease and dementia in later life and for accelerated aging in midlife (Belsky et al., 2017a; Deary and Batty, 2006). Therefore, to evaluate whether associations between mPoA and cognitive test performance at age 45 might reflect reverse causation instead of early cognitive decline, we next conducted analysis of cognitive decline between adolescence and midlife. We evaluated cognitive decline by comparing Study-members' cognitive-test performance at age 45 to their cognitive-test performance three decades earlier when they were ages 7-13 years. Cognitive performance was measured from composite scores on the Wechsler Intelligence Scales (the Wechsler Adult Intelligence Scales Version IV at the age-45 assessment and the Wechsler Intelligence Scales for Children Version R at the earlier timepoints). On average, Study members showed a decline of 2.00 IQ points (95% CI [1.31-2.70]) across the follow-up interval. We conducted two analyses to test if participants with faster mPoA experienced more cognitive decline. First, we computed difference scores (age-45 IQ – childhood baseline IQ) and regressed these difference scores on mPoA. Second, we conducted analysis of residualized change by regressing age-45 IQ on mPoA and childhood IQ. Both analyses found that Study members with faster mPoA experienced more decline (difference-score r=0.12, [0.05-0.19]; residualized change r=0.20 [0.13-0.27]). The mPoA association with cognitive decline is graphed in the bottom-left cell of **Figure 1, Panel B**.

Subjective Signs of Aging. We evaluated subjective signs of aging from Study members' ratings of their current health status (excellent, very-good, good, fair, poor) and from ratings of perceived age made by undergraduate raters based on facial photographs. Study members with faster age-38 mPoA rated themselves to be in worse health at age 45 (r=0.27, 95% CI [0.20-0.34]). These Study members were also rated as looking older (r=0.35 [0.28-0.43]). Effect-sizes are graphed in the orange bars in **Figure 1, Panel A**.

Subjective Signs of Decline with Aging. We next analyzed change in subjective signs of aging. Across the 7-year follow-up interval, an increasing number of Study members rated themselves as being in fair or poor health (6% rated their health as fair or poor at age 38, as compared to 8% 7 years later at age 45). Those with faster age-38 mPoA were more likely to transition to the fair/poor categories (Incidence Rate Ratio (IRR)=1.79 95% CI [1.48-2.18]). We

tested if Study members with faster mPoA experienced more rapid facial aging by subtracting the age-45 score from the age 38-score and regressing this difference on mPoA. This analysis tested if Study members with faster mPoA experienced upward rank mobility within the cohort in terms of how old they looked. Study members with faster age-38 mPoA were rated as looking older relative to peers at age 45 than they had been at age 38 (r=0.10 [0.03-0.18]). Effect-sizes are graphed in the bottom-right two panels of **Figure 1, Panel B**.

Comparing mPoA versus Pace of Aging. We compared mPoA effect-sizes to effect-sizes for the original, 18-biomarker 3-time-point Pace of Aging. Across the domains of physical function, cognitive function, and subjective signs of aging, mPoA effect-sizes were similar to and sometimes larger than effect-sizes for the original Pace of Aging measure (**Supplemental Table 1, Supplemental Figure 1**).

Evaluating mPoA and other methylation clocks in the Understanding Society study

To test variation in mPoA and to compare it with published methylation measures of biological aging, we conducted analysis using data on N=1,175 participants aged 28-95 years (M=58, SD=15; 42% male) in the UK Understanding Society cohort. In this mixed-age sample, the mean mPoA was 1.03 years of biological aging per each calendar year (SD=0.07).

We first tested if higher mPoA levels, which indicate faster aging, were correlated with older chronological age. Mortality rates increase with advancing chronological age, although there may be some slowing at the oldest ages (Barbi et al., 2018). This suggests the hypothesis that the rate of aging increases across much of the adult lifespan. Consistent with this hypothesis, Understanding Society participants who were of older chronological age tended to have faster mPoA (r=0.11, [0.06-0.17], **Figure 2 Panel A**). We also compared mPoA with three methylation measures of biological age: the epigenetic clocks proposed by Horvath, Hannum, and Levine (Hannum et al., 2013; Horvath, 2013; Levine et al., 2018). These epigenetic clocks were highly correlated with chronological age in the Understanding Society sample (Horvath Clock r=0.91, Hannum Clock r=0.92, Levine Clock r=0.88).

Next, to test if mPoA captured similar information about aging to published epigenetic clocks, we regressed each of the published clocks on chronological age and predicted residual

values, following the procedure used by the developers of the clocks. These residuals are referred to in the literature as measures of "epigenetic age acceleration." None of the 46 CpGs included in the mPoA algorithm overlapped with CpGs in these epigenetic clocks. Nevertheless, mPoA was moderately correlated with epigenetic age acceleration measured from the clocks proposed by Hannum (r=0.24) and Levine (r=0.30). mPoA was less-well correlated with acceleration measured from the Horvath clock (r=0.06). Associations among mPoA and the epigenetic clocks in the Understanding Society sample are shown in **Figure 2 Panel B.**

Finally, we tested correlations of mPoA with (a) a measure of biological age derived from blood chemistry and blood pressure data, and (b) a measure of self-rated health. We computed biological age from Understanding Society blood chemistry and blood pressure data following the Klemera and Doubal method (KDM) (Klemera and Doubal, 2006) and the procedure described by Levine (Levine, 2013). KDM Biological Age details are reported in the **Supplemental Methods**. Participants with faster mPoA had more advanced KDM Biological Age (r=0.20 95% CI [0.15-0.26]) and worse self-rated health (r=-0.22 [-0.28,-0.16]). Covariate adjustment to models for estimated cell counts (Houseman et al., 2012) and smoking status did not change results. Results for all models are reported in **Supplemental Table 2**.

In comparison to mPoA, effect-sizes for associations with self-rated health and KDM Biological Age were smaller for the epigenetic clocks and, in the cases of the Horvath and Hannum clocks, were not statistically different from zero at the alpha=0.05 threshold. Effect-sizes are plotted in **Supplemental Figure 3**.

Childhood exposure to poverty and victimization were associated with faster mPoA in young adults in the E-Risk Study

To test if mPoA indicated faster aging in young people with histories of exposure thought to shorten healthy lifespan, we analyzed data from N=1,658 members of the E-Risk Longitudinal Study. The E-Risk Study follows a 1994-95 birth cohort of same-sex twins. Blood DNA methylation data were collected when participants were aged 18 years. We analyzed two exposures associated with shorter healthy lifespan, childhood low socioeconomic status and childhood victimization. Socioeconomic status was measured from data on their parents'

education, occupation, and income (Trzesniewski et al., 2006). Victimization was measured from exposure dossiers compiled from interviews with the children's mothers and home-visit assessments conducted when the children were aged 5, 7, 10, and 12 (Fisher et al., 2015). The dossiers recorded children's exposure to domestic violence, peer bullying, physical and sexual harm by an adult, and neglect. 72% of the analysis sample had no victimization exposure, 21% had one type of victimization exposure, 4% had two types of exposure, and 2% had three or more types of exposure.

Mean mPoA in E-Risk adolescents at age 18 was 0.92 years of biological aging per calendar year (SD=0.06), suggesting that this younger cohort is aging more slowly than the older cohorts. E-Risk adolescents who grew up in lower socioeconomic-status families exhibited faster mPoA (Cohen's d for comparison of low to moderate SES =0.21 [0.06-0.35]; Cohen's d for comparison of low to high SES =0.44 [0.31-0.56]; Pearson r=0.19 [0.13-0.24]). In parallel, E-Risk adolescents with exposure to more types of victimization exhibited faster mPoA (Cohen's d for comparison of never victimized to one type of victimization =0.28 [0.15-0.41]; Cohen's d for comparison of never victimized to two types of victimization =0.48 [0.23-0.72]; Cohen's d for comparison of never victimized to three or more types of victimization =0.53 [0.25-0.81]; Pearson r=0.15 [0.10-0.20]). Covariate adjustment to models for estimated cell counts (Houseman et al., 2012) did not change results. Adjustment for smoking status attenuated effect-sizes by about half, but most associations remained statistically different from zero at the alpha=0.05 level. Results for all models are reported in **Supplemental Table 3**. Differences in mPoA across strata of childhood socioeconomic status and victimization are graphed in **Figure 3**.

mPoA measured at baseline in the CALERIE randomized trial predicted future rate of aging measured from clinical-biomarker data

The CALERIE Trial is the first randomized trial of long-term caloric restriction in non-obese adult humans. CALERIE randomized N=220 adults on a 2:1 ratio to treatment of 25% caloric restriction (CR-treatment) or control ad-libitum (AL-control, as usual) diet for two years (Ravussin et al., 2015). We previously reported that CALERIE participants who were randomized

to CR-treatment experienced a slower rate of biological aging as compared to participants in the AL-control arm based on longitudinal change analysis of clinical-biomarker data from the baseline, 12-month, and 24-month follow-up assessments (Belsky et al., 2017b). Among control participants, the rate of increase in biological age measured using the Klemera-Doubal method (KDM) Biological Age algorithm was 0.71 years of biological age per 12-month follow-up interval. (This slower-than-expected rate of aging could reflect differences between CALERIE Trial participants, who were selected for being in good health, and the nationally representative NHANES sample in which the KDM algorithm was developed (Belsky et al., 2017b).) In contrast, among treatment participants, the rate of increase was only 0.11 years of biological age per 12-month follow-up interval (difference b=-0.60 [-0.99, -0.21]). We subsequently generated DNA methylation data from blood DNA that was collected at the baseline assessment of the CALERIE trial for a sub-sample (N=68 AL-control participants and 118 CR-treatment participants). We used these methylation data to calculate participants' mPoA values at study baseline. We then tested if baseline mPoA could predict participants' future rate of biological aging as they progressed through the trial.

We first replicated our original analysis within the methylation sub-sample. Results were the same as in the full sample (**Supplemental Table 4**). Next, we compared mPoA between CR-treatment and AL-control participants. As expected, there was no group difference at baseline (AL M=1.00, SD=0.05; CR M=1.01, SD=0.06, p-value for difference =0.440). Finally, we tested if participants' baseline mPoA was associated with their rate of biological aging over the 24 months of follow-up, and if this association was modified by randomization to caloric restriction as compared to ad libitum diet. For AL-control participants, faster baseline mPoA predicted faster biological aging over the 24-month follow-up, although in this small group this association was not statistically significant at the alpha=0.05 level (b=0.22 [-0.05, 0.49], p=0.104). For CR-treatment participants, the association of baseline mPoA with future rate of aging was sharply reduced, (b=-0.08 [-0.24, 0.09], p=0.351), although the difference between the rate of aging in the AL-control and CR-treatment groups did not reach the alpha=0.05 threshold for statistical significance (interaction-term testing difference in slopes b=-0.30 [-0.61, 0.01], p-value=0.060). Slopes of change in KDM Biological Age for participants in the AL-control

and CR-treatment groups are plotted for fast baseline mPoA (1 SD above the mean) and slow baseline mPoA (1 SD below the mean) in **Figure 4**. CALERIE DNA methylation data are not yet available to test if the intervention altered post-treatment mPoA.

DISCUSSION

Breakthrough discoveries in the new field of geroscience suggest opportunities to extend healthy lifespan through interventions that slow biological processes of aging (Campisi et al., 2019). To advance translation of these interventions, measures are needed that can detect changes in a person's rate of biological aging (Moffitt et al., 2016). We previously showed that the rate of biological aging can be measured by tracking change over time in multiple indicators of organ-system integrity (Belsky et al., 2015).Here, we report data illustrating the potential to streamline measurement of Pace of Aging to an exportable, inexpensive and non-invasive blood test, and thereby ease implementation of Pace of Aging measurement in studies of interventions to slow processes of biological aging.

We conducted machine-learning analysis of the original Pace of Aging measure using elastic-net regression and whole-genome blood DNA methylation data. We trained the algorithm to predict how fast a person was aging. We called the resulting algorithm "mPoA" for "(m)ethylation (P)ace (o)f (A)ging". There were four overall findings:

First, while mPoA was not a perfect proxy of Pace of Aging, it nevertheless captured critical information about Dunedin Study members' healthspan-related characteristics. Across the domains of physical function, cognitive function, and subjective signs of aging, Study members with faster mPoA at age 38 were worse off seven years later at age 45 and, in repeated-measures analysis of change, they showed signs of more rapid decline. Effect-sizes were equal to or greater than those for the 18-biomarker 3-time point measure of Pace of Aging. In sum, mPoA showed promise as an easy-to-implement alternative to Pace of Aging. Emerging technologies for deep-learning analysis (Zhavoronkov et al., 2019) may improve methylation measurement of Pace of Aging. Alternatively, integration of methylation data with additional molecular datasets (Hasin et al., 2017; Zierer et al., 2015) may be needed to achieve precise measurement of Pace of Aging from a single time-point blood sample.

Second, mPoA analysis of the Understanding Society sample provided proof-of-concept for using mPoA to quantify biological aging. Age differences in mPoA parallel population demographic patterns of mortality risk. In the Understanding Society sample, older adults had faster mPoA as compared to younger ones. This observation is consistent with the well-documented acceleration of mortality risk with advancing chronological age (Robine, 2011). However, it sets mPoA apart from other indices of biological aging, which are not known to register this acceleration (Finch and Crimmins, 2016). mPoA may therefore provide a novel tool for testing how the rate of aging changes across the life course and whether, as demographic data documenting so-called "mortality plateaus" suggest, processes of aging slow down at the oldest chronological ages (Barbi et al., 2018).

mPoA is related to but distinct from alternative approaches to quantification of biological aging. In the Understanding Society sample, mPoA was moderately correlated with aging rates measured by the epigenetic clocks proposed by Hannum et al. and Levine et al. (Hannum et al., 2013; Levine et al., 2018) as well as KDM Biological Age derived from clinical biomarker data (Klemera and Doubal, 2006; Levine, 2013), and with self-rated health. Consistent with findings for the measured Pace of Aging (Belsky et al., 2018), mPoA was only weakly correlated with the multi-tissue clock proposed by Horvath and was more strongly correlated with a clinical-biomarker measure of biological age and with self-rated health as compared to the epigenetic clocks.

Third, mPoA is already variable by young adulthood and is accelerated in young people at risk for eventual shortened healthspan. E-Risk young adults who grew up in socioeconomically disadvantaged families or who were exposed to victimization early in life already showed accelerated mPoA by age 18, consistent with epidemiological observations of shorter healthy lifespan for individuals with these exposures (Adler and Rehkopf, 2008; Danese and McEwen, 2012). We previously found that Dunedin Study members with histories of early-life adversity showed accelerated Pace of Aging in their 30s (Belsky et al., 2017a). mPoA analysis of the E-Risk cohort suggests effects may be already manifest at least a decade earlier. mPoA may therefore provide a useful index that can be applied to evaluate prevention programs to buffer at-risk youth against health damaging effects of challenging circumstances.

Fourth, mPoA analysis of the CALERIE trial provided proof-of-concept for using mPoA to quantify biological aging in anti-aging intervention studies. mPoA measures the rate of aging over the recent past. Control-arm participants' baseline mPoA correlated positively with their clinical-biomarker pace of aging over the two years of the trial, consistent with the hypothesis that their rate of aging was not altered. In contrast, there was no relationship between mPoA and clinical-biomarker pace of aging for caloric-restriction-arm participants, consistent with the hypothesis that caloric restriction altered participants' rate of aging. Ultimately, data on mPoA for all CALERIE participants (and participants in other anti-aging trails) at trial baseline and follow-up will be needed to establish utility of mPoA as a surrogate endpoint. In the mean-time, these data establish potential to use mPoA as a pre-treatment covariate in geroprotector trials to boost statistical power (Kahan et al., 2014) or to screen participants for enrollment, e.g. to identify those who are aging more rapidly and may therefore show larger effects of treatment.

We acknowledge limitations. Foremost, mPoA is a first step toward a single-assay cross-sectional measurement of Pace of Aging. The relatively modest size of the Dunedin cohort and the lack of other cohorts that have the requisite 3 or more waves of repeated biomarkers to measure the Pace of Aging limited sample size for our machine-learning analysis to develop methylation algorithms. As Pace of Aging is measured in additional cohorts, more refined analysis to develop mPoA-type algorithms will become possible. In addition, our work thus far has not addressed population diversity in biological aging. The Dunedin cohort in which mPoA was developed and the Understanding Society and E-Risk cohorts and CALERIE trial sample in which it was tested were mostly of white European-descent. Follow-up of mPoA in more diverse samples is needed to establish cross-population validity. Further, none of the datasets we analyzed included follow-up to establish prospective associations of mPoA with onset of disease, disability, or mortality. Effect-sizes from this type of analysis will be important to interpret changes in mPoA produced by intervention. Finally, because methylation data are not yet available from CALERIE follow-up assessments, we could not test if intervention modified mPoA at outcome.

Within the bounds of these limitations, our analysis establishes proof-of-concept for mPoA as a single-time-point measure that quantifies Pace of Aging from a blood test. It can be

implemented in Illumina 450k and EPIC array data, making it immediately available for testing in a wide range of existing datasets as a complement to existing methylation measures of aging. Critically, mPoA offers a unique measurement for intervention trials and natural experiment studies investigating how the rate of aging may be changed by behavioral or drug therapy, or by environmental modification. mPoA may be especially valuable to studies that collect data outside of clinical settings and lack blood chemistry, hematology, and other data needed to measure aging-related changes to physiology.

REFERENCES

Adler NE, Rehkopf DH. 2008. U.S. Disparities in Health: Descriptions, Causes, and Mechanisms. *Annu Rev Public Health* **29**:235–252. doi:10.1146/annurev.publhealth.29.020907.090852

Barbi E, Lagona F, Marsili M, Vaupel JW, Wachter KW. 2018. The plateau of human mortality: Demography of longevity pioneers. *Science* **360**:1459–1461. doi:10.1126/science.aat3119

Bell CG, Lowe R, Adams PD, Baccarelli AA, Beck S, Bell JT, Christensen BC, Gladyshev VN, Heijmans BT, Horvath S, Ideker T, Issa J-PJ, Kelsey KT, Marioni RE, Reik W, Relton CL, Schalkwyk LC, Teschendorff AE, Wagner W, Zhang K, Rakyan VK. 2019. DNA methylation aging clocks: challenges and recommendations. *Genome Biol* **20**:249. doi:10.1186/s13059-019-1824-y

Belsky DW, Caspi A, Cohen HJ, Kraus WE, Ramrakha S, Poulton R, Moffitt TE. 2017a. Impact of early personal-history characteristics on the Pace of Aging: implications for clinical trials of therapies to slow aging and extend healthspan. *Aging Cell* **16**:644–651. doi:10.1111/acel.12591

Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE. 2015. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A* **112**:E4104-4110. doi:10.1073/pnas.1506264112

Belsky DW, Huffman KM, Pieper CF, Shalev I, Kraus WE. 2017b. Change in the Rate of Biological Aging in Response to Caloric Restriction: CALERIE Biobank Analysis. *J Gerontol Ser A* **73**:4–10. doi:10.1093/gerona/glx096

Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, Schaefer J, Sugden K, Williams B, Poulton R, Caspi A. 2018. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? *Am J Epidemiol* **187**:1220–1230. doi:10.1093/aje/kwx346

Birney E, Smith GD, Greally JM. 2016. Epigenome-wide Association Studies and the Interpretation of Disease -Omics. *PLOS Genet* **12**:e1006105. doi:10.1371/journal.pgen.1006105

Bolund ACS, Starnawska A, Miller MR, Schlünssen V, Backer V, Børglum AD, Christensen K, Tan Q, Christiansen L, Sigsgaard T. 2017. Lung function discordance in monozygotic twins and associated differences in blood DNA methylation. *Clin Epigenetics* **9**:132. doi:10.1186/s13148-017-0427-2

Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. 2019. From discoveries in ageing research to therapeutics for healthy ageing. *Nature* **571**:183. doi:10.1038/s41586-019-1365-2

Chambers JC, Loh M, Lehne B, Drong A, Kriebel J, Motta V, Wahl S, Elliott HR, Rota F, Scott WR, Zhang W, Tan S-T, Campanella G, Chadeau-Hyam M, Yengo L, Richmond RC, Adamowicz-Brice M, Afzal U, Bozaoglu K, Mok ZY, Ng HK, Pattou F, Prokisch H, Rozario MA, Tarantini L, Abbott J, Ala-Korpela M, Albetti B, Ammerpohl O, Bertazzi PA, Blancher C, Caiazzo R, Danesh J, Gaunt TR, de Lusignan S, Gieger C, Illig T, Jha S, Jones S, Jowett J, Kangas AJ, Kasturiratne A, Kato N, Kotea N, Kowlessur S, Pitkäniemi J, Punjabi P, Saleheen D, Schafmayer C, Soininen P, Tai E-S, Thorand B, Tuomilehto J, Wickremasinghe AR,

Kyrtopoulos SA, Aitman TJ, Herder C, Hampe J, Cauchi S, Relton CL, Froguel P, Soong R, Vineis P, Jarvelin M-R, Scott J, Grallert H, Bollati V, Elliott P, McCarthy MI, Kooner JS. 2015. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. *Lancet Diabetes Endocrinol* **3**:526–534. doi:10.1016/S2213-8587(15)00127-8

Chu AY, Tin A, Schlosser P, Ko Y-A, Qiu C, Yao C, Joehanes R, Grams ME, Liang L, Gluck CA, Liu C, Coresh J, Hwang S-J, Levy D, Boerwinkle E, Pankow JS, Yang Q, Fornage M, Fox CS, Susztak K, Köttgen A. 2017. Epigenome-wide association studies identify DNA methylation associated with kidney function. *Nat Commun* **8**:1286. doi:10.1038/s41467-017-01297-7

Danese A, McEwen BS. 2012. Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol Behav* **106**:29–39. doi:10.1016/j.physbeh.2011.08.019

Deary IJ, Batty GD. 2006. Commentary: pre-morbid IQ and later health–the rapidly evolving field of cognitive epidemiology. *Int J Epidemiol* **35**:670–2. doi:10.1093/ije/dyl053

Elliott ML, Belsky DW, Knodt AR, Ireland D, Melzer TR, Poulton R, Ramrakha S, Caspi A, Moffitt TE, Hariri AR. 2019. Brain-age in midlife is associated with accelerated biological aging and cognitive decline in a longitudinal birth-cohort. *bioRxiv* 712851. doi:10.1101/712851

Finch CE, Crimmins EM. 2016. Constant molecular aging rates vs. the exponential acceleration of mortality. *Proc Natl Acad Sci* **113**:1121–1123. doi:10.1073/pnas.1524017113

Fisher HL, Caspi A, Moffitt TE, Wertz J, Gray R, Newbury J, Ambler A, Zavos H, Danese A, Mill J, Odgers CL, Pariante C, Wong CCY, Arseneault L. 2015. Measuring adolescents' exposure to victimization: The Environmental Risk (E-Risk) Longitudinal Twin Study. *Dev Psychopathol* **27**:1399–1416. doi:10.1017/S0954579415000838

Gladyshev VN. 2016. Aging: progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes. *Aging Cell* **15**:594–602. doi:10.1111/acel.12480

Goldman DP, Cutler D, Rowe JW, Michaud P-C, Sullivan J, Peneva D, Olshansky SJ. 2013. Substantial health and economic returns from delayed aging may warrant a new focus for medical research. *Health Aff (Millwood)* **32**:1698–1705. doi:10.1377/hlthaff.2013.0052

Hannon E, Gorrie-Stone TJ, Smart MC, Burrage J, Hughes A, Bao Y, Kumari M, Schalkwyk LC, Mill J. 2018. Leveraging DNA-Methylation Quantitative-Trait Loci to Characterize the Relationship between Methylomic Variation, Gene Expression, and Complex Traits. *Am J Hum Genet* **103**:654–665. doi:10.1016/j.ajhg.2018.09.007

Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan J-B, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* **49**:359–367. doi:10.1016/j.molcel.2012.10.016

Harper S. 2014. Economic and social implications of aging societies. *Science* **346**:587–591. doi:10.1126/science.1254405

Hartshorne JK, Germine LT. 2015. When does cognitive functioning peak? The asynchronous rise and fall of different cognitive abilities across the life span. *Psychol Sci* **26**:433–443. doi:10.1177/0956797614567339

Hasin Y, Seldin M, Lusis A. 2017. Multi-omics approaches to disease. *Genome Biol* **18**:83. doi:10.1186/s13059-017-1215-1

Hedman Åsa K., Mendelson Michael M., Marioni Riccardo E., Gustafsson Stefan, Joehanes Roby, Irvin Marguerite R., Zhi Degui, Sandling Johanna K., Yao Chen, Liu Chunyu, Liang Liming, Huan Tianxiao, McRae Allan F., Demissie Serkalem, Shah Sonia, Starr John M., Cupples L. Adrienne, Deloukas Panos, Spector Timothy D., Sundström Johan, Krauss Ronald M., Arnett Donna K., Deary Ian J., Lind Lars, Levy Daniel, Ingelsson Erik. 2017. Epigenetic Patterns in Blood Associated With Lipid Traits Predict Incident Coronary Heart Disease Events and Are Enriched for Results From Genome-Wide Association Studies. *Circ Cardiovasc Genet* **10**:e001487. doi:10.1161/CIRCGENETICS.116.001487

Horvath S. 2013. DNA methylation age of human tissues and cell types. *Genome Biol* **14**:R115. doi:10.1186/gb-2013-14-10-r115

Horvath S, Raj K. 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet* **19**:371–384. doi:10.1038/s41576-018-0004-3

Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. 2012. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* **13**:86. doi:10.1186/1471-2105-13-86

Justice J, Miller JD, Newman JC, Hashmi SK, Halter J, Austad SN, Barzilai N, Kirkland JL, Justice J, Miller JD, Newman JC, Hashmi SK, Halter J, Austad SN, Barzilai N. 2016. Frameworks for Proof-of-Concept Clinical Trials of Interventions That Target Fundamental Aging Processes. *J Gerontol A Biol Sci Med Sci* **71**:1415–1423. doi:10.1093/gerona/glw126

Justice JN, Ferrucci L, Newman AB, Aroda VR, Bahnson JL, Divers J, Espeland MA, Marcovina S, Pollak MN, Kritchevsky SB, Barzilai N, Kuchel GA. 2018. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. *GeroScience* **40**:419–436. doi:10.1007/s11357-018-0042-y

Kaeberlein M. 2013. Longevity and aging. *F1000Prime Rep* **5**. doi:10.12703/P5-5

Kaeberlein M, Rabinovitch PS, Martin GM. 2015. Healthy aging: The ultimate preventative medicine. *Science* **350**:1191–1193. doi:10.1126/science.aad3267

Kahan BC, Jairath V, Doré CJ, Morris TP. 2014. The risks and rewards of covariate adjustment in randomized trials: an assessment of 12 outcomes from 8 studies. *Trials* **15**:139. doi:10.1186/1745-6215-15-139

Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T, Sierra F. 2014. Geroscience: linking aging to chronic disease. *Cell* **159**:709–713. doi:10.1016/j.cell.2014.10.039

Kirkwood TBL. 2005. Understanding the Odd Science of Aging. *Cell* **120**:437–447. doi:10.1016/j.cell.2005.01.027

Klemera P, Doubal S. 2006. A new approach to the concept and computation of biological age. *Mech Ageing Dev* **127**:240–248. doi:10.1016/j.mad.2005.10.004

Levine ME. 2013. Modeling the rate of senescence: Can estimated biological age predict mortality more accurately than chronological age? *J Gerontol A Biol Sci Med Sci* **68**:667–674. doi:10.1093/gerona/gls233

Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L, Horvath S.

2018. An epigenetic biomarker of aging for lifespan and healthspan. *Aging* **10**:573–591. doi:10.18632/aging.101414

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* **153**:1194–1217. doi:10.1016/j.cell.2013.05.039

Ma J, Nano J, Ding J, Zheng Y, Hennein R, Liu C, Speliotes EK, Huan T, Song C, Mendelson MM, Joehanes R, Long MT, Liang L, Smith JA, Reynolds L, Ghanbari M, Muka T, Meurs J van, Alferink LJM, Franco OH, Dehghan A, Ratliff S, Zhao W, Bielak L, Kardia SL, Peyser PA, Ning H, VanWagner LB, Lloyd-Jones DM, Carr JJ, Greenland P, Lichtenstein AH, Hu FB, Liu Y, Hou L, Murad SD, Levy D. 2019. A Peripheral Blood DNA Methylation Signature of Hepatic Fat Reveals a Potential Causal Pathway for Non-Alcoholic Fatty Liver Disease. *Diabetes* db181193. doi:10.2337/db18-1193

Marzi SJ, Sugden K, Arseneault L, Belsky DW, Burrage J, Corcoran DL, Danese A, Fisher HL, Hannon E, Moffitt TE, Odgers CL, Pariante C, Poulton R, Williams BS, Wong CCY, Mill J, Caspi A. 2018. Analysis of DNA Methylation in Young People: Limited Evidence for an Association Between Victimization Stress and Epigenetic Variation in Blood. *Am J Psychiatry* appi.ajp.2017.17060693. doi:10.1176/appi.ajp.2017.17060693

Mill J, Heijmans BT. 2013. From promises to practical strategies in epigenetic epidemiology. *Nat Rev Genet* **14**:585–594. doi:10.1038/nrg3405

Moffitt TE, Belsky DW, Danese A, Poulton R, Caspi A. 2016. The Longitudinal Study of Aging in Human Young Adults: Knowledge Gaps and Research Agenda. *J Gerontol A Biol Sci Med Sci* glw191. doi:10.1093/gerona/glw191

Morris JA, Tsai P-C, Joehanes R, Zheng J, Trajanoska K, Soerensen M, Forgetta V, Castillo-Fernandez JE, Frost M, Spector TD, Christensen K, Christiansen L, Rivadeneira F, Tobias JH, Evans DM, Kiel DP, Hsu Y-H, Richards JB, Bell JT. 2017. Epigenome-wide Association of DNA Methylation in Whole Blood With Bone Mineral Density. *J Bone Miner Res* **32**:1644–1650. doi:10.1002/jbmr.3148

Moskalev A, Chernyagina E, Tsvetkov V, Fedintsev A, Shaposhnikov M, Krut'ko V, Zhavoronkov A, Kennedy BK. 2016. Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic. *Aging Cell*. doi:10.1111/acel.12463

Park DC, Lautenschlager G, Hedden T, Davidson NS, Smith AD, Smith PK. 2002. Models of visuospatial and verbal memory across the adult life span. *Psychol Aging* **17**:299.

Ravussin E, Redman LM, Rochon J, Das SK, Fontana L, Kraus WE, Romashkan S, Williamson DA, Meydani SN, Villareal DT, Smith SR, Stein RI, Scott TM, Stewart TM, Saltzman E, Klein S, Bhapkar M, Martin CK, Gilhooly CH, Holloszy JO, Hadley EC, Roberts SB, CALERIE Study Group. 2015. A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. *J Gerontol A Biol Sci Med Sci* **70**:1097–1104. doi:10.1093/gerona/glv057

Robine J-M. 2011. Age Patterns in Adult Mortality In: Rogers RG, Crimmins EM, editors. International Handbook of Adult Mortality, International Handbooks of Population. Dordrecht: Springer Netherlands. pp. 207–226. doi:10.1007/978-90-481-9996-9_10

Schaie KW. 1967. Age changes and age differences. *The Gerontologist* **7**:128–132.

Singer JD, Willett JB. 2003. Applied Longitudinal Data Analysis. New York: Oxford University Press.

Trzesniewski KH, Moffitt TE, Caspi A, Taylor A, Maughan B. 2006. Revisiting the association between reading achievement and antisocial behavior: New evidence of an environmental explanation from a twin study. *Child Dev* **77**:72–88.

Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai P-C, Ried JS, Zhang W, Yang Y, Tan S, Fiorito G, Franke L, Guarrera S, Kasela S, Kriebel J, Richmond RC, Adamo M, Afzal U, Ala-Korpela M, Albetti B, Ammerpohl O, Apperley JF, Beekman M, Bertazzi PA, Black SL, Blancher C, Bonder M-J, Brosch M, Carstensen-Kirberg M, de Craen AJM, de Lusignan S, Dehghan A, Elkalaawy M, Fischer K, Franco OH, Gaunt TR, Hampe J, Hashemi M, Isaacs A, Jenkinson A, Jha S, Kato N, Krogh V, Laffan M, Meisinger C, Meitinger T, Mok ZY, Motta V, Ng HK, Nikolakopoulou Z, Nteliopoulos G, Panico S, Pervjakova N, Prokisch H, Rathmann W, Roden M, Rota F, Rozario MA, Sandling JK, Schafmayer C, Schramm K, Siebert R, Slagboom PE, Soininen P, Stolk L, Strauch K, Tai E-S, Tarantini L, Thorand B, Tigchelaar EF, Tumino R, Uitterlinden AG, van Duijn C, van Meurs JBJ, Vineis P, Wickremasinghe AR, Wijmenga C, Yang T-P, Yuan W, Zhernakova A, Batterham RL, Smith GD, Deloukas P, Heijmans BT, Herder C, Hofman A, Lindgren CM, Milani L, van der Harst P, Peters A, Illig T, Relton CL, Waldenberger M, Järvelin M-R, Bollati V, Soong R, Spector TD, Scott J, McCarthy MI, Elliott P, Bell JT, Matullo G, Gieger C, Kooner JS, Grallert H, Chambers JC. 2017. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature* **541**:81–86. doi:10.1038/nature20784

Zhavoronkov A, Mamoshina P, Vanhaelen Q, Scheibye-Knudsen M, Moskalev A, Aliper A. 2019. Artificial intelligence for aging and longevity research: Recent advances and perspectives. *Ageing Res Rev* **49**:49–66. doi:10.1016/j.arr.2018.11.003

Zierer J, Menni C, Kastenmüller G, Spector TD. 2015. Integration of 'omics' data in aging research: from biomarkers to systems biology. *Aging Cell* **14**:933–944. doi:10.1111/acel.12386

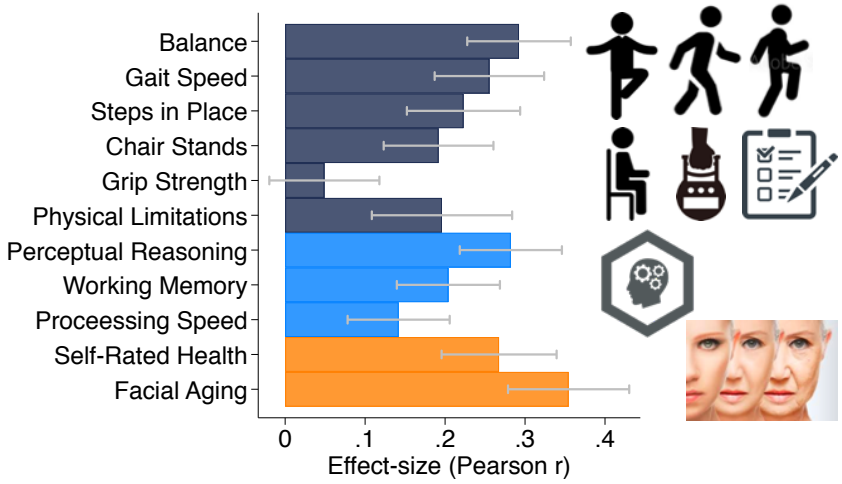
Zou H, Hastie T. 2005. Regularization and variable selection via the elastic net. *J R Stat Soc Ser B Stat Methodol* **67**:301–320. doi:10.1111/j.1467-9868.2005.00503.x

Table 1. Probes and associated weights composing the mPoA algorithm. The mPoA algorithm is a linear combination of 46 CpG methylation beta values weighted by coefficients estimated in the elastic net regression and added to the model intercept value of -0.06. (For sensitivity analyses addressing normalization method, and specific probes, see Supplement section 3.)

CpG list withheld pending acceptance for publication
Contact corresponding author for details

Figure 1. Faster age-38 mPoA is associated with poorer physical and cognitive functioning and subjective signs of aging at age 45 years, and with physical, cognitive, and subjective decline in the Dunedin Study. Panel A graphs effect-sizes for age-38 mPoA associations with age-45 measures of physical and cognitive functioning and subjective signs of aging in the Dunedin Study. Effect-sizes are standardized regression coefficients interpretable as Pearson r. Models included covariate adjustment for sex. Panel B graphs associations between mPoA and change in physical functioning between age 38 and age 45 (top row), change in cognitive functioning between age 13 and age 45 (bottom row, left-side cell), and incident fair/poor health and accelerated facial aging between ages 38 and 45 (bottom row, center and right-side cells). Graphs for changes in balance, grip-strength, physical limitations, cognition, and facial aging are binned scatterplots. Plotted points reflect average x- and y-coordinates for "bins" of approximately ten Study members. Fitted slopes show the association estimated from the raw, un-binned data. The y-axis scale on graphs of balance, grip-strength, and physical limitations shows change scores (age 45 – age 38) scaled in terms of age-38 standard deviation units. The y-axis scale on the graph of cognitive change shows the difference in IQ score (age 45 – baseline). The graph of change in facial aging shows the change in z-score between measurement intervals (age 45 – age 38). Effect-sizes reported on the graphs are standardized regression coefficients interpretable as Pearson r. Models included covariate adjustment for sex. The graph for self-rated health plots the predicted probability (fitted slope) and 95% confidence interval (shaded area) of incident fair/poor health at age 45. The effect-size reported on the graph is the incidence-rate ratio (IRR) associated with a 1-SD increase in mPoA estimated from Poisson regression. The model included covariate adjustment for sex.

Panel A.



Panel B.

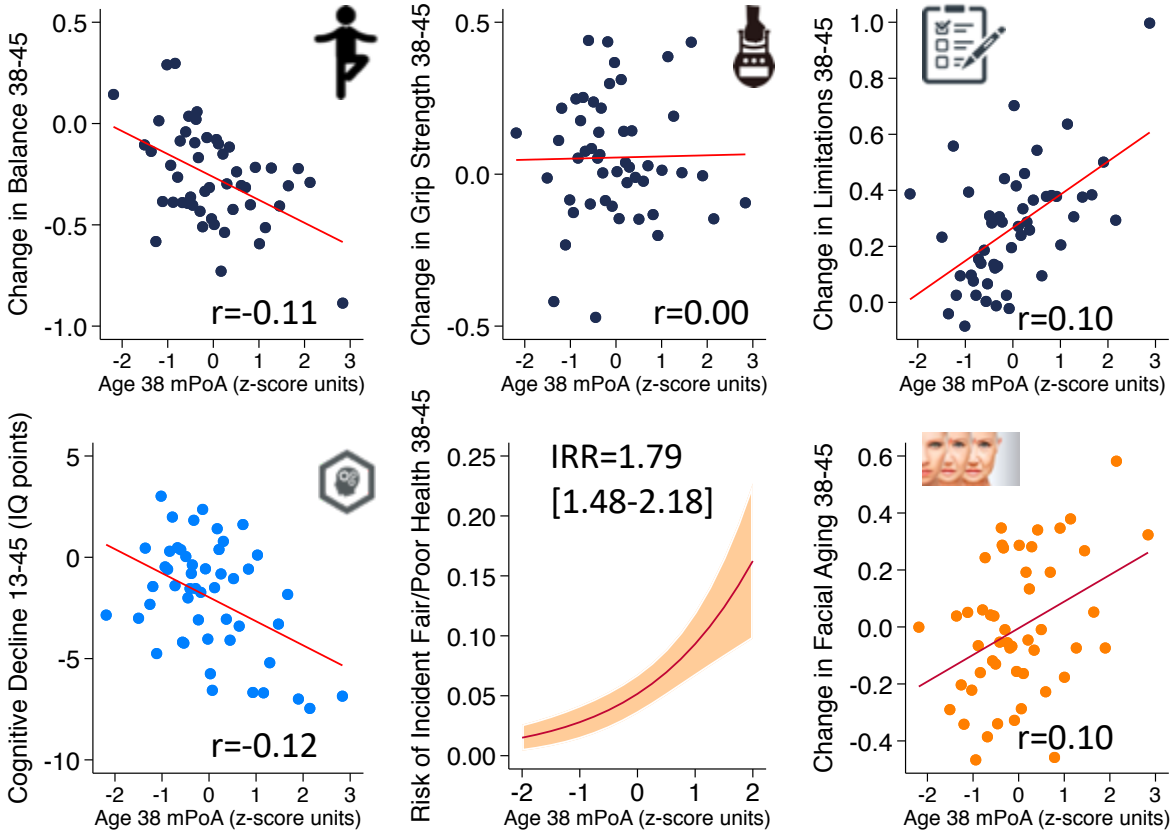
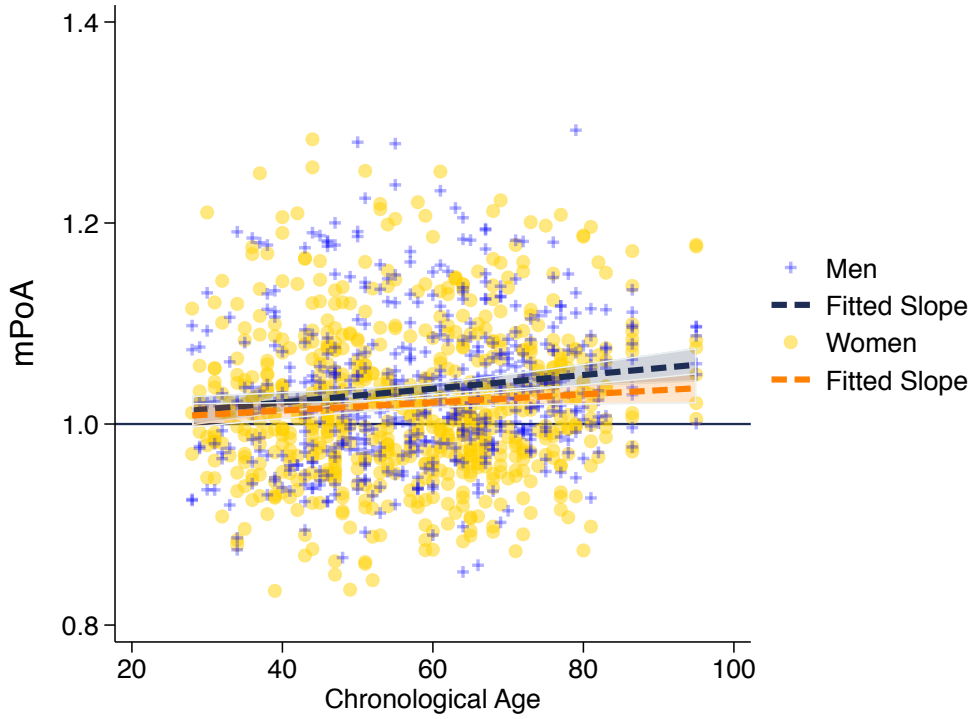


Figure 2. Associations among mPoA, and chronological age in the Understanding Society Study. Panel A shows a scatterplot and fitted slopes illustrating the association between chronological age (x-axis) and mPoA (y-axis) in women and men in the Understanding Society sample. Data for women are plotted with yellow dots (orange slope) and for men with blue crosses (navy slope). The figure illustrates a positive association between chronological age an mPoA (Pearson r=0.11 95% CI [0.06-0.17]). Panel B shows a matrix of association plots among mPoA, the methylation clocks proposed by Horvath, Hannum, and Levine, and chronological age. The figure shows analysis of age "acceleration" (accel) values of the clocks, i.e. the residual value from a regression of the clock on chronological age. These values are thought to reflect how much faster or more slowly a person has aged up to the time of measurement as compared to the population norm. The diagonal cells of the matrix list the variables included in the analysis. The lower half of the matrix shows scatter plots of associations. For each scatter-plot cell, the x-axis corresponds to the variable named along the matrix diagonal to the right of the plot and the y-axis corresponds to the variable named along the matrix diagonal above the plot. The upper half of the matrix lists Pearson correlations between the variables. For each correlation cell, the value listed reflects the correlation of the variables named along the matrix diagonal to the left of the cell and below the cell.

A.



B.

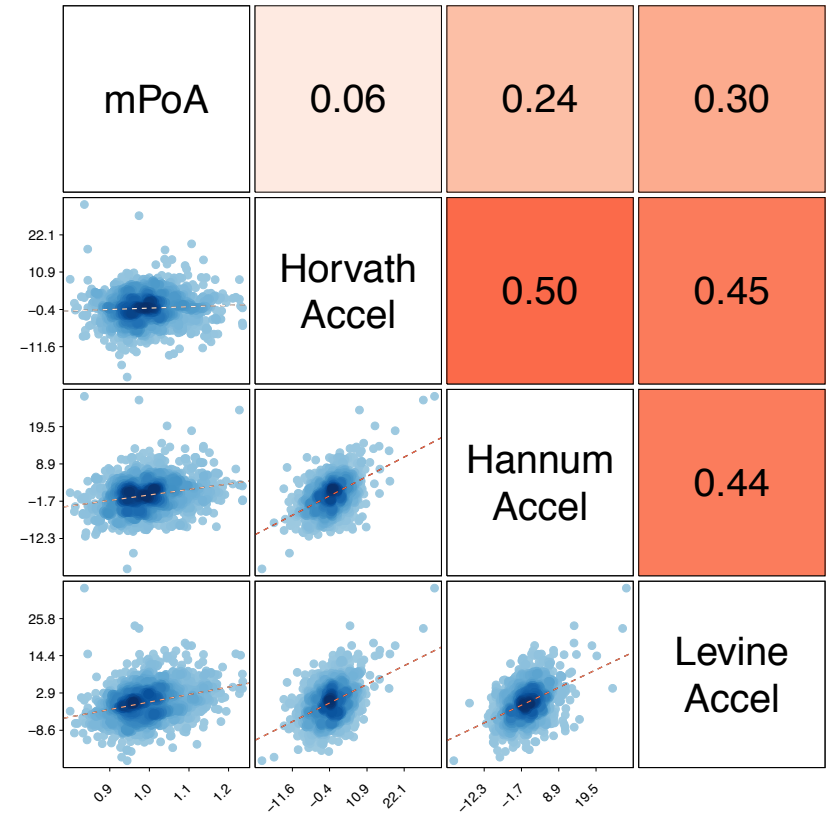

mPoA
0.06
0.24
0.30
Horvath Accel
0.50
0.45
Hannum Accel
0.44
Levine Accel
22.1
10.9
−0.4
−11.6
19.5
8.9
−1.7
−12.3
25.8
14.4
2.9
−8.6
0.9
1.0
1.1
1.2
−11.6
−0.4
10.9
22.1
−12.3
−1.7
8.9
19.5

Figure 3. mPoA levels by strata of childhood socioeconomic status (SES) and victimization in the E-Risk Study. Panel A (left side) plots means and 95% CIs for mPoA measured at age 18 among E-Risk participants who grew up low, middle, and high socioeconomic status households. Panel B (right side) plots means and 95% CIs for mPoA measured at age 18 among E-Risk participants who experienced 0, 1, 2, or 3 or more types of victimization through age 12 years.

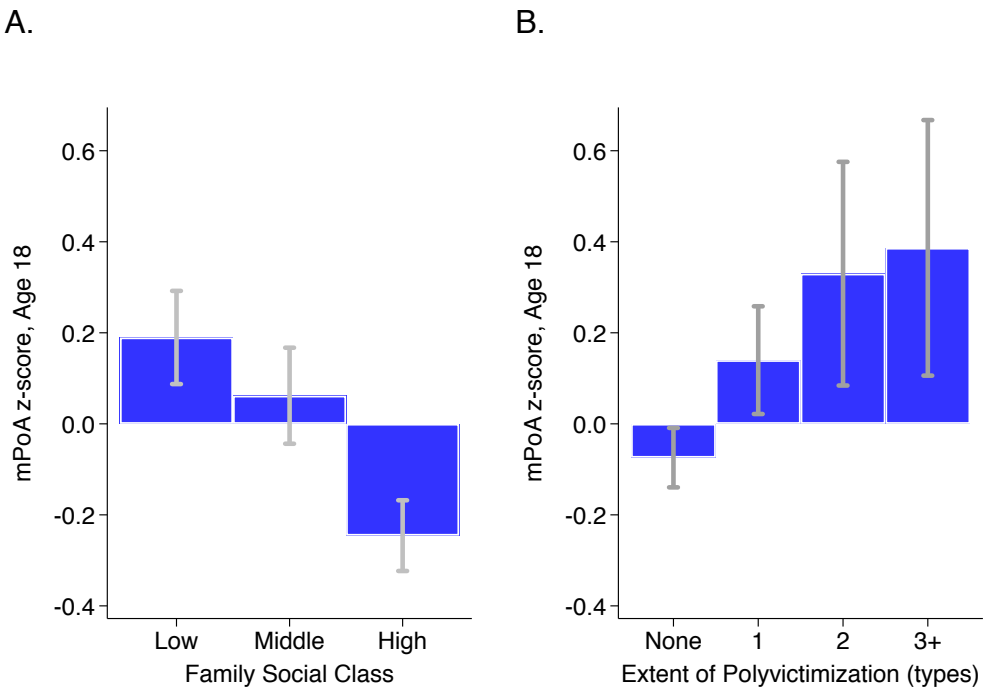
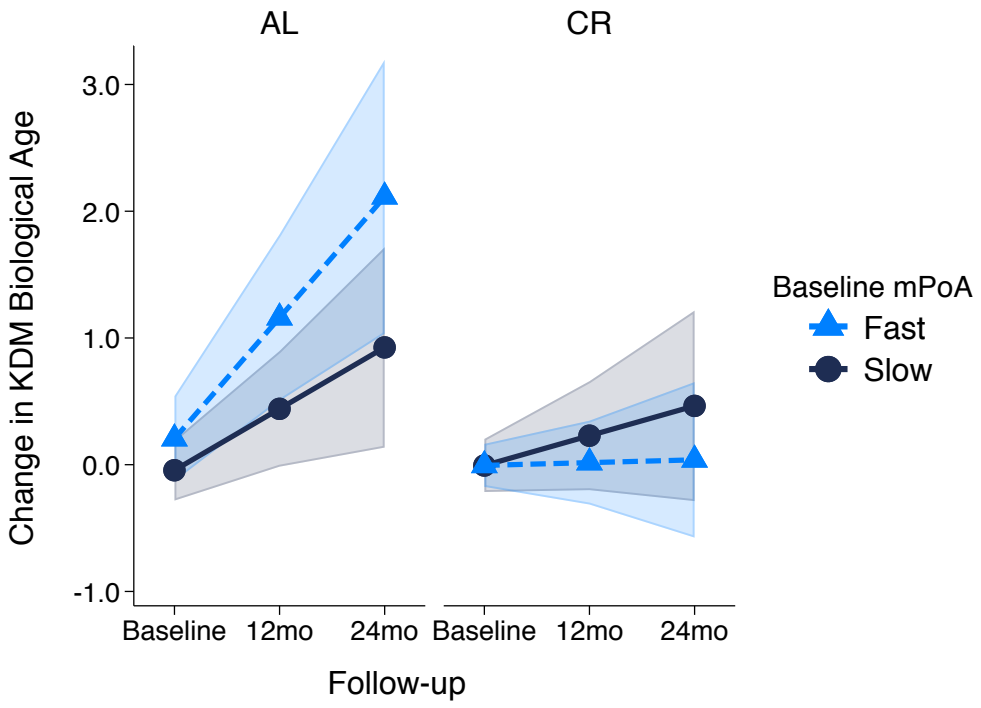


Figure 4. Change in KDM Biological Age over 24-month follow-up by treatment condition and baseline mPoA in the CALERIE Trial. Fast mPoA is defined as 1 SD above the sample mean. Slow mPoA is defined as 1 SD below the cohort mean. Slopes are predicted values from mixed effects regression including a 3-way interaction between trial condition, time, and continuous mPoA at baseline. The figure shows that in the Ad Libitum (AL) arm of the trial, participants with fast mPoA at baseline experience substantially more change in KDM Biological Age from baseline to follow-up as compared to AL participants with slow mPoA. In contrast, there was little difference between participants with fast as compared to slow mPoA in the Caloric Restriction (CR) arm of the trial.



SUPPLEMENTAL MATERIALS

1. Data.

The Dunedin Study is a longitudinal investigation of health and behavior in a complete birth cohort. Study members (N=1,037; 91% of eligible births; 52% male) were all individuals born between April 1972 and March 1973 in Dunedin, New Zealand (NZ), who were eligible based on residence in the province and who participated in the first assessment at age 3. The cohort represents the full range of socioeconomic status on NZ's South Island and matches the NZ National Health and Nutrition Survey on key health indicators (e.g., BMI, smoking, GP visits) (Poulton et al., 2015). The cohort is primarily white (93%) (Poulton et al., 2015). Assessments were carried out at birth and ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, 38 and, most recently, 45 years, when 94% of the 997 study members still alive took part. At each assessment, each study member is brought to the research unit for a full day of interviews and examinations. Study data may be accessed through agreement with the Study investigators (https://moffittcaspi.trinity.duke.edu/research-topics/dunedin).

Dunedin Study analysis to develop the Pace of Aging algorithm included 18 biomarkers measured at the age 26, 32, and 38 assessments: (in order of listing in Figure 3 of (Belsky et al., 2015)) glycated hemoglobin, cardiorespiratory fitness, waist-hip ratio, FEV_1/FVC ratio, FEV_1, mean arterial pressure, body mass index, leukocyte telomere length, creatinine clearance, blood urea nitrogen, lipoprotein (a), triglycerides, gum health, total cholesterol, white blood cell count, high-sensitivity C-reactive protein, HDL cholesterol, ApoB100/ApoA1 ratio.

Supplemental Table 1. Physical and cognitive functioning and subjective signs of aging measures in the Dunedin Study

Physical Functioning (N=800 with mPoA data)	
Balance	Balance was measured using the Unipedal Stance Test as the maximum time achieved across three trials of the test with eyes closed (Bohannon et al., 1984; Springer et al., 2007; Vereeck et al., 2008).
Gait Speed	Gait speed (meters per second) was assessed with the 6-m-long GAITRite Electronic Walkway (CIR Systems, Inc) with 2-m acceleration and 2-m deceleration before and after the walkway, respectively. Gait speed was assessed under 3 walking conditions: usual gait speed (walk at normal pace from a standing start, measured as a mean of 2 walks) and 2 challenge paradigms, dual-task gait speed (walk at normal pace while reciting alternate letters of the alphabet out loud, starting with the letter "A," measured as a mean of 2 walks) and maximum gait speed (walk as fast as safely possible, measured as a mean of 3 walks). We calculated the mean of the 3 individual walk conditions to generate our primary measure of composite gait speed (Rasmussen et al., 2019).
Steps in Place	The 2-min step test was measured as the number of times a participant lifted their right knee to mid-thigh height (measured as the height half-way between the knee cap and the iliac crest) in 2 minutes at a self-directed pace (Jones and Rikli, 2002; Rikli and Jones, 1999).

Chair Stands	Chair rises were measured as the number of stands a participant completed in 30 seconds from a seated position (Jones et al., 1999; Jones and Rikli, 2002).
Grip Strength	Handgrip strength was measured for the dominant hand (elbow held at 90°, upper arm held tight against the trunk) as the maximum value achieved across three trials using a Jamar digital dynamometer (Mathiowetz et al., 1985; Rantanen T et al., 1999).
Physical Limitations	Physical limitations were measured with the 10-item RAND 36-Item Health Survey 1.0 physical functioning scale (Ware and Sherbourne, 1992). Participant responses ("limited a lot", "limited a little", "not limited at all") assessed their difficulty with completing various activities, e.g., climbing several flights of stairs, walking more than 1 km, participating in strenuous sports, etc. Scores were reversed to reflect physical limitations so that a high score indicates more limitations.
Decline in Physical Functioning	Tests of balance and grip strength and interviews about physical limitations were completed at both the age-38 and age-45 Dunedin Study assessments. We measured decline across the 7-year measurement interval by subtracting the age-38 test score from the age-45 test score.
Cognitive Functioning (N=795 with mPoA data)	
Cognitive Functioning	The Wechsler Adult Intelligence Scale-IV (WAIS-IV) (Wechsler, 2008) was administered to the participants at age 45 years, yielding the IQ. In addition to full scale IQ, the WAIS-IV measures four specific domains of cognitive function: Processing Speed, Working Memory, Perceptual Reasoning, and Verbal Comprehension.
Cognitive Decline	IQ is a highly reliable measure of general intellectual functioning that captures overall ability across differentiable cognitive functions. We measured IQ from the individually administered Wechsler Intelligence Scale for Children-Revised (WISC-R; averaged across ages 7, 9, 11, and 13)(Wechsler, 2003) and the Wechsler Adult Intelligence Scale-IV (WAIS-IV; age 45) (Wechsler, 2008). We measured IQ decline by comparing scores from the WISC-R and the WAIS-IV.
Subjective Signs of Aging (N=802 with mPoA data)	
Self-rated Health	Study members rated their health on a scale of 1-5 (poor, fair, good, very good, or excellent).
Facial Aging	Facial Aging was based on ratings by an independent panel of 8 raters of each participant's facial photograph. Facial Aging was based on two measurements of perceived age. First, Age Range was assessed by an independent panel of 4 raters, who were presented with standardized (non-smiling) facial photographs of participants and were kept blind to their actual age. Raters used a Likert scale to categorize each participant into a 5-year age range (i.e., from 20-24 years old up to 70+ years old) (interrater reliability = .77). Scores for each participant were averaged across all raters. Second, Relative Age was assessed by a different panel of 4 raters, who were told that all photos were of people aged 45 years old. Raters then used a 7-item Likert scale to assign a "relative age" to each participant (1="young looking", 7="old looking") (interrater

	reliability = .79). The measure of perceived age at 45 years, Facial Age, was derived by standardizing and averaging Age Range and Relative Age scores.
Subjective Decline	Self-rated Health and Facial Aging were measured at both the age-38 and age-45 assessments. We measured decline in self-rated health as incident fair/poor health reported at the age-45 assessment. We measured acceleration in Facial Aging by computing the difference in Facial Aging Z-scores between the age-45 and age-38 assessments.

Understanding Society is an ongoing panel study of the United Kingdom population (https://www.understandingsociety.ac.uk/). During 2010-12, participants were invited to take part in a nurse's exam involving a blood draw. Of the roughly 20,000 participants who provided clinical data in this exam, methylation data have been generated for just under 1,200. We analyzed data from 1,175 participants with available methylation and blood chemistry data. Documentation of the methylation (University of Essex, n.d.) and blood chemistry (University of Essex, n.d.) data resource is available online (https://www.understandingsociety.ac.uk/sites/default/files/downloads/documentation/health/user-guides/7251-UnderstandingSociety-Biomarker-UserGuide-2014.pdf).

Methylation Clocks. We computed the methylation clocks proposed by Horvath, Hannum, and Levine using the methylation data provided by Understanding Society and published algorithms (Hannum et al., 2013; Horvath, 2013; Levine et al., 2018).

Klemera-Doubal method (KDM) Biological Age. We measured KDM Biological age from blood chemistry, systolic blood pressure, and lung-function data using the algorithm proposed by Klemera and Doubal (Klemera and Doubal, 2006) trained in data from the NHANES following the method originally described by Levine (Levine, 2013) and using the dataset compiled by Hastings (Hastings et al., 2019). We included 8 of Levine's original 10 biomarkers in the algorithm: albumin, alkaline phosphatase (log), blood urea nitrogen, creatinine (log), C-reactive protein (log), HbA1C, systolic blood pressure, and forced expiratory volume in 1 second (FEV_1). We omitted total cholesterol because of evidence this biomarker shows different directions of association with aging in younger and older adults (Arbeev et al., 2016). Cytomegalovirus optical density was not available in the Understanding Society database.

Self-rated Health. Understanding Society participants rated their health as excellent, very-good, good, fair, or poor. We standardized this measure to have Mean=0, Standard Deviation=1 for analysis.

The Environmental Risk Longitudinal Twin Study tracks the development of a birth cohort of 2,232 British participants. The sample was drawn from a larger birth register of twins born in England and Wales in 1994-1995. Full details about the sample are reported elsewhere (Moffitt and E-risk Team, 2002). Briefly, the E-Risk sample was constructed in 1999-2000, when 1,116 families (93% of those eligible) with same-sex 5-year-old twins participated in home-visit assessments. This sample comprised 56% monozygotic (MZ) and 44% dizygotic (DZ) twin pairs; sex was evenly distributed within zygosity (49% male). Families were recruited to represent the

UK population of families with newborns in the 1990s, on the basis of residential location throughout England and Wales and mother's age. Teenaged mothers with twins were over-selected to replace high-risk families who were selectively lost to the register through non-response. Older mothers having twins via assisted reproduction were under-selected to avoid an excess of well-educated older mothers. The study sample represents the full range of socioeconomic conditions in the UK, as reflected in the families' distribution on a neighborhood-level socioeconomic index (ACORN [A Classification of Residential Neighborhoods], developed by CACI Inc. for commercial use): 25.6% of E-Risk families lived in "wealthy achiever" neighborhoods compared to 25.3% nationwide; 5.3% vs. 11.6% lived in "urban prosperity" neighborhoods; 29.6% vs. 26.9% lived in "comfortably off" neighborhoods; 13.4% vs. 13.9% lived in "moderate means" neighborhoods, and 26.1% vs. 20.7% lived in "hard-pressed" neighborhoods. E-Risk underrepresents "urban prosperity" neighborhoods because such households are likely to be childless.

Home-visits assessments took place when participants were aged 5, 7, 10, 12 and, most recently, 18 years, when 93% of the participants took part. At ages 5, 7, 10, and 12 years, assessments were carried out with participants as well as their mothers (or primary caretakers); the home visit at age 18 included interviews only with participants. Each twin was assessed by a different interviewer. These data are supplemented by searches of official records and by questionnaires that are mailed, as developmentally appropriate, to teachers, and co-informants nominated by participants themselves. The Joint South London and Maudsley and the Institute of Psychiatry Research Ethics Committee approved each phase of the study. Parents gave informed consent and twins gave assent between 5-12 years and then informed consent at age 18. Study data may be accessed through agreement with the Study investigators (https://moffittcaspi.trinity.duke.edu/research-topics/erisk).

Childhood Socioeconomic Status (SES) was defined through a standardized composite of parental income, education, and occupation (Trzesniewski et al., 2006). The three SES indicators were highly correlated (r=0.57–0.67) and loaded significantly onto one factor. The population-wide distribution of the resulting factor was divided in tertiles for analyses.

Childhood Victimization. As previously described (Danese et al., 2016), we assessed exposure to six types of childhood victimization between birth to age 12: exposure to domestic violence between the mother and her partner, frequent bullying by peers, physical and sexual harm by an adult, and neglect.

CALERIE. The CALERIE trial is described in detail elsewhere (Ravussin et al., 2015). Briefly, N=220 normal-weight ($22.0 \leq$ BMI < 28 kg/m^2) participants (70% female, 77% white) aged 21-50 years at baseline were randomized to caloric restriction or ad libitum conditions with a 2:1 ratio (n=145 to caloric restriction, n=75 to ad libitum). "*Ad libitum*" (normal) caloric intake was determined from two consecutive 14-day assessments of total daily energy expenditure using doubly labeled water (Redman et al., 2014). Average percent caloric restriction over six-month intervals was retrospectively calculated by the intake-balance method with simultaneous measurements of total daily energy expenditure using doubly labeled water and changes in body composition (Racette et al., 2012; Wong et al., 2014). Over the course of the trial, participants in the caloric-restriction arm averaged 12% reduction in caloric intake (about half

the prescribed reduction). Participants in the ad libitum condition reduced caloric intake by <2% (Ravussin et al., 2015). CALERIE data are available at https://calerie.duke.edu/samples-data-access-and-analysis.

Klemera-Doubal method (KDM) Biological Age. KDM Biological age was measured according to the procedure described in our previous article (Belsky et al., 2017). Briefly, we computed KDM Biological Age from CALERIE blood chemistry and blood pressure data using the algorithm proposed by Klemera and Doubal (Klemera and Doubal, 2006) trained in data from the NHANES following the method originally described by Levine (Levine, 2013) and NHANES data from years matched to the timing of the CALERIE Trial. We included 8 of Levine's original 10 biomarkers in the algorithm: albumin, alkaline phosphatase (log), blood urea nitrogen, creatinine (log), C-reactive protein (log), HbA1C, systolic blood pressure, and total cholesterol. Cytomegalovirus optical density and lung function were not measured in CALERIE. We supplemented the algorithm with data on uric acid and white blood cell count.

2. Sensitivity analyses.

We tested sensitivity of mPoA to alternative methods of normalizing DNA methylation data. We normalized data using the 'methylumi' and 'minfi' packages and computed correlations between mPoA measures derived from these two datasets. The correlation was r=0.94.

The elastic net model selected 46 CpGs to compose the mPoA. One of these CpGs, cg11897887, has been identified as an mQTL (Volkov et al., 2016). To evaluate sensitivity of results to the exclusion of this SNP, we computed a version of the mPoA excluding this CpG and repeated analysis. This version of the score was correlated with the full mPoA at r=1. Results were the same in analyses with both versions (available from the authors upon request).

Another CpG selected in the elastic net, cg05575921, is located within the gene *AHRR*, previously identified as a methylation site modified by tobacco exposure and associated with lung cancer and other chronic disease, e.g. (Fasanelli et al., 2015; Reynolds et al., 2015). We tested sensitivity of results to the exclusion of this probe using the method described above. This version of the score was correlated with the full mPoA at r=0.94. Again, results were the same in analyses with both versions (available from the authors upon request).

3. Bootstrap repetition analysis to estimate out-of-sample correlation between mPoA and longitudinal Pace of Aging.

The Dunedin Study is the only dataset to include measured 12-year longitudinal Pace of Aging. To estimate the out-of-sample correlation between mPoA and the original Pace of Aging measure, we conducted 90/10 crossfold validation analysis. We randomly selected 90% of the cohort to serve as the "training" sample in which the mPoA algorithm was developed. We used the remaining 10% to form a "test" sample to estimate the correlation between mPoA and Pace of Aging. We repeated this analysis across 100 bootstrap repetitions. In each repetition, we randomly sampled 90% of the cohort to use in the training analysis and reserved the remaining 10% for testing.

The mPoA algorithms developed across the 100 bootstrap repetitions included different sets of CpGs (range of 21-209 CpGs selected, M=54, SD=27 CpGs). However, the resulting

algorithms were highly correlated (mean pairwise r=0.90, SD=0.14). The average correlation between the 90%-trained mPoA and longitudinal Pace of Aging in the 10% test samples was (r=0.33, SD=0.10). Details are reported in **Supplemental Figure 2**.

SUPPLEMENTAL MATERIALS REFERENCES

Arbeev KG, Ukraintseva SV, Yashin AI. 2016. Dynamics of biomarkers in relation to aging and mortality. *Mechanisms of Ageing and Development* **156**:42–54. doi:10.1016/j.mad.2016.04.010

Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE. 2015. Quantification of biological aging in young adults. *Proc Natl Acad Sci USA* **112**:E4104-4110. doi:10.1073/pnas.1506264112

Belsky DW, Huffman KM, Pieper CF, Shalev I, Kraus WE. 2017. Change in the Rate of Biological Aging in Response to Caloric Restriction: CALERIE Biobank Analysis. *J Gerontol A Biol Sci Med Sci* **73**:4–10. doi:10.1093/gerona/glx096

Bohannon RW, Larkin PA, Cook AC, Gear J, Singer J. 1984. Decrease in timed balance test scores with aging. *Phys Ther* **64**:1067–1070.

Danese A, Moffitt TE, Arseneault L, Bleiberg BA, Dinardo PB, Gandelman SB, Houts R, Ambler A, Fisher HL, Poulton R. 2016. The origins of cognitive deficits in victimized children: implications for neuroscientists and clinicians. *American journal of psychiatry* **174**:349–361.

Fasanelli F, Baglietto L, Ponzi E, Guida F, Campanella G, Johansson Mattias, Grankvist K, Johansson Mikael, Assumma MB, Naccarati A, Chadeau-Hyam M, Ala U, Faltus C, Kaaks R, Risch A, Stavola BD, Hodge A, Giles GG, Southey MC, Relton CL, Haycock PC, Lund E, Polidoro S, Sandanger TM, Severi G, Vineis P. 2015. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. *Nature Communications* **6**:10192. doi:10.1038/ncomms10192

Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan J-B, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* **49**:359–367. doi:10.1016/j.molcel.2012.10.016

Hastings WJ, Shalev I, Belsky DW. 2019. Comparability of biological aging measures in the National Health and Nutrition Examination Study, 1999–2002. *Psychoneuroendocrinology* **106**:171–178. doi:10.1016/j.psyneuen.2019.03.012

Horvath S. 2013. DNA methylation age of human tissues and cell types. *Genome Biology* **14**:R115. doi:10.1186/gb-2013-14-10-r115

Jones CJ, Rikli RE. 2002. Measuring functional fitness in older adults. *The Journal on active aging* **1**:24–30.

Jones CJ, Rikli RE, Beam WC. 1999. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Research quarterly for exercise and sport* **70**:113–119.

Klemera P, Doubal S. 2006. A new approach to the concept and computation of biological age. *Mechanisms of Ageing and Development* **127**:240–248. doi:10.1016/j.mad.2005.10.004

Levine ME. 2013. Modeling the rate of senescence: Can estimated biological age predict mortality more accurately than chronological age? *J Gerontol A Biol Sci Med Sci* **68**:667–674. doi:10.1093/gerona/gls233

Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L, Horvath S. 2018. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)* **10**:573–591. doi:10.18632/aging.101414

Mathiowetz V, Kashman N, Volland G, Weber K, Dowe M, Rogers S. 1985. Grip and pinch strength: normative data for adults. *Arch Phys Med Rehabil* **66**:69–74.

Moffitt TE, E-risk Team. 2002. Teen-aged mothers in contemporary Britain. *Journal of Child Psychology and Psychiatry* **43**:727–742.

Poulton R, Moffitt TE, Silva PA. 2015. The Dunedin Multidisciplinary Health and Development Study: overview of the first 40 years, with an eye to the future. *Soc Psychiatry Psychiatr Epidemiol* **50**:679–693. doi:10.1007/s00127-015-1048-8

Racette SB, Das SK, Bhapkar M, Hadley EC, Roberts SB, Ravussin E, Pieper C, DeLany JP, Kraus WE, Rochon J, Redman LM, Group TCS. 2012. Approaches for quantifying energy intake and %calorie restriction during calorie restriction interventions in humans: the multicenter CALERIE study. *American Journal of Physiology - Endocrinology and Metabolism* **302**:E441–E448. doi:10.1152/ajpendo.00290.2011

Rantanen T, Guralnik JM, Foley D, et al. 1999. MIdlife hand grip strength as a predictor of old age disability. *JAMA* **281**:558–560. doi:10.1001/jama.281.6.558

Rasmussen LJH, Caspi A, Ambler A, Broadbent JM, Cohen HJ, d'Arbeloff T, Elliott M, Hancox RJ, Harrington H, Hogan S, Houts R, Ireland D, Knodt AR, Meredith-Jones K, Morey MC, Morrison L, Poulton R, Ramrakha S, Richmond-Rakerd L, Sison ML, Sneddon K, Thomson WM, Hariri AR, Moffitt TE. 2019. Association of Neurocognitive and Physical Function With Gait Speed in Midlife. *JAMA Netw Open* **2**:e1913123–e1913123. doi:10.1001/jamanetworkopen.2019.13123

Ravussin E, Redman LM, Rochon J, Das SK, Fontana L, Kraus WE, Romashkan S, Williamson DA, Meydani SN, Villareal DT, Smith SR, Stein RI, Scott TM, Stewart TM, Saltzman E, Klein S, Bhapkar M, Martin CK, Gilhooly CH, Holloszy JO, Hadley EC, Roberts SB, CALERIE Study Group. 2015. A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. *J Gerontol A Biol Sci Med Sci* **70**:1097–1104. doi:10.1093/gerona/glv057

Redman LM, Kraus WE, Bhapkar M, Das SK, Racette SB, Martin CK, Fontana L, Wong WW, Roberts SB, Ravussin E, Group for the CS. 2014. Energy requirements in nonobese men and women: results from CALERIE. *Am J Clin Nutr* **99**:71–78. doi:10.3945/ajcn.113.065631

Reynolds LM, Wan M, Ding J, Taylor JR, Lohman K, Su D, Bennett BD, Porter DK, Gimple R, Pittman GS, Wang X, Howard TD, Siscovick D, Psaty BM, Shea S, Burke GL, Jacobs DR, Rich SS, Hixson JE, Stein JH, Stunnenberg H, Barr RG, Kaufman JD, Post WS, Hoeschele I, Herrington DM, Bell DA, Liu Y. 2015. DNA Methylation of the Aryl Hydrocarbon Receptor Repressor Associations With Cigarette Smoking and Subclinical Atherosclerosis. *Circ Cardiovasc Genet* **8**:707–716. doi:10.1161/CIRCGENETICS.115.001097

Rikli RE, Jones CJ. 1999. Functional fitness normative scores for community-residing older adults, ages 60-94. *Journal of aging and physical activity* **7**:162–181.

Springer BA, Marin R, Cyhan T, Roberts H, Gill NW. 2007. Normative values for the unipedal stance test with eyes open and closed. *J Geriatr Phys Ther* **30**:8–15.

Trzesniewski KH, Moffitt TE, Caspi A, Taylor A, Maughan B. 2006. Revisiting the association between reading achievement and antisocial behavior: New evidence of an environmental explanation from a twin study. *Child Development* **77**:72–88.

University of Essex. n.d. Institute for Social and Economic Research, NatCen Social Research, and University of Exeter Medical School. (2017). Understanding Society: DNA methylation data based on Illumina methylation EPIC array.

University of Essex. n.d. Institute for Social and Economic Research and National Centre for Social Research, Understanding Society: Waves 2 and 3 Nurse Health Assessment, 2010-2012, 3rd Edition. ed. UK Data Service.

Vereeck L, Wuyts F, Truijen S, Van de Heyning P. 2008. Clinical assessment of balance: Normative data, and gender and age effects. *International Journal of Audiology* **47**:67–75. doi:10.1080/14992020701689688

Volkov P, Olsson AH, Gillberg L, Jørgensen SW, Brøns C, Eriksson K-F, Groop L, Jansson P-A, Nilsson E, Rönn T, Vaag A, Ling C. 2016. A Genome-Wide mQTL Analysis in Human Adipose Tissue Identifies Genetic Variants Associated with DNA Methylation, Gene Expression and Metabolic Traits. *PLOS ONE* **11**:e0157776. doi:10.1371/journal.pone.0157776

Ware JE Jr, Sherbourne CD. 1992. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item Selection. *Medical Care* **30**:473–483.

Wechsler D. 2008. Wechsler Adult Intelligence Scale, 4th ed. San Antonio, TX: Pearson Assessment.

Wechsler D. 2003. Wechsler Intelligence Scale for Children, 4th (UK Version). ed. San Antonio, TX: Harcourt Assessment.

Wong WW, Roberts SB, Racette SB, Das SK, Redman LM, Rochon J, Bhapkar MV, Clarke LL, Kraus WE. 2014. The doubly labeled water method produces highly reproducible longitudinal results in nutrition studies. *J Nutr* **144**:777–783. doi:10.3945/jn.113.187823

Supplemental Table 1. Comparison of age-38 mPoA and age 26-38 Pace of Aging effect-sizes for analysis of healthspan-related characteristics. The table shows effect-sizes for analysis of healthspan-related characteristics at age 45 years. mPoA was measured from blood DNA methylation collected when Study members were aged 38 years. Pace of Aging was measured from longitudinal change in 18 biomarkers across measurements made at ages 26, 32, and 38 years. Sample restricted to N=810 Study members with data on mPoA and Pace of Aging. Effect-sizes correspond to the analysis reported in **Supplemental Figure 1B** and are reported in terms of standard deviation differences in the age-45 outcome associated with a 1 standard deviation increase in mPoA (i.e. effect-sizes are interpretable as Pearson r). All models were adjusted for sex.

	mPoA Effect-Sizes			**Pace of Aging Effect-Sizes**		
	r	**95% CI**	**p-value**	**r**	**95% CI**	**p-value**
Balance	-0.29	[-0.36, -0.23]	5.13E-18	-0.21	[-0.27, -0.15]	9.32E-11
Gait Speed	-0.27	[-0.33, -0.20]	2.15E-14	-0.18	[-0.25, -0.11]	4.77E-07
Steps in Place	-0.22	[-0.29, -0.15]	1.70E-09	-0.16	[-0.23, -0.08]	3.98E-05
Chair Stands	-0.19	[-0.26, -0.12]	8.96E-08	-0.18	[-0.25, -0.11]	5.01E-07
Grip Strength	-0.05	[-0.12, 0.02]	0.138	-0.07	[-0.14, 0.00]	0.060
Physical Limitations	0.21	[0.12, 0.30]	3.08E-06	0.15	[0.07, 0.22]	1.77E-04
Perceptual Reasoning	-0.29	[-0.35, -0.22]	2.51E-17	-0.19	[-0.26, -0.11]	6.60E-07
Working Memory	-0.21	[-0.28, -0.15]	4.35E-10	-0.16	[-0.23, -0.10]	1.49E-06
Processing Speed	-0.15	[-0.22, -0.09]	6.86E-06	-0.16	[-0.23, -0.08]	3.46E-05
Self-rated Health	-0.28	[-0.35, -0.20]	1.86E-13	-0.24	[-0.31, -0.16]	6.55E-10
Facial Aging	0.35	[0.28, 0.43]	9.94E-19	0.26	[0.19, 0.34]	1.34E-11

Supplemental Table 2. Effect-sizes for associations of mPoA and measures of epigenetic age acceleration with KDM Biological Age, and self-rated health in the Understanding Society Study. The table reports standardized regression coefficients and their standard errors from linear regression models in which the predictor was the methylation measure listed in the far-left column and the dependent variable was either KDM Biological Age (left side coefficients) or self-rated health (right side coefficients). All models included sex and chronological age as covariates. Model 2 included covariates for cell counts estimated from the methylation data. Model 3 included covariates adjusting for smoking status. Model 4 included nonsmokers only. Prior to analysis, Horvath, Hannum, and Levine Clock values were residualized for chronological age. All methylation variables were residualized for plate number.

	KDM Biological Age			**Self-rated Health**		
	b	**SE**	**p-value**	**b**	**SE**	**p-value**
M1. Models adjusted for age and sex						
mPoA	0.200	0.028	1.67E-12	-0.217	0.029	1.04E-13
Horvath Clock	0.050	0.030	0.090	-0.003	0.028	0.921
Hannum Clock	0.051	0.034	0.130	-0.042	0.029	0.143
Levine Clock	0.150	0.030	8.26E-07	-0.088	0.027	1.20E-03
N	1164			1175		
M2. Models adjusted for age, sex, and estimated cell counts						
mPoA	0.186	0.031	2.37E-09	-0.183	0.032	1.91E-08
Horvath Clock	0.061	0.030	0.045	-0.006	0.029	0.839
Hannum Clock	0.034	0.042	0.425	-0.006	0.036	0.867
Levine Clock	0.151	0.033	5.53E-06	-0.070	0.029	0.016
N	1164			1175		
M3. Models adjusted for age, sex, and smoking						
mPoA	0.207	0.037	2.78E-08	-0.155	0.034	7.44E-06
Horvath Clock	0.044	0.029	0.134	0.004	0.028	0.881
Hannum Clock	0.046	0.034	0.167	-0.036	0.028	0.196
Levine Clock	0.135	0.031	1.15E-05	-0.067	0.026	0.011
N	1160			1171		
M4. Models adjusted for age, sex; non-smokers						
mPoA	0.252	0.057	1.05E-05	-0.141	0.057	0.014
Horvath Clock	0.051	0.041	0.219	-0.061	0.041	0.141
Hannum Clock	0.082	0.049	0.092	-0.043	0.045	0.343
Levine Clock	0.101	0.040	0.012	-0.079	0.039	0.043
N	495			498		

Effect-sizes are standardized regression coefficients. Effect-sizes in gray text indicate p-value >0.05. Ns reflect samples with complete data for regression analysis.

Supplemental Table 3. Effect sizes for associations of socioeconomic status (SES) and victimization exposure with mPoA at age 18 in the E-Risk Study. The table shows effect-sizes reported as standardized regression coefficients (b) and 95% confidence intervals (CIs) from models in which childhood family socioeconomic status and victimization were predictor variables and the dependent variable was the mPoA. Model 1 included covariate adjustment for sex. Model 2 additionally included covariates for estimated cell counts. (Chronological age was the same for all twins in the birth cohort.) Model 3 included covariates adjusting for smoking status. Model 4 included nonsmokers only. mPoA was residualized for plate number prior to analysis. Standard errors were clustered at the family level to account for non-independence of twin data.

	b	95% CI	p-value
SES			
M1 adjusted for sex and batch	-0.18	[-0.23, -0.13]	4.70E-11
M2 adjusted for sex, batch, and estimated cell counts	-0.17	[-0.21, -0.12]	9.45E-14
M3 adjusted for sex, batch, and smoking status	-0.09	[-0.14, -0.04]	3.77E-04
M4 adjusted for sex and batch, nonsmokers only	-0.08	[-0.13, -0.03]	3.31E-03
Victimization			
M1 adjusted for sex and batch	0.13	[0.08, 0.18]	1.42E-06
M2 adjusted for sex, batch, and estimated cell counts	0.12	[0.07, 0.16]	1.48E-10
M3 adjusted for sex, batch, and smoking status	0.04	[-0.01, 0.09]	0.083
M4 adjusted for sex and batch, nonsmokers only	0.07	[0.01, 0.12]	1.82E-02

Supplemental Table 4. Characteristics of participants in the CALERIE Trial and subsample with baseline methylation data. The top panel of the table shows demographic characteristics and measured rate of aging for participants in the Ad Libitum (usual diet) and Caloric Restriction arms of the CALERIE Trial. The bottom panel shows the same data for the subset of participants for whom methylation data were available from the baseline CALERIE assessment.

CALERIE Trial

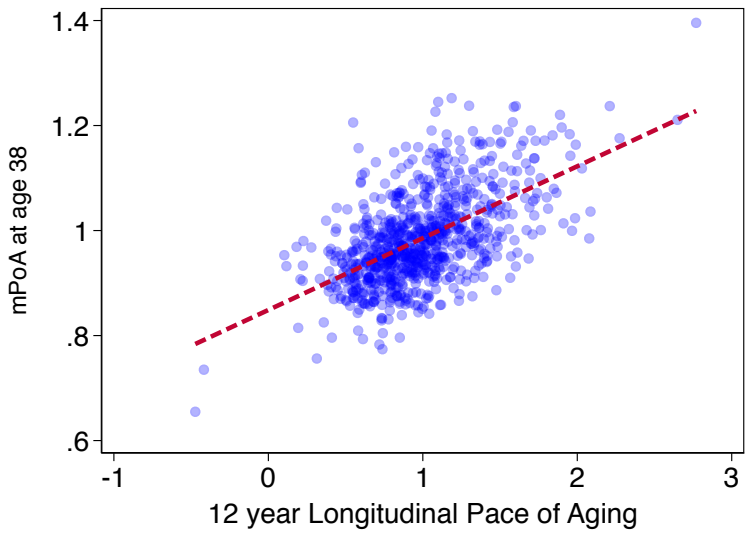
	Ad Libitum (n=75)		**Caloric Restriction (n=145)**	
	M	**SD**	**M**	**SD**
Age	37.86	6.94	37.89	7.32
Male (%)	0.29	0.46	0.31	0.46
KDM Biological Age	36.31	6.98	36.59	6.92
Annual Change in KDM Biological Age	0.72	0.95	0.11	0.96

Sub-sample with baseline methylation data

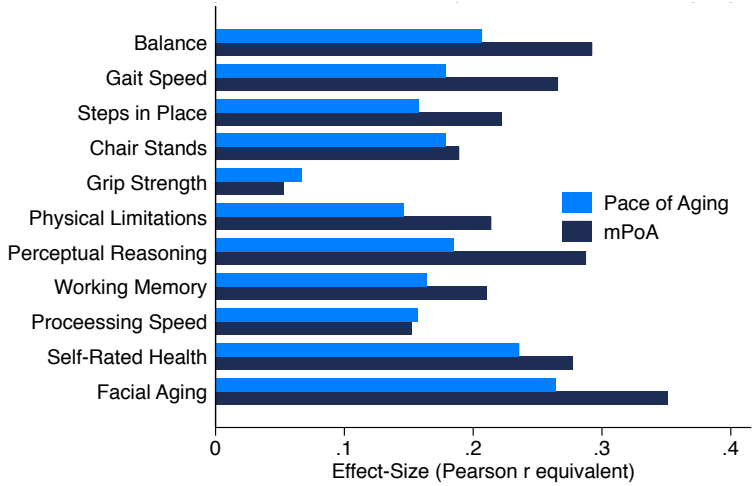
	Ad Libitum (n=68)		**Caloric Restriction (n=118)**	
	M	**SD**	**M**	**SD**
Age	38.16	7.07	38.37	7.30
Male (%)	0.31	0.47	0.31	0.47
KDM Biological Age	36.46	6.99	37.19	6.73
Annual Change in KDM Biological Age	0.73	0.95	0.12	1.03
mPoA	1.00	0.05	1.01	0.06

Supplemental Figure 1. Association of mPoA with Pace of Aging in the Dunedin Study and Comparison of Effect-sizes for mPoA and Pace of Aging. Panel A shows a scatterplot and fitted regression slope for the association between the methylation Pace of Aging (mPoA) trained in the full Dunedin sample and the Pace of Aging measure that was used as the criterion in the training analysis. The correlation of r=0.56 is a within-training sample estimate of association and therefore reflects the upper-bound of possible true association between the mPoA and Pace of Aging. The figure shows data for the N=810 Dunedin Study members included in the training analysis. Panel B graphs effect-sizes for associations of age-38 mPoA and 12-year longitudinal Pace of Aging with measures of physical and cognitive functioning and subjective signs of aging measured at the Dunedin Study age-45 assessment. Effect-sizes and 95% Cis are also reported in Supplemental Table 1.

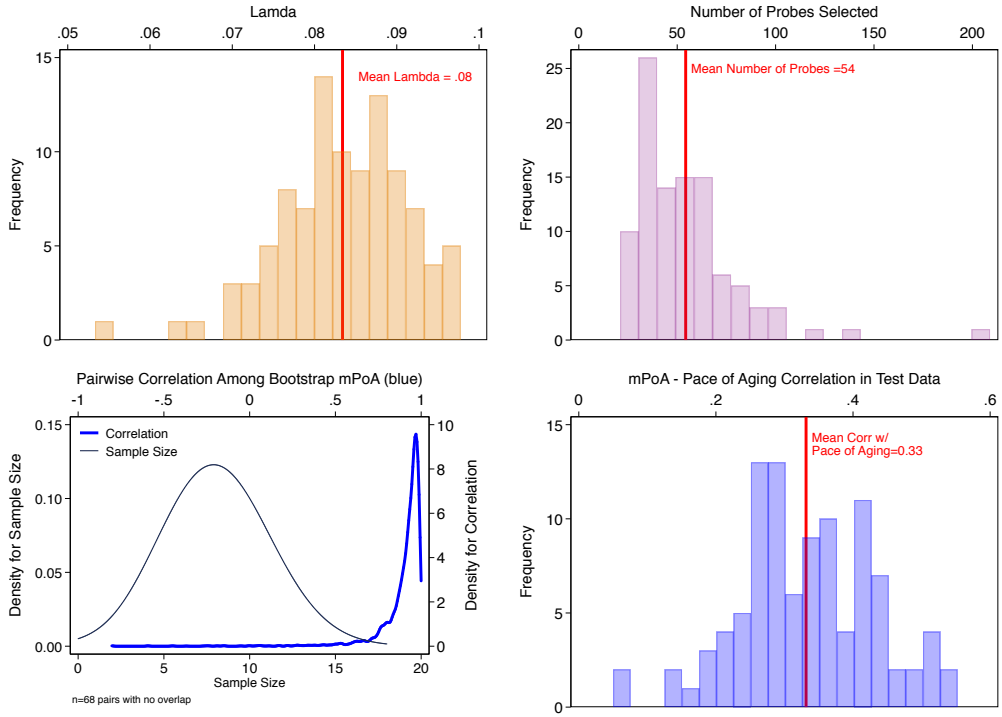
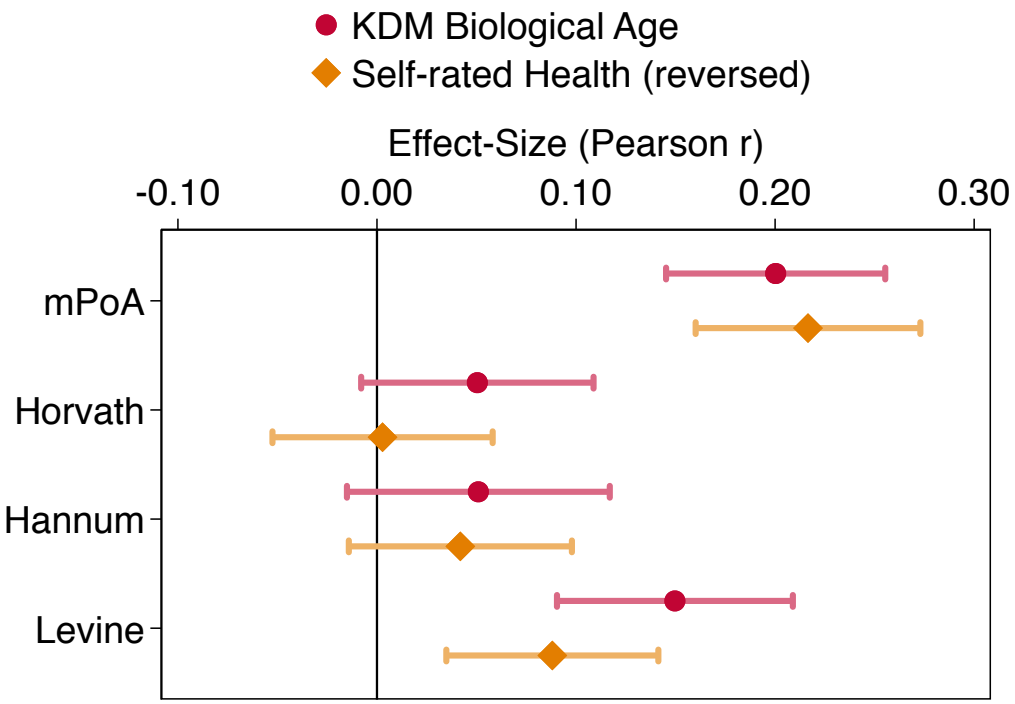
A.



B.



Supplemental Figure 2. Bootstrap repetition analysis to estimate out-of-sample correlation between mPoA and longitudinal Pace of Aging. The figure shows results from our Bootstrap crossfold validation analysis to evaluate mPoA within the Dunedin Study. Panel A (top left) shows the distribution of elastic-net regression Lambda values estimated across the 100 bootstrap training samples. Panel B (top right) shows the distribution of the number of probes selected by the elastic net regression to compose mPoA across the 100 bootstrap training samples. Panel C (bottom left) graphs two densities illustrating analysis of intercorrelations among the different mPoA algorithms estimated across the bootstrap repetitions. The first density (thin gray line, left side Y axis) shows the distribution of sample sizes for calculation of correlations between pairs of mPoA algorithms. There were 68 pairs of mPoA algorithms for which there were no overlapping samples (i.e. sample size=0). The second density (thick blue line, right side Y axis) shows the distribution of pairwise correlations. Nearly all of the correlations were r>0.95. Panel D (bottom right) shows the distribution of correlations between mPoA and Pace of Aging in the 100 bootstrap test samples.



Supplemental Figure 3. Effect-sizes for association of mPoA and the epigenetic clocks proposed by Horvath, Hannum et al., and Levine et al. with KDM Biological Age and self-rated health. Effect-sizes are standardized regression coefficients (interpretable as Pearson r). Effect-sizes are computed for "epigenetic-age acceleration" values of the clocks (i.e. epigenetic ages residualized for chronological age). The dependent variable in analysis of KDM Biological Age was the difference between KDM Biological Age and chronological age. Self-rated health scores were reversed for analysis so that higher values correspond to ratings of poorer health. All models were adjusted for chronological age and sex.