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# Comparability of biological aging measures in the National Health and Nutrition Examination Study, 1999–2002

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ABSTRACT

*Background:* Biological processes of aging are thought to be modifiable causes of many different chronic diseases. Measures of biological aging could provide sensitive endpoints for studies of risk factors hypothesized to shorten healthy lifespan and/or interventions that extend it. But uncertainty remains about how to measure biological aging and if proposed measures assess the same thing.

*Method:* We tested four proposed measures of biological aging that could be quantified with available data from the National Health and Nutrition Examination Survey (NHANES), Klemera-Doubal method (KDM) Biological Age, homeostatic dysregulation, Levine Method (LM) Biological Age, and leukocyte telomere length.

*Results:* We analyzed data collected during 1999–2002, when all four biological aging meausres could be taken. Participants' KDM biological ages, homeostatic dysregulation levels, LM biological ages, and telomere length were all correlated with their chronological ages. KDM Biological Age, homeostatic dysregulation, and LM Biological Age were all correlated with one another, but these measures were uncorrelated with telomere length. Participants' with more advanced biological aging performed worse on tests of physical, cognitive, and perceptual functioning and reported more limitations to their daily activities and more pain, and rated themselves as being in worse health. In parallel, participants with risk factors for shorter healthy lifespan exhibited more advanced biological aging. In both sets of analyses, effect-sizes tended to be larger for KDM Biological Age, homeostatic dysregulation, and LM Biological Age as compared to telomere length.

*Discussion:* The cellular-level aging biomarker telomere length may measure different aspects of the aging process as compared to the patient-level physiological composite measures KDM Biological Age, homeostatic dysregulation, and LM Biological Age. Studies aiming to test if risk factors accelerate aging or if interventions may slow aging should not treat proposed measures of aging as interchangeable.

## 1. Introduction

The global population is aging. Advances in medical care and public health have led to increases in expected human lifespans (Christensen et al., 2009). By 2050, the proportion of the population over age 60 is expected to double, and the number of individuals aged 80 and above is expected to triple (DeSA, 2017). This population aging threatens increased burden of disease and disability (Vos et al., 2012), posing systemic risks to societies around the world (Harper, 2014). Treatment of individual diseases is likely to generate only modest improvements in overall healthy lifespan (hereafter "healthspan") because individuals who avoid one disease are often sickened by another (Goldman et al., 2013). Instead, it is proposed that the most effective means to extend healthspan is to slow aging itself (Burch et al., 2014; Olshansky and Carnes, 2017).

Aging is increasingly understood as a biological process, a gradual and progressive decline in system integrity with the passage of time that is mediated by an accumulation of molecular changes (Kennedy et al., 2014; Kirkwood, 2005; Lopez-Otin et al., 2013). Evidence from animal studies suggests these molecular changes can be slowed or reversed, generating increases in healthy lifespan (Fontana et al., 2014; Kaeberlein et al., 2015; Kirkwood, 2005). A key challenge in translating these therapies to humans is that humans live too long for trials to test effects of therapies on healthspan extension. An appealing possibility is that trials could focus on surrogate endpoints indicative of long-term effects on healthspan, but measurable within timescales of years rather than decades (Justice et al., 2016). One potential source of such surrogate endpoints are recently proposed methods to quantify the rate of

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biological aging (Belsky et al., 2017a).

Several approaches to developing measures of biological aging have been proposed (Belsky et al., 2017b). Two broad categories of measures are (i) those that assess specific cellular-level changes hypothesized to mediate the biological process of aging, such as epigenetic modifications and telomere erosion (Harley et al., 1992; Horvath and Raj, 2018); and (ii) those that assess patient-level declines in system integrity hypothesized to arise from molecular changes in aging, such as indices that combine information from multiple clinical tests (Fried et al., 2001; Sebastiani et al., 2017). Biological aging measures from both categories correlate with chronological age and relate to deficits in functional capacities, disease status, and mortality (Jylhava et al., 2017). But it is not clear that different measures quantify the same process of biological aging. In a recent analysis of the Dunedin birth cohort at age 38 years, we found little evidence that cellular-level measures such as telomere length and epigenetic clock ages reflected similar aging processes as patient-level measures composed of multiple clinical biomarkers (Belsky et al., 2017b). A parallel finding was reported from the Framingham Heart Study (Murabito et al., 2018).

To build on findings from these two place-based cohort studies, we took up the same question using data from a large, US-based sample of mixed chronological age drawn from the National Health and Nutrition Examination Surveys (NHANES). NHANES data allowed us to examine biological aging across the adult lifespan in a sample drawn from many different geographic locations in the US. We analyzed four biological aging measures that could be quantified with available NHANES data, Klemera-Doubal method (KDM) Biological Age, homeostatic dysregulation, Levine-method (LM) Biological Age, and leukocyte telomere length. Our analysis proceeded in three steps. First, we tested associations among chronological age and the different biological aging measures. Second, we tested associations between biological aging measures and measures of functional capacities that mediate age-related disability, hereafter referred to as “healthspan-related characteristics”. Third, we tested the extent to which biological aging measures were associated with risk factors for shorter healthy lifespan, including low educational attainment, material and social resource deficits, and mental health problems.

## 2. Methods

### 2.1. Sample

Data were from the National Health and Nutrition Examination Surveys (NHANES), an ongoing nationally-representative, cross-sectional survey conducted by the US Centers for Disease Control and Prevention. NHANES administers questionnaires during in-home interviews and conducts health examinations, including blood draws, in a mobile examination center. Details of recruitment procedures and study design are available from the Centers for Disease Control and Prevention (Centers for Disease Control and Prevention, 2018). We conducted analyses to test hypotheses about measures of biological aging using data from adults aged 20 and older participating in the NHANES panels collected during 1999–2002 and for whom all biological aging measures could be computed (N = 6731, 52% male). These NHANES panels were selected for analysis because they are the only panels for which telomere length was measured.

### 2.2. Biological aging measures

We analyzed four biological aging measures. The first three, Klemera-Doubal method (KDM) Biological Age, homeostatic dysregulation, and Levine-method (LM) Biological Age are patient-level measures that combine information from multiple clinical biomarkers to quantify decline in system integrity. The fourth, leukocyte telomere length, is a cellular-level measure reflecting processes of senescence.

***KDM Biological Age and homeostatic dysregulation*** are algorithm-based measures that combine information on the integrity of multiple organ systems in the body (Cohen et al., 2013; Klemera and Doubal, 2006; Levine, 2013). Biological aging measurements made with these algorithms are predictive of morbidity, mortality, and indicators of healthspan in young and older populations (Belsky et al., 2015, 2017b; Brown et al., 2017; Cohen et al., 2014; Levine, 2013; Levine and Crimmins, 2018; Li et al., 2015; Murabito et al., 2018).

KDM Biological Age is computed from an algorithm derived from a series of regressions of individual biomarkers on chronological age in a reference population. Following previous work (Belsky et al., 2017a; Levine, 2013), we formed this reference population from NHANES participants aged 30–75 years who were excluded from our analysis sample (N = 38,028, 49% male; see Appendix Table A.1). An individual’s KDM Biological Age prediction corresponds to the chronological age at which her/his physiology would be approximately normal in the NHANES reference sample.

Homeostatic dysregulation is computed from an algorithm based on Mahalanobis distance (Mahalanobis, 1936) of a panel of biomarkers computed relative to a reference population. Following previous work (Belsky et al., 2017a), we defined our reference population as NHANES participants aged 20–30 years who were not obese and for whom all biomarkers fell within the clinically normal range (N = 1,138, 43% male; see Appendix Table A.4). An individual’s homeostatic dysregulation value quantifies how different their physiology is from this young, healthy NHANES sample.

We calculated KDM Biological Age and homeostatic dysregulation based on a panel of 12 biomarkers measuring system integrity, including cardiovascular, renal, hepatic, immune, and metabolic function: albumin, alkaline phosphatase, blood urea nitrogen, creatinine, C-reactive protein, glycated hemoglobin, uric acid, white blood cell count, lymphocyte percent, mean corpuscular volume, red-cell distribution width, and systolic blood pressure. Biomarkers we selected on the basis of their inclusion in published analyses of biological age (Belsky et al., 2017a; Levine, 2013; Levine et al., 2018) and availability in the NHANES 1999–2002 data. Details on biomarker measurements are available from the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

We conducted analysis to estimate parameters for the KDM Biological Age and homeostatic dysregulation algorithms in data from NHANES III and continuous NHANES panels spanning 2003-2016. Analysis to estimate parameters for the KDM Biological Age and homeostatic dysregulation algorithms are described in detail in Appendix A. Biomarker summary statistics for the final analytical sample (N = 6731) are provided in Appendix Table A.6.

***LM Biological Age*** is computed from an algorithm derived from multivariate analysis of mortality hazards (Levine et al., 2018; Liu et al., 2018). Briefly, Levine and colleagues used machine learning analysis of data from NHANES III to select a parsimonious panel of biomarkers for mortality prediction. The biomarkers selected were albumin, alkaline phosphatase, creatinine, C-reactive protein (log), glucose, white blood cell count, lymphocyte percent, mean corpuscular volume, and red cell distribution width. Next, they fitted a multivariate Gompertz model of mortality hazard to the selected biomarkers and chronological age. To compute the LM Biological Age, parameters from the multivariate model are combined with biomarker and chronological age data from a test sample to compute a predicted hazard of mortality, called a “mortality score.” The mortality score is then converted to a biological age value. This conversion is made based on a univariate Gompertz regression of the mortality hazard on chronological age. Thus, the LM Biological Age is equivalent to the chronological age at which the predicted hazard of mortality would be approximately normal in the NHANES III population. We applied parameters published in Levine and colleagues’ article to compute LM Biological Ages for participants in the 1999–2002 NHANES.

***Telomere length*** is a cellular-level measure of repetitive nucleoprotein regions at chromosome ends (TTAGGG in humans) which

prevent end-to-end fusions and protect against DNA degradation. Successive cell divisions, combined with processes of "wear and tear" gradually erode telomere length across the lifespan (Shalev and Hastings, 2019). This progressive age-dependent shortening of telomeres contributes to cellular senescence, one of the hallmarks of aging (Lopez-Otin et al., 2013; Xu et al., 2018). Among individuals of the same age, those with shorter telomeres are more likely to develop chronic disease and are at increased risk for death (D'Mello et al., 2015; Haycock et al., 2014; Ma et al., 2011; Rode et al., 2015).

Leukocyte Telomere Length was assessed from DNA extracted from whole blood for all participants aged 20 or older which also provided consent to future genetic research (N = 7,827, 48% male). Whole blood comprises two types of cells, leukocytes and red blood cells. Red blood cells lack nuclei and thus lack nuclear DNA. As a result, telomere length assessed in DNA extracted from whole blood is commonly referred to as leukocyte telomere length (LTL). Extracted DNA was stored at −80 °C until it was shipped to the laboratory of Dr. Elizabeth Blackburn at the University of California San Francisco for telomere length analysis. Telomere length was quantified as previously described (Lin et al., 2010) using quantitative polymerase chain reaction to measure telomere length relative to standard reference DNA (T/S ratio) (Cawthon, 2002). The T/S ratio is a semi-quantitative measure which captures average telomere length across all DNA molecules. In other words, the T/S ratio is an approximation of the average telomere length across the 92 chromosome ends of all cells in the sample. Each DNA sample was assayed three times on three different days. For each assay, samples were run in duplicate, resulting in a total of six measurements per sample. Telomere length was calculated as the mean T/S ratio, telomeric content relative to a single-copy gene, across measurements. To reduce skew in the distribution, we log transformed T/S ratio to construct the measure of telomere length used in analyses.

### 2.3. Healthspan-related characteristics

We tested associations of biological aging measures with functional assessments of capacities thought to mediate age-related disability, referred to as "healthspan-related characteristics". Healthspan-related characteristics encompassed three domains: *Physical functioning* was measured with tests of balance, muscle strength (knee extensor peak force), gait speed, and cardiorespiratory fitness ($VO_2$ Max). *Cognitive and perceptual functioning* was measured with the digit symbol coding task from the Wechsler Adult Intelligence Scale (WAIS III), audiometry, and a test of visual acuity. *Subjective functioning and pain* was assessed with measures of self-rated general health, self-reported disability (activities of daily living, ADLs; instrumental activities of daily living, IADLs), self-reports of joint pain and a composite chronic pain variable defined according to American College of Rheumatology criteria (Hardt et al., 2008; Wolfe et al., 1990). Healthspan-related characteristics measures are described in Appendix B. Advanced chronological age was associated with worse performance on all healthspan-related characteristics (Appendix Table B.1).

### 2.4. Life-course risk factors for shorter healthspan

We tested associations of biological aging measures with risk factors known to predict shorter healthspan: low educational attainment, material and social resource deficits, and mental health problems. We measured *educational attainment* using an ordered categorical variable encoding the highest level of education completed by the participant (none, high school graduate, some college coursework, and college graduate). We assessed *material resources* using poverty-income ratio calculated from self-reported family income and food insecurity measured using the Federal Department of Agriculture Food Insecurity instrument (Bickel et al., 2000). We assessed *social resources* using a composite variable derived from social network size and availability of emotional support. We assessed *mental health* problems using the NHANES CIDI, an adapted version of three modules from the World Health Organization Composite International Diagnostic Interview, Version 2.1 (CIDI-Auto 2.1) which addresses diagnoses of panic disorder, generalized anxiety disorder, and depressive disorders (Kessler et al., 1998). Life-course risk factor measures are described in Appendix B and Appendix Table B.2.

### 2.5. Statistical analyses

We focused analysis on the subset of NHANES 1999–2002 participants with available telomere length, KDM Biological Age, homeostatic dysregulation, and LM Biological Age data and who were aged 20–84 (N = 6731, 52% male). Maximum age was set at 84 years because public NHANES data does not differentiate chronological ages for participants aged 85 years and older. Pregnant women were excluded from analyses.

We tested associations among biological aging measures using Pearson correlations. For analysis of healthspan-related characteristics and life-course risk factors, we tested associations with continuous outcomes using linear regression to compute standardized effect-sizes (interpretable as Pearson's r) and with dichotomous outcomes using logistic regression to compute odds ratios (ORs). For models testing associations between biological aging measures and healthspan-related characteristics, biological aging measures were specified as independent variables and healthspan-related characteristics were specified as dependent variables. For models testing associations between risk factors and biological aging, risk factors were specified as independent variables and biological aging measures were specified as dependent variables. Models included participants with available data on all four biological aging measures and the healthspan-related characteristic or risk factor under analysis. All models included covariate adjustment for chronological age and sex. Sensitivity analysis added models showing covariate adjustment for body-mass index (BMI), race/ethnicity and education.

## 3. Results

### 3.1. NHANES participants' chronological ages were correlated with their biological ages

For all biological aging measures, chronologically older NHANES participants also had older biological ages, Fig. 1 Panel A, Appendix Table C.1 Panel A. NHANES participants' chronological ages were positively correlated with their KDM and LM Biological Ages (KDM r = 0.91, LM r = 0.96). NHANES participants' with older chronological ages also tended to have higher homeostatic dysregulation scores (r = 0.56) and shorter telomere length (r = −0.42).

### 3.2. KDM Biological Age, homeostatic dysregulation, and LM Biological Age were correlated with one another, but not with leukocyte telomere length

To investigate whether KDM Biological Age, homeostatic dysregulation, LD Biological Age and leukocyte telomere length capture similar or distinct aspects of the aging process, we computed correlations among these four measures of biological aging. To focus on biological aging, we computed associations controlling for variation due to chronological age. Participants' KDM and LM Biological Ages and their homeostatic dysregulation scores were correlated ($r > 0.5$ for all); but these measures were not correlated with telomere length ($|r| \leq 0.01$). Age-adjusted associations among measures of biological aging are shown in Fig. 1 Panel B.

### 3.3. Biological aging measures predicted differences in healthspan-related characteristics

We tested associations between biological aging measures and

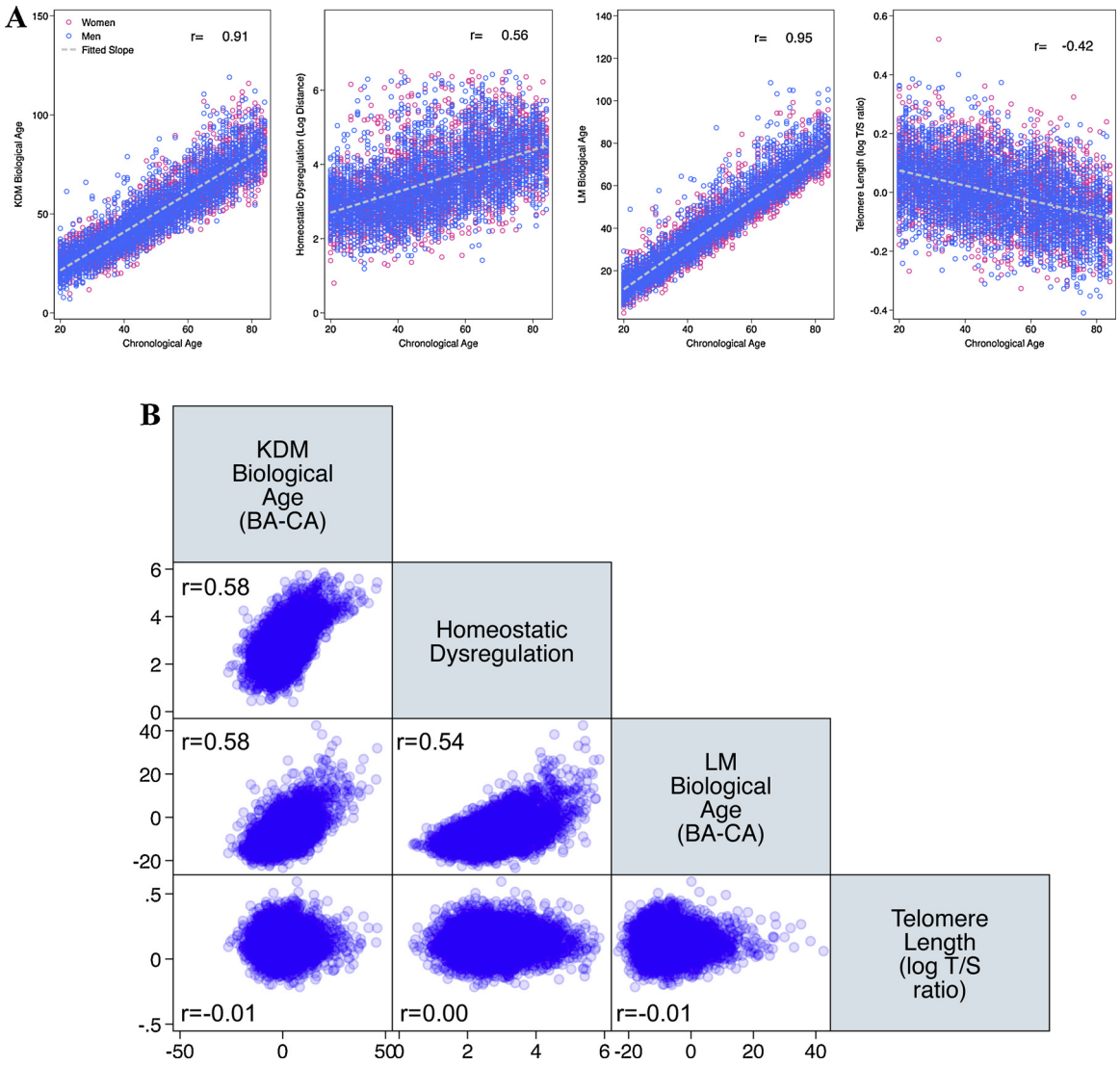


**Fig. 1. Panel A. Associations of chronological age with KDM Biological Age, Homeostatic Dysregulation, LM Biological Age, and Telomere Length.** Correlation coefficient (Pearson r) shown at top-right was computed after adjusting for sex for sample aged 20–84 with available data for all four measures of biological aging (N = 6731). Men and women are indicated by blue and pink circles respectively. Dotted line reflects linear approximation of the relationship between select measure and chronological age. **Panel B. Associations among biological aging measures.** NHANES participants' KDM Biological Ages, homeostatic dysregulation scores, and LM Biological Ages were correlated with one another, but not with their telomere length. Correlation coefficient (Pearson r) shown at top-right was computed after adjustment for chronological age and sex. For KDM and LM Biological Age measures, Biological Aging was computed as the difference between Biological Age and Chronological Age. For Homeostatic Dysregulation and Telomere Length, values were regressed on chronological age and the residuals were used to estimate associations (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

healthspan-related characteristics across three healthspan-related domains: physical functioning, cognitive and perceptual functioning, and subjective functioning and pain. Because of the high correlation of KDM and LM Biological Age measures with chronological age, we focused analysis on the difference between these measures and chronological age (computed as biological age – chronological age) in this and subsequent analyses.

NHANES participants with more advanced biological aging as measured by KDM Biological Age, homeostatic dysregulation, and LM Biological Age performed more poorly on tests of physical functioning, with the exception that KDM Biological Age was not associated with muscle strength. Participants with shorter telomeres also tended to perform more poorly on tests of physical function, but effect-sizes were much smaller and were statistically significant at alpha = 0.05 only for balance and cardiorespiratory fitness. Effect-sizes are shown in Fig. 2 and reported in Appendix Table C.2.

NHANES participants with more advanced biological aging as measured by KDM Biological Age, homeostatic dysregulation, and LM Biological Age performed more poorly on tests of cognitive functioning, hearing, and vision. Participants with shorter telomeres performed more poorly on tests of cognitive and perceptual functioning, but effect-sizes were smaller and statistically significant at alpha = 0.05 for vision only. Effect-sizes are shown in Fig. 2 and reported in Appendix Table C.2.

NHANES participants who were aging faster as measured by KDM Biological Age, homeostatic dysregulation, and LM Biological Age rated themselves as being in poorer health and were more likely to report ADL and IADL limitations. Participants with shorter telomeres also rated themselves as being in poorer health, but were not more likely to report ADL and IADL limitations. Effect-sizes are shown in Fig. 2 and reported in Appendix Table C.2.

NHANES participants with more advanced biological aging as measured by KDM Biological Age and LM Biological Age were more likely to report joint pain and widespread chronic pain. Participants

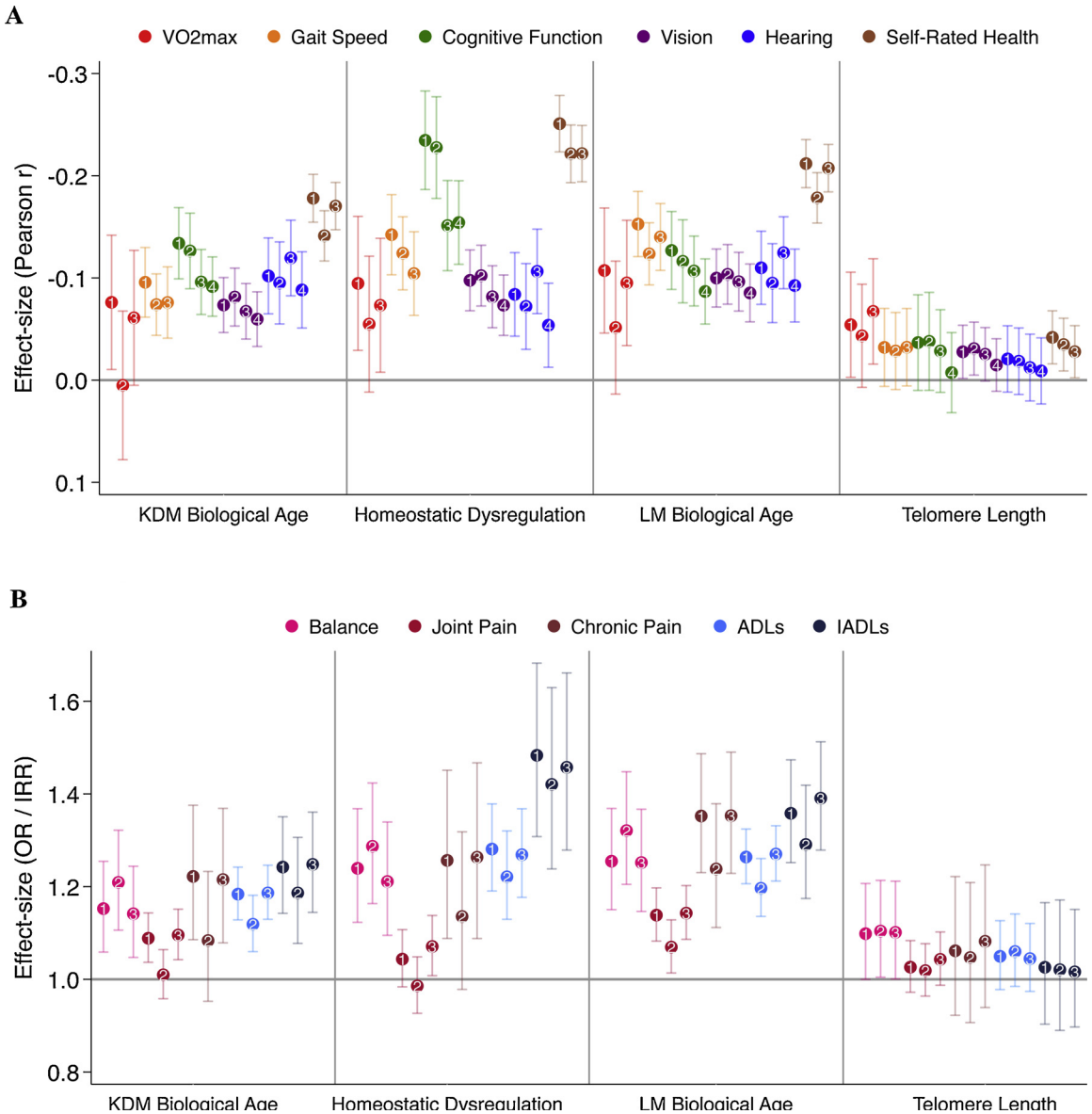


**Fig. 2. Effect-sizes for associations of Biological Aging Measures with continuously-distributed healthspan-related characteristics.** Panel A graphs effect-sizes for continuously distributed healthspan-related characteristics (Pearson r). Panel B graphs effect-sizes for dichotomous and count healthspan-related characteristics (odds ratios (OR) for dichotomous measures of balance and pain, and incidence-rate-ratios (IRRs) for counts of Activities of Daily Living (ADLs) and Instrumental Activities of Daily Living (IADLs)). Effect-sizes for each healthspan-related characteristic are graphed in a different color. Plotted points are numbered to reflect each of three or four models from which effect-sizes were estimated: Model 1 included covariates for chronological age and sex. Model 2 included Model-1 covariates and body-mass index. Model 3 included Model-1 covariates and race/ethnicity. For healthspan-related characteristics in the cognitive domain, we also show effect-sizes for Model 4, which included Model-1 covariates and educational attainment. Error bars reflect 95% confidence intervals. For this analysis, telomere length was reverse coded so that higher values indicate shorter telomeres. For Panel A analysis, each continuous outcome was coded such that a higher score reflected better functioning. Thus, the expected direction for all associations in Panel A is negative. The Y-axis of Panel A is reversed so that values run from positive to negative moving from the bottom of the Y-axis to the top. In Panel B analysis, which graphs risk associated with more advanced biological aging, the expected direction of all associations is positive. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

exhibiting greater homeostatic dysregulation were more likely to report widespread chronic pain but not joint pain. NHANES participants' telomere length was not associated with their self-reported pain. Effect-sizes are shown in Fig. 2 and reported in Appendix Table C.2.

We conducted sensitivity analyses to evaluate sex-differences in associations and sensitivity of results to covariate adjustment for BMI and race/ethnicity. Associations between measures of biological aging and healthspan-related characteristics were mostly similar for men and women. An exception was muscle strength, for which KDM Biological Age, homeostatic dysregulation, and LM Biological Age all showed stronger associations in men as compared to women. Tests of sex differences are reported in Appendix Table C.3. Covariate adjustment for BMI attenuated associations of biological aging measures with some healthspan-related characteristics, in particular $VO_2$ max and chronic pain; all associations with $VO_2$ max were attenuated below the alpha = 0.05 threshold of statistical significance and only LM Biological age remained associated with chronic pain at the alpha = 0.05 threshold. Covariate adjustment for race/ethnicity attenuated some associations between biological aging measures and healthspan-related characteristics; the associations of KDM Biological Age with $VO_2$ max and of homeostatic dysregulation with muscle-strength were attenuated below the alpha = 0.05 threshold of statistical significance. Sensitivity analyses evaluating covariate adjustment for BMI and race-ethnicity are reported in Appendix Tables C.4 and C.5 and shown in

Fig. 2. We conducted a final sensitivity analysis of biological-aging-measure associations with cognitive and perceptual functioning, adding covariate adjustment for educational attainment. Effect-sizes were modestly attenuated, but all associations with KDM Biological Age, homeostatic dysregulation, and LM Biological Age remained statistically significant at the alpha = 0.05 threshold. Results are reported in Appendix Table C.6 and shown in Fig. 2.

### 3.4. Life-course risk factors are associated with advanced biological aging

We next tested whether participants with life-course risk factors for shorter healthspan exhibited more advanced biological aging as measured by the four biological aging measures. Specifically, we investigated low educational attainment, material and social resource deficits, and mental health problems. Analysis controlled for chronological age and sex.

NHANES participants with lower educational attainment and fewer material and social resources tended to manifest advanced biological aging as measured by KDM Biological Age, homeostatic dysregulation, and LM Biological age. Consistent with a previous analysis, participants with lower educational attainment and those with fewer material resources also tended to have shorter telomeres (Needham et al., 2013). For social support, telomere associations were in the expected direction, but effect-sizes were smaller and were not statistically significant at alpha = 0.05. NHANES participants' mental health problems were not associated with any of the measures of their biological aging. Effect-sizes for associations with life-course risk factors are shown in Fig. 3 and reported in Appendix Table C.7. Sensitivity analyses testing effects of covariate adjustment for BMI and race/ethnicity are reported in Appendix Tables C.8 and C.9 and in Fig. 3. Covariate adjustment for BMI and race/ethnicity moderately attenuated some associations between life-course risk factors and measures of biological aging; associations between low social support and KDM Biological age and between food insecurity and telomere length were attenuated below the alpha = 0.05 level of statistical significance.

### 3.5. Integrating telomere length into KDM Biological Age and homeostatic dysregulation measures of biological aging

KDM Biological Age, homeostatic dysregulation, and telomere length were all associated with healthspan-related characteristics and with risk factors for shorter healthspan. But while KDM and LM Biological Ages and homeostatic dysregulation were correlated with each other, these measures were not associated with telomere length. We therefore tested if integrating telomere information into the KDM Biological Age and homeostatic dysregulation algorithms could produce a measure of aging that would be more strongly associated with healthspan-related characteristics and risk factors for shorter healthspan. (We could not test how integration of telomere length might affect performance of the LM Biological Age because this measure was constructed in the NHANES III dataset in which telomere length was not measured.) Overall, effect-sizes for analysis of healthspan-related characteristics and risk factors were similar between the original versions of KDM Biological Age and homeostatic dysregulation and versions created with telomere length included in the biomarker panel. Although effect-sizes for the versions including telomere length tended to be very modestly larger. Effect-sizes for the original KDM Biological Age and homeostatic dysregulation measures and the versions composed telomere length included in the biomarker panel are reported in Appendix Tables C.10 and C.11.

Finally, to compare the KDM Biological Age and homeostatic dysregulation methods to the LM Biological Age, we constructed versions of the KDM Biological Age and homeostatic dysregulation measures using the same biomarkers included in the LM Biological Age. We then repeated analysis of healthspan-related characteristics and risk factors. Overall, effect-sizes were very similar to original KDM Biological Age and homeostatic dysregulation measures (Appendix Tables C.12 and C.13).

## 4. Discussion

We studied four proposed measures of biological aging in a cohort of 6731 individuals of mixed chronological age drawn from the National Health and Nutrition Examination Study. We quantified participants' leukocyte telomere length, a cellular-level measure reflecting proximity to senescence, as well as KDM Biological Age, homeostatic dysregulation, and LM Biological Age, patient-level measures that combine information from multiple clinical biomarkers to quantify decline in system integrity. All four measures were correlated with chronological age, with chronologically older NHANES participants exhibiting older KDM and LM Biological Ages, higher levels of homeostatic dysregulation, and shorter telomeres (Fig. 1A). Participants' exhibiting more advanced biological aging performed worse on tests of physical, cognitive, and perceptual functioning and reported more limitations to their daily activities and more pain, and rated themselves as being in worse health as compared to peers of the same chronological age with less advanced biological aging (i.e. younger biological ages, lower levels of homeostatic dysregulation, or longer telomeres (Fig. 2A and B). In parallel, participants with risk factors for shorter healthy lifespan exhibited more advanced biological aging as

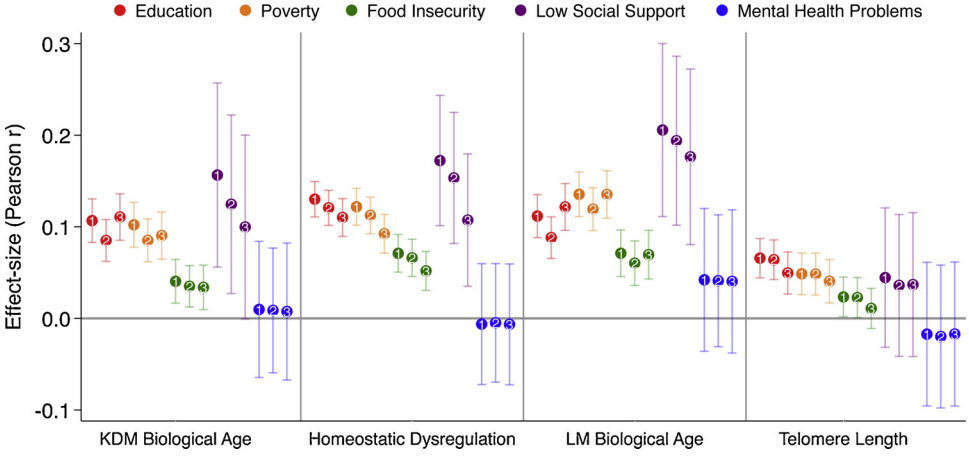


**Fig. 3. Effect-sizes for associations of exposures hypothesized to accelerate biological aging with Biological Aging Measures.** The figure graphs effect-sizes (Pearson r) for associations of risk factors with measures of biological aging. Effect-sizes for each risk factor are graphed in a different color. Plotted points are numbered to reflect each of three models from which effect-sizes were estimated: Model 1 included covariates for chronological age and sex. Model 2 included Model-1 covariates and body-mass index. Model 3 included Model-1 covariates and race/ethnicity. Error bars reflect 95% confidence intervals. For this analysis, telomere length was reverse coded so that higher values indicate shorter telomeres. Thus, the expected direction for all associations is positive. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

compared to peers of the same chronological age who did not carry risk factors (Fig. 3). Covariate adjustment for BMI, race/ethnicity, and education did not fully explain most associations between measures of biological aging and healthspan-related characteristics. Effect-sizes were relatively consistent across the KDM Biological Age, homeostatic dysregulation, and LM Biological Age measures, but were markedly smaller for telomere length.

Our findings replicate and extend previously reported differences between cellular- and patient-level measures of biological aging (Belsky et al., 2017b; Murabito et al., 2018). Although participants' KDM Biological Ages, homeostatic dysregulation scores and LM Biological Ages were correlated, these measures were not correlated with telomere length (Fig. 1B). Previous studies using the same NHANES data we analyzed reported associations between telomere length and biomarkers of cardiometabolic disease (Mazidi et al., 2018; Rehkopf et al., 2016). Those data, together with other findings (D'Mello et al., 2015), suggest that telomere length could provide a biomarker of cardiometabolic system integrity. Our findings suggest that telomere length may be less-well correlated with the integrity of other systems in the body.

KDM Biological Age, homeostatic dysregulation, and LM Biological Age were more consistently associated with measures of healthspan-related characteristics as compared to telomere length. Although effect-sizes for most associations were small, effect-sizes for telomere length were consistently smaller than for the composite measures and were sometimes in the opposite direction expected (Appendix Table C.2). These findings echo results recently published from the US Health and Retirement Study, in which salivary telomere length was not consistently associated with measures of physical functioning in older adults (Brown et al., 2018).

Parallel to findings for healthspan-related characteristics, associations of life-course risks with biological aging were stronger for KDM Biological Age, homeostatic dysregulation, and LM Biological Age as compared to telomere length (Fig. 3). Telomere literature in the social and behavioral sciences has focused heavily on telomere associations with risk factors for shorter healthy lifespan (Mitchell et al., 2014; Shalev et al., 2013). Our findings suggest that, in studies of adults, composite measures of biological aging derived from clinical biomarkers may be more sensitive to personal histories of risk exposure and could complement measures to telomere length in profiling individuals' risk for adverse outcomes in aging.

The lack of association between any measure of biological aging and mental health is notable. Mental health problems are strongly linked with shorter healthy lifespan (Prince et al., 2007). The NHANES data we analyzed measured mental health using a field standard instrument, the World Health Organization CIDI, a diagnostic interview adapted for use by laypersons (Kessler et al., 1998). The sample size for mental health analyses was sufficiently large to detect even relatively small effect-sizes ($N > 1000$). There is also evidence from other studies to support a link between poor mental health and advanced biological aging. Although findings for mental health and shorter telomere length are mixed (reviewed in (Lindqvist et al., 2015)), meta-analyses tend to support a positive association between mental health problems and shorter telomere length, e.g. (Darrow et al., 2016). And at least one previous study observed a positive association between depression and KDM Biological Age in older adults (Brown et al., 2017). Even so, null findings from analysis of mental health and telomere length have been reported in analysis of the same NHANES data we analyzed (Needham et al., 2015). Our analysis reproduces that observation and adds evidence for null associations with KDM Biological Age, homeostatic dysregulation, and LM Biological Age. A possible explanation for null findings in the NHANES data may be that the CIDI measure of mental health problems was only implemented in a subsample of young adults aged 20–39. Associations between mental health problems and biological aging may not manifest until later in the life course. NHANES mental health data also lack detail regarding symptom severity and persistence. Replication in studies including somewhat older persons and with measures of symptom severity and persistence is needed.

Our findings were equivocal on the question of whether combining information on telomeres with information on clinical biomarkers could improve quantification of biological aging. Versions of the KDM Biological Age and homeostatic dysregulation algorithms that included telomere data had similar effect-sizes as compared to the original versions of the measures (Appendix Tables C.10 and C.11). Telomere data were only available in the 1999–2000 and 2001–2002 NHANES. So that the data used to compute algorithm parameters and the data used to test hypotheses were independent, we restricted algorithm development analysis to the 2001–2002 NHANES and conducted testing in the 1999–2000 NHANES. Although effect-sizes were slightly larger for the versions of the measures including telomere data, these differences were not statistically different from zero. Thus, the value added from integrating telomere data into patient-level measures of biological aging may be modest. To the extent telomeres quantify processes of cellular senescence, telomere data could be more valuable for understanding mechanisms through which interventions or exposures modify patient-level measures of biological aging.

We acknowledge limitations. First, our analysis did not include epigenetic clocks, which are an increasingly popular method to quantify biological aging (Horvath and Raj, 2018). DNA methylation data necessary to measure epigenetic clocks are not yet available from the NHANES. In two previous analyses, epigenetic clocks were not correlated with clinical biomarker composite measures of biological aging and showed weaker associations with healthspan-related characteristics and mortality (Belsky et al., 2017b; Murabito et al., 2018). Replication of these observations is needed, as are analyses that consider newly published epigenetic clocks that may be more strongly correlated with healthspan-related characteristics (Levine et al., 2018). Second, our analyses were conducted in cross-sectional data without longitudinal repeated-measures assessment of biological aging. This limits our ability to distinguish true differences in aging from cohort differences arising from physical, social, and cultural differences in the early environments of younger as compared to older participants (Finch and Crimmins, 2004). Cross sectional measurements also cannot distinguish differences present from early life from differences that accumulate during adulthood (Moffitt et al., 2017). Third, only a limited set of life-course risk factors were tested, all of which were derived from self-reports collected at the same time biological aging was measured. Finally, our analyses included adults aged 20–84 and therefore excluded both children and the very oldest members of the population.

The aging population and the growing understanding of aging biology make therapies to extend healthspan both increasingly necessary and increasingly plausible. To test if therapies suggested by animal studies can extend human healthspan, measures that can accurately assess changes in the rate of human aging within timescales of years rather than decades are needed. Cellular-level measures like telomeres attempt to directly assess molecular changes at the core of the biological process of aging. However, technical challenges associated with assaying telomere length may limit the utility of telomere length for testing geroprotective interventions (Hastings et al., 2017). For example, commonly employed methods to measure telomere length provide an average for a population of cells and may not reliably quantify cells' proximity to senescence, which may be driven by the single shortest telomere in a given cell (Hemann et al., 2001). Furthermore, telomere length in the current study was assessed in one tissue, leukocytes. By contrast, patient-level measures like KDM Biological Age and homeostatic dysregulation are composed of biomarkers released into the bloodstream by a variety of tissues in response to allostatic demands of the environment and, as such, may better measure global declines in system integrity, allowing for better-powered tests of interventions. However, these measures provide little information about mechanism.

We tested associations of telomeres and patient-level measures with healthspan-related characteristics and indicators of life-course risk

exposures. Findings highlight potential differences between measures of biological aging implemented at the cellular- and patient-level. In sum, consistent with results of a previous study (Belsky et al., 2017b), our findings suggest that patient-level clinical biomarker composite measures may be superior to telomere length for testing effectiveness of geroprotective therapies. Our study does not rule out the possibility that alternative methods to assay telomere length, such as those which provide a distribution of telomere lengths within a sample, could yield more powerful measures of aging processes. Moreover, analysis of patient-level measures will need to be combined with analysis of cellular-level measures to clarify mechanisms through which geroprotective effects operate. To translate geroprotective therapies to extend human healthspan, studies are needed that include measures of both patient- and cellular-level measures of aging.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.03.012.

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