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Research Article

Conceptual and Analytical Overlap Between Allostatic Load and Systemic Biological Aging Measures: Analyses From the National Survey of Midlife Development in the United States

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Abstract

Background: Indices quantifying allostatic load (AL) and biological aging (BA) have independently received widespread use in epidemiological literature. However, little attention has been paid to their conceptual and quantitative overlap. By reviewing literature utilizing measures of AL and BA, and conducting comparative analysis, we highlight similarities and differences in biological markers employed and approach toward scale construction. Further, we outline opportunities where both types of indices might be improved by adopting methodological features of the other.
Methods: Using data from the National Survey of Midlife Development in the United States (N = 2055, age = 26–86), we constructed 3 AL indices: 1 common literature standard and 2 alternative formulations informed by previous work with measures of BA. The performance of AL indices was juxtaposed against 2 commonly employed BA indices: Klemera-Doubal Method Biological Age and Homeostatic Dysregulation.
Results: All indices correlated with chronological age. Participants with higher AL and older BA performed worse on tests of physical and subjective functioning. Further, participants with increased life-course risk exposure exhibited higher AL and BA. Notably, alternative AL formulations tended to exhibit effect sizes equivalent to or larger than those observed for BA measures, and displayed superior mortality prediction.
Conclusions: In addition to their conceptual similarity, AL and BA indices also exhibit significant analytical similarity. Further, BA measures are robust to construction using a panel of biomarkers not observed in previous iterations, including carotenoids indexing antioxidant capacity. In turn, AL indices could benefit by adopting the methodological rigor formalized within BA composites, such as applying biomarker down-selection criteria.

Keywords: Biomarkers, Epidemiology, Functional performance, Healthspan, Risk factors

Biological aging (BA), in a broad sense, describes "the time-dependent functional decline that affects most living organisms" (1) (p1194). The dependency of aging processes on time is subject to robust interindividual variation, with individuals of identical chronological age presenting a variety of endophenotypes. As a result, there is increased interest in identifying measures which quantify differences in the rate of aging across individuals and populations.

Various indices quantifying BA have been implemented at the clinical (2), physiological (3,4), and molecular levels (5,6). Composite indices integrating a blood-chemistry panel of systemic biomarkers that collectively assess the integrity of major organ systems have shown promise as biomarkers of aging. Measures of this sort are predictive of morbidity and mortality (7), show variation by young adulthood (8), and appear responsive to intervention (9). These systemic biomarker indices are predicated on the rationale that the molecular changes underlying aging processes within individual cells ultimately manifest as organ-level physiological dysregulation, which in turn are linked to age-related disease and disability.

Allostatic load (AL) is a related term employed within gerontological literature to describe the "wear and tear on the body and brain resulting from chronic overactivity or inactivity of physiological systems that are normally involved in adaptation to environmental challenge" (10). Similar to blood-chemistry indices of systemic BA, AL measures integrate systemic biomarkers to form a measure of cumulative dysregulation resulting from life-course environmental risk exposures. AL indices show individual differences in longitudinal trajectories (11), are predictive of mortality above and beyond the functioning of individual organ systems (12), and are responsive to differences in exposures known to increase risk for morbidity and mortality (13).

Despite conceptual overlap between the 2 processes, explicit application of AL as a BA metric are limited (14,15). The apparent separation of these fields may be in part due to differences in motivation surrounding the development of these measures. BA composites are goal-oriented, developed with the specific intent to serve as surrogate endpoints to test for intervention effects on processes of aging. By contrast, AL indices are process-oriented, developed to test theories about physiological dysregulation resulting from differences in exposure to life-course risk factors.

These differences in motivation are reflected in the type of biomarkers employed within BA and AL indices. We conducted a nonexhaustive review for studies employing biomarker indices of systemic BA (from 2013 to 2020), identifying 24 studies utilizing Klemera-Doubal Method (KDM) Biological Age, Homeostatic Dysregulation (HD), or Phenotypic Biological Age (PhenoAge). Study details are provided in Supplementary Table D1. Among these studies, a total of 88 unique biomarkers were employed, with a range of 6–40 biomarkers across studies (median = 10.00). Most commonly employed were biomarkers indexing hepatic (albumin; 75%), immune (C-reactive protein [CRP]; 71%), renal (creatinine; 67%), and cardiovascular functioning (systolic blood pressure; 63%). Lipoproteins (total cholesterol; 71%) and blood sugar indicators (glucose and/or glycated hemoglobin [HbA1c]; 71%) also tended to be included. Notably, these biomarkers are (i) associated with the functioning of a major organ system or disease process, and (ii) measured via standardized methods as part of a blood panel during a physician's visits, which align with the goal of BA indices as ready-to-implement surrogate endpoints.

It is important to highlight that biomarker selection methods among the different types of BA indices are not homogenous. Specifically, KDM Biological Age and PhenoAge methods rely on explicit rules for biomarker selection. For KDM Biological Age, only biomarkers correlated with chronological age at a magnitude of $|r| = .1$ or greater are included (4,16). For PhenoAge, biomarkers are selected based on associations with mortality (17). By contrast, biomarker selection for HD measures tends to be less straightforward, relying upon a combination of theory, availability, or in some cases, change in variance with chronological age (3). This lack of structure tends to increase the variety of biomarkers employed for HD measures relative to studies utilizing KDM Biological Age or PhenoAge. In fact, 7 of the 24 (29.2%) studies in our review that utilize only the HD measure account for 56 of the 88 (63.6%) unique biomarkers identified (Supplementary Table D1).

Similar to HD measures, biomarker selection for AL indices relies on theory and/or availability in a given cohort. In a comparative nonexhaustive review spanning 1997 to 2020, we identified 61 studies implementing measures of AL (Supplementary Table D2). For this collection, there were a total of 63 unique biomarkers with a range of 6–24 per study (median = 10.00). The most commonly employed biomarkers were cardiovascular (systolic/diastolic blood pressure; 92%), blood sugar (HbA1c and/or glucose; 92%), body mass indicators (BMI/waist-hip ratio/waist circumference; 90%), lipoproteins (HDL cholesterol; 85%), and immune functioning (CRP; 66%). Although there was a significant quantitative overlap among the biomarkers employed, diagnostic biomarkers commonly utilized in BA composites such as creatinine and alkaline phosphatase are rarely observed in AL indices (11% and 5%, respectively).

AL theory is influenced by stress-related literature situating humans as sensory creatures constantly responding to external stimuli (18). In line with this, 41% of the studies in our review included proxies for the functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Supplementary Table D2). AL theory is also conceptually relevant for BA processes. Psychosocial stress can accelerate the progression of several aging hallmarks including mitochondrial dysfunction, telomere shortening, and cellular senescence (19). Furthermore, CpG sites located within glucocorticoid response elements make up as much as 24% of the CpG sites included in epigenetic clock algorithms (20). Thus, an emphasis on stress-related domains may strengthen, rather than weaken, AL indices' ability to approximate aging processes.

Theoretical differences affecting norms of biomarker inclusion between indices approximating BA and AL are also reflected in the statistical operations implemented during scale construction. A common practice across BA indices is the use of a reference population to define model parameters prior to their implementation in an analytical sample. There are distinct benefits and drawbacks to this approach. For example, using one or several large-scale external referents reduces cohort-specific biases that may limit the reliability and reproducibility of findings. At the same time, using historical cohorts introduces biases related to generational effects and mortality selection (21–23). Moreover, an agnostic reliance upon correlations with chronological age to identify a biomarker panel (*or CPGs for epigenetic clocks)* may influence selection in a manner favoring those with little to no mechanistic links to aging processes (24).

Mathematical operations used to construct AL indices also vary (25), but often involve estimating physiological risk based upon the distribution of biomarker values *within the analytical sample*. Variation exists on whether risk is partitioned to distinct physiological domains (ie, cardiovascular, immune, etc.) or assessed collectively across all biomarkers, but a common interpretation is the operationalizing of AL as the accumulation of changes that become impactful only when they reach a critical threshold. When constructed in this manner, AL indices are susceptible to spurious associations driven by characteristics unique to that particular cohort. For example, the National Survey of Midlife Development in the United States (MIDUS) cohort, in which AL has been extensively studied, tends to be healthier and wealthier than U.S. population averages (26). As such, the critical threshold for risk, commonly defined as the highest or lowest quartile of the biomarker distribution, may end up falling at a point well within what would be considered clinically healthy for the general U.S. population.

To investigate the analytical overlap between indices approximating AL and BA, we utilized data from the MIDUS cohort. We analyzed 2 indices of systemic BA that could be quantified with MIDUS data, KDM Biological Age and HD, as well as 3 approaches toward the calculation of AL, including a common literature standard and 2 alternative approaches informed by our experiences with BA indices. Our analyses proceeded in 4 steps. First, we tested associations among chronological age and the different biomarker indices. Second, we tested associations between biomarker indices

and tests of functional capacities mediating age-related decline, hereafter referred to as healthspan-related characteristics, including physical, perceptual, and subjective functioning. Third, we tested the extent to which biomarker indices were associated with life-course risk factors for shorter healthspan including childhood adversity, low educational attainment, material resource deficits, and mental health problems. Fourth, we analyzed the extent to which biomarker indices were predictive of mortality.

Method

Sample

Data were from the MIDUS II and MIDUS Refresher Biomarker Projects of The MIDUS National Survey: a longitudinal survey of more than 7000 midlife adults in the United States. The MIDUS II Biomarker Project was a follow-up project involving a subsample (n = 1255) of the original cohort initiated in 2004, wherein participants traveled to Georgetown University, UCLA, or the University of Wisconsin for a 2-day visit protocol including blood draws, psychometric assessments, and health examinations. Sample collection procedures were standardized across the 3 sites (27). The MIDUS Refresher Survey was implemented in 2011 with the intent to replenish the original MIDUS cohort with a similar age stratification. The Refresher Biomarker Project follows the same design of the MIDUS II Biomarker Project. All MIDUS data sets and documentation are available at the ICPSR website (http://www.icpsr.umuch.edu/).

We conducted analyses to test hypotheses about measures of AL and BA using data from adults aged 26–86 years participating in MIDUS II or MIDUS Refresher Biomarker Projects and for whom all measures could be estimated (N = 2055, 45% male).

Allostatic Load Indices

We analyzed 3 different indices of AL. The first, $AL_{STANDARD}$, was computed in line with the most frequent implementation identified in our literature search, and utilized 24 biomarkers to quantify cumulative risk across 7 physiological domains (28–36). The 7 physiological domains represented were sympathetic nervous system, parasympathetic nervous system, HPA axis, inflammation, cardiovascular system, glucose metabolism, and lipid metabolism. Within each domain participants were assigned a score ranging from 0 to 1, calculated as the number of biomarkers at risk divided by the total number of biomarkers within that domain. Risk was defined independently for men and women as the highest or lowest quartile for a given biomarker, depending on whether a higher or lower biomarker value conferred greater risk for morbidity and mortality. Scores across the 7 domains were summed to produce an AL score ranging from 0 to 7. Descriptive statistics for the biomarkers included in the calculation of $AL_{STANDARD}$ are detailed in Table 1. Risk cutoffs by sex are detailed in Supplementary Table A2.

The second and third AL indices were alternative formulations which sequentially integrated practices commonly employed within systemic composites of BA. Namely, imposing selection criteria prior to defining which biomarkers are employed in the algorithm, and secondly, the use of a reference population. Before applying these criteria, we extracted only those biomarkers which were measured using the same tissue/method in both MIDUS and NHANES (n = 20). In this manner, we ensured the same panel of biomarkers could be used for a comparative AL and BA analysis. Next, we applied down-selection criteria to decide which biomarkers to utilize in the second AL score, AL_{MIDUS}. Following previous work (4), Pearson correlations were used to assess the association among biomarkers and chronological age. An initial set of 10 biomarkers were selected based on their correlation with chronological age ($|r| > .1$). Subsequently, associations among these 10 biomarkers were inspected to ensure independence from other items in the panel ($|r| < .4$). When 2 biomarkers were found to correlated at $|r| > .4$ we selected the biomarker with the larger sample size, or stronger association with chronological age if the sample size was equivalent. A final panel of 8 biomarkers was selected, which collectively assess the integrity of cardiovascular, renal, immune, and antioxidant systems. AL_{MIDUS} scores were calculated as the number of biomarkers that fell within high-risk quartiles divided by the total number of biomarkers to produce AL_{MIDUS} scores ranging from 0 to 1. For $AL_{MIDUS,}$ risk quartiles were defined according to biomarker distributions within the MIDUS analytical sample. Descriptive statistics for the biomarkers included in the calculation of AL_{MIDUS} are provided in Table 1.

The final AL score, AL_{NHANES}, utilized the same panel of 8 biomarkers as AL_{MIDUS}, but integrated the use of a reference population. Specifically, risk cutoffs for each biomarker were defined according to their distribution in a reference population instead of the analytical sample. We formed this reference population from nonpregnant participants aged 26–84 years in NHANES III and continuous NHANES panels spanning 1999–2016 (N = 56 615, 49% male; Supplementary Table A4). Risk cutoffs for biomarkers used in the calculation of AL_{MIDUS} and AL_{NHANES} are provided in Supplementary Table A5. Analyses to construct AL scores are described in greater detail in Supplementary Appendix A.

Systemic Biological Aging Indices

We analyzed 2 BA indices that could be quantified with MIDUS data: KDM Biological Age and HD.

KDM Biological Age was computed from an algorithm derived from a series of regressions of individual biomarkers onto chronological age in a reference population. Following previous work (37), we formed this reference populations from participants in NHANES III and continuous NHANES panels 1999–2016 aged 30–75 years who were nonpregnant at the time of biomarker data collection (N = 46 038, 49% male; Supplementary Table A7). An individual's KDM Biological Age prediction corresponds to the chronological age at which their physiology would be approximately normal in the NHANES reference population. Final parameters forming the KDM Biological Age algorithm are reported in Supplementary Table A7.

Homeostatic Dysregulation was computed from an algorithm based on Mahalanobis distance (38) for a panel of biomarkers relative to a reference population. Following previous work (37), we formed this reference populations from participants in NHANES III and continuous NHANES panels 1999–2016 aged 20–30 years who were not obese, nonpregnant, and for whom all biomarkers fell within clinically normal ranges (N = 350, 57% male; Supplementary Tables A8 and A9). An individual's HD score quantifies how different their physiology is from this young, healthy norm.

Importantly, we calculated KDM Biological Age and HD using the same panel of 8 biomarkers used to calculated AL_{MIDUS} and AL_{NHANES}, namely creatinine, CRP, HbA1c, lutein/zeaxanthin, lycopene, retinol, systolic blood pressure, and urinary creatinine (Table 1). Details on biomarker measurements for the reference population are available from the NHANES website (https://www.cdc.gov/nchs/nhanes/).

Table 1. Descriptive Statistics for Biomarkers Used to Construct Systemic Biomarker Composites Within the Final Analysis Sample (n = 2055)

Biomarkers by Physiological Domain	Men			Women		
	N	*M*	*SD*	*N*	*M*	*SD*
Chronological age	931	56.43	12.90	1124	54.78	12.24
Cardiovascular						
Resting systolic blood pressure (mmHg)	931	132.04	15.05	1124	127.78	18.74
Resting diastolic blood pressure (mmHg)	931	79.10	9.77	1124	74.16	10.10
Resting heart rate (bpm)	762	79.01	12.51	963	82.45	13.20
Metabolic—lipids						
Body mass index	931	29.80	5.83	1124	29.96	7.71
Waist-hip ratio	930	0.96	0.08	1123	0.84	0.08
Triglycerides (mg/dL)	931	137.45	78.46	1124	110.21	60.25
HDL cholesterol (mg/dL)	928	50.00	15.91	1124	62.58	18.22
LDL cholesterol (mg/dL)	928	100.97	33.68	1124	103.83	34.10
Metabolic—glucose metabolism						
HbA1c (%)	931	5.91	1.05	1124	5.93	0.97
Fasting glucose (mg/dL)	929	104.18	23.40	1121	98.65	19.08
Insulin resistance (HOMA-IR)	929	4.29	4.44	1121	3.60	3.69
Inflammation						
CRP (mg/L)	931	2.13	2.82	1124	3.49	4.75
IL6 (pg/mL)	931	2.77	2.40	1124	2.98	2.76
Fibrinogen (mg/dL)	931	331.25	74.47	1124	358.83	80.53
sE-Selectin (ng/mL)	931	43.86	20.94	1124	40.48	19.76
sICAM-1 (ng/mL)	931	272.46	96.50	1124	276.54	107.88
Sympathetic Nervous System						
Urine Epinephrine (µg/g creatinine)	910	24.57	55.71	1102	26.82	62.95
Urine Norepinephrine (µg/g creatinine)	926	132.15	207.18	1120	146.29	265.88
Hypothalamic Pituitary Adrenal Axis						
Urine Cortisol (µg/g creatinine)	927	17.53	12.83	1116	21.01	16.70
Blood DHEA-S (µg/dL)	945	138.60	87.17	1122	89.46	62.28
Parasympathetic Nervous System						
SDRR (msec)	813	36.99	17.44	1027	35.15	16.60
RMSSD	813	22.34	14.95	1027	24.19	16.64
Low-frequency spectral power	813	487.59	574.73	1027	376.66	457.82
High-frequency spectral power	813	263.71	383.03	1027	355.38	558.75
Renal						
Creatinine (mg/dL)	931	0.99	0.19	1124	0.77	0.14
Urinary Creatinine (mg/dL)	931	106.05	61.45	1124	73.44	48.72
Antioxidant						
Lutein+Zeaxanthin (mg/dL)	931	0.34	0.18	1124	0.37	0.24
Lycopene (µmol/L)	931	0.77	0.52	1124	0.71	0.52
Retinol (µmol/L)	931	2.16	0.78	1124	1.91	0.73

Notes: Biomarkers in bold represent the set of 8 biomarkers included in AL_{MIDUS}, AL_{NHANES}, KDM, and HD measures. The $AL_{STANDARD}$ measure was constructed using all biomarkers except those 5 markers in the *Renal* and *Antioxidant* domains. CRP = C-reactive protein; DHEA-S = dehydroepiandrosterone sulfate; HDL =high-density lipoprotein; HbA1c = hemoglobin A1c; HOMA-IR = homeostatic model assessment of insulin resistance; IL6 = interleukin 6; LDL = low-density lipoprotein; M = mean; *N* = sample size; RMSSD = root mean square of differences between successive heartbeats; SD = standard deviation; SDRR = standard deviation of intervals between heartbeats; sICAM = soluble intercellular adhesion molecule-1.

Healthspan-related Characteristics

We tested associations of BA and AL indices with functional assessments of capacities thought to mediate age-related disability, referred to here as "healthspan-related characteristics." The functional capacities included upper body strength (grip strength using a handheld dynameter), gait speed (time to complete 50ft walk), respiratory rate (breaths per minute), and visual acuity (without correction), all assessed at the same time as biomarker data collection. We also tested associations with a MIDUS composite variable constructed as a cumulative count of chronic symptoms and conditions such as heart disease, high blood pressure, diabetes, thyroid disease, depression, and alcoholism. Associations with measures of self-reported physical and emotional health assessed during baseline visits were also tested. On average, 23.7 months had passed between baseline visits and biomarker collection. Healthspan-related characteristics, including variable names of all items used in their construction, are described in detail in Supplementary Appendix B. Advanced chronological age was associated with worse performance on most healthspan-related characteristics except self-rated physical health, which was not associated with chronological age, and self-rated emotional health, which tended to increase in quality with advancing chronological age (Supplementary Table B1).

Life-course Risk Factors for Shorter Healthspan

We tested associations of BA and AL indices with life-course risk factors known to predict shorter healthspan: early-life adversity, low educational attainment, material resource deficits, and mental health problems. We assessed early-life adversity using

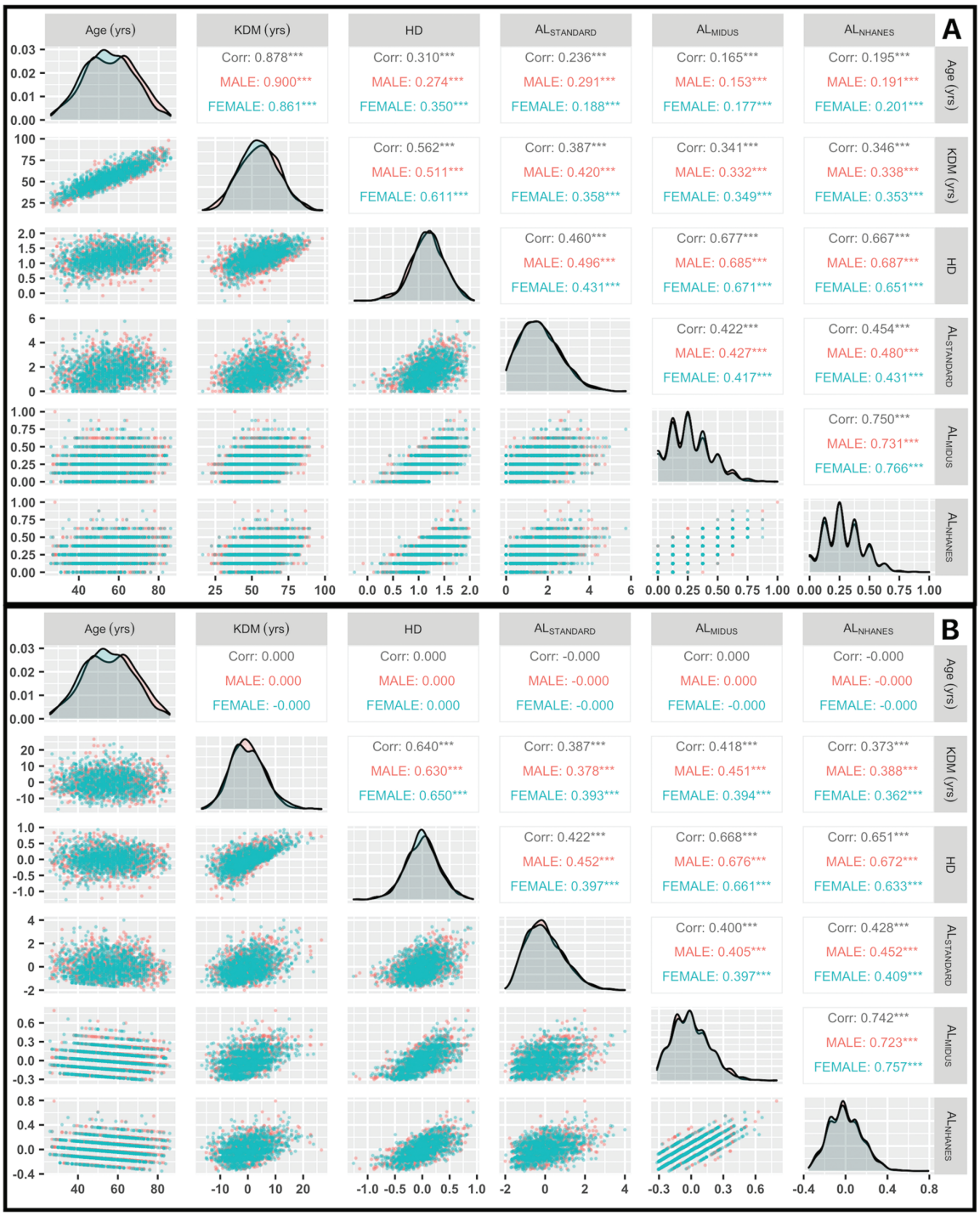

Figure 1. (**A**) Associations between chronological age biomarker indices approximating biological aging and allostatic load. Lower panel illustrates scatter plots for full sample (n = 2055). Density distributions for each variable are illustrated on the diagonal. Upper panel provides Pearson's correlation coefficients for the full sample, as well as for males and females independently. (**B**) Associations between chronological age and biomarker indices approximating biological aging and allostatic load after adjustment for chronological age. Age adjustment was performed by extracting residuals from regression models of each index onto chronological age. Lower panel illustrates scatter plots for full sample (n = 2055). Density distributions for each variable are illustrated on the diagonal. Upper panel provides Pearson's correlation coefficients for the full sample, as well as for males and females independently. AL = allostatic load; HD = homeostatic dysregulation; KDM = Klemera-Doubal Biological Age. Full color version is available within the online issue.

the Childhood Trauma Questionnaire (39). We measured educational attainment using a 5-level categorical variable describing the highest level of education completed by the participant. We assessed material resources using household-adjusted poverty to income ratio. We assessed mental health problems using the Center for Epidemiologic Studies Depression Scale (40) and the Perceived Stress Scale (41). Life-course risk factors and computations are described in Supplementary Appendix B and Supplementary Table B2.

Mortality Data

Longitudinal mortality data were drawn from the third wave of The MIDUS National Survey (ie, MIDUS III). Information on vital status of participants in MIDUS II was obtained from data available at the ICPSR website. This data set includes all known MIDUS decedents as of March 2018, but does not include data on MIDUS Refresher participants.

Statistical Analyses

Our analyses involved MIDUS participants with all covariate information and sufficient biomarker data to calculate all 5 systemic biomarker composites (N = 2055, 45% male; Supplementary Table C1). Women who were pregnant or breastfeeding at the time of biomarker collection were excluded from analyses.

We tested cross-sectional associations among biomarker composites and chronological age using Pearson correlations. For cross-sectional analyses of healthspan-related characteristics and life-course risk factors, we tested associations using linear regression (*lm function in R statistical package*) to compute standardized effect sizes (interpretable as Pearson's r). For models testing associations between biomarker indices and healthspan-related characteristics, biomarker indices were specified as independent variables. For models testing associations between risk factors and biomarker indices, risk factors were specified as independent variables. To allow for comparability across biomarker indices and outcomes, we standardized each index, healthspan characteristic, and risk factor by sex within each subsample to compute standardized effect sizes in all models.

Primary models include covariate adjustment for chronological age and sex. In addition to primary models, we also conducted analyses using a set of fully-adjusted models with additional covariate control for race/ethnicity and BMI. Fully-adjusted models were added to address observed differences in the rate of aging among different racial/ethnic groups (42), as well as concerns that change in biomarker composites are driven by differences in body mass rather than aging (43,44). The degree of attenuation was calculated as the proportional decrease of the standardized effect size in fully-adjusted models relative to base models. Associations with mortality were tested via Cox proportional hazard models using the *coxph* function from the "survival" package in R (45). Cox models included covariate adjustment for chronological age and sex.

Results

Associations Among Indices of Allostatic Load, Systemic Biological Aging, and Chronological Age

Chronologically older MIDUS participants had older BA and higher AL (Figure 1A, Supplementary Table C2A). MIDUS participants' chronological ages were most strongly associated with their KDM Biological Ages (r = .88). Participants' chronological ages were moderately associated with their HD scores (r = .31) and all 3 indices of AL ($AL_{STANDARD}$ r = .24, AL_{MIDUS} r = .17, AL_{NHANES} r = .20).

To investigate similarity across biomarker indices, we computed correlations among these 5 indices. To focus on their ability to index BA, we computed associations controlling for variation due to chronological age using residuals of each measure regressed onto chronological age independently by sex. After adjusting for chronological age, correlations among indices were largely unchanged relative to raw, unadjusted versions. Age-adjusted associations among indices are shown in Figure 1B and Supplementary Table C2B.

Associations With Healthspan-Related Characteristics

We tested associations between age-adjusted biomarker indices and healthspan-related characteristics across 3 domains: physical functioning, perceptual functioning, and subjective functioning.

MIDUS participants with more advanced AL and higher BA performed more poorly on tests of physical functioning. Participants' KDM Biological Ages were associated with grip strength in the opposite direction than expected, replicating previous associations seen between KDM Biological Age and muscle strength in NHANES (46). MIDUS participants with more advanced BA and AL also tended to report increased chronic symptoms and conditions, as well as worse physical and emotional health. Participants' visual acuity was not associated with any measures of BA or AL. Effect sizes for primary

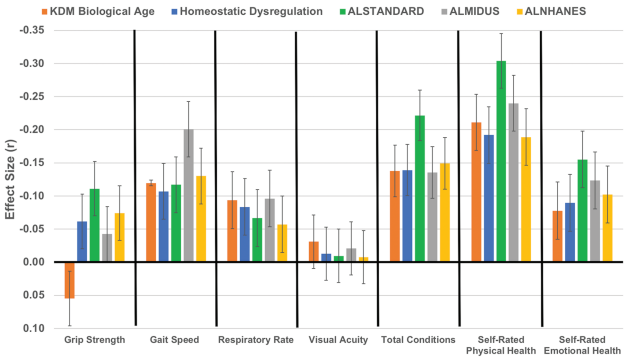

Figure 2. Associations between healthspan-related characteristics and biomarker indices hypothesized to index biological age and allostatic load. Plotted bars reflect standardized effect sizes for associations between biomarker indices and healthspan-related characteristics. Left to right order of bars within each characteristic corresponds to left to right order of biomarker indices listed at top of figure. Prior to analyses, all characteristics were recoded such that higher values indicate better performance. Thus, the expected direction for all associations is negative. Error bars reflect 95% confidence interval of effect-size estimate. AL = allostatic load; KDM = Klemera-Doubal Biological Age. Full color version is available within the online issue.

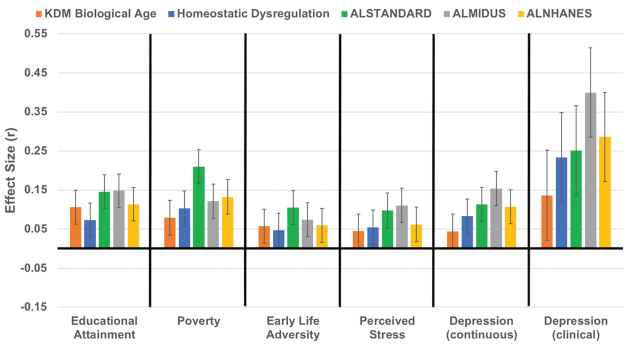

Figure 3. Associations between life-course risk factors and biomarker indices hypothesized to index biological age and allostatic load. Plotted bars reflect standardized effect sizes for associations between biomarker indices and life-course risk factors. Left to right order of bars within each risk factor corresponds to left to right order of biomarker indices listed at top of figure. All factors were coded such that higher values indicate greater exposure. Thus, the expected direction for all associations is positive. Error bars reflect 95% confidence interval of effect-size estimate. AL = allostatic load; KDM = Klemera-Doubal Biological Age. Full color version is available within the online issue.

models are shown in Figure 2 and reported in Supplementary Table C3.

Given the small percentage of participants reporting "Poor" physical or emotional health (3% and 1%, respectively), we conducted sensitivity analyses to ensure associations between biomarker indices and subjective functioning were not biased by this small sample. We retested associations after integrating fair or worse physical and emotional health categories. Effect sizes were unchanged relative to original models (Supplementary Table C3).

We conducted analyses using fully-adjusted models to evaluate the robustness of findings to covariate adjustment for BMI and race/ethnicity. Adjustment for BMI and race/ethnicity attenuated associations between biomarker indices and most healthspan-related characteristics, with observed effect sizes decreasing by 15%–97% depending upon the composite and characteristic tested. Associations between $AL_{STANDARD}$ were particularly attenuated, with an average effect size decrease of 50%, and was no longer associated with gait speed or respiratory rate at an $\alpha = 0.05$. The smallest degree of attenuation was observed for AL_{MIDUS}, which decreased by 25% on average. With respect to specific healthspan characteristics, the largest degree of attenuation was observed for associations with gait speed and respiratory rate, which decreased by 50% and 52%, respectively. The smallest degree of attenuation was observed for associations with self-reported morbidity, which decreased by only 20% on average. By contrast, associations with grip strength increased by an average of 15% in fully-adjusted models. Effect sizes for fully-adjusted models testing associations between biomarker indices and healthspan-related characteristics are reported in Supplementary Table C4.

To further explore the AL_{MIDUS} measure constructed using a limited biomarker panel, we conducted a leave-one-out analysis for associations with healthspan-related characteristics, reconstructing AL_{MIDUS} 5 different times, each time removing one of the novel biomarkers included in the original index which was not included in the $AL_{STANDARD}$ measure (ie, lutein+zeaxanthin, lycopene, retinol, creatinine, urinary creatinine). Overall, no single biomarker significantly accounted for the utility of AL_{MIDUS} as a proxy for functional capacity. The most consistent trends were observed for the AL_{MIDUS} index constructed without creatinine, for which effect sizes decreased by an average of 11%.

Table 2. Hazard Ratios From Cox Regression Models Investigating Differences in Mortality as a Function of Biological Age and Allostatic Load

Predictor	Hazard Ratio* (95% CI)	*p* Value
KDM	1.12 (0.95, 1.33)	.18
HD	1.57 (1.29, 1.90)	5.56E−06
$AL_{STANDARD}$	1.45 (1.21, 1.74)	4.70E−05
AL_{MIDUS}	1.64 (1.37, 1.96)	7.40E−08
AL_{NHANES}	1.60 (1.34, 1.91)	2.20E−07

Notes: AL = allostatic load; CI = confidence interval; HD = homeostatic dysregulation; KDM = Klemera-Doubal Biological Age.

*All composite indices were standardized prior to running models. Thus, hazard ratios reflect the increased odds of mortality per standard deviation increase in allostatic load or biological age after adjustment for chronological age and sex.

Associations With Life-course Risk Factors

We next tested whether participants with life-course risk factors for shorter healthspan exhibited more advanced BA and AL. Specifically, we investigated low educational attainment, material resource deficits, early-life adversity, perceived stress, and mental health problems.

MIDUS participants with lower educational attainment and fewer material resources tended to have higher AL and increased BA. Participants' reporting increased early-life adversity and perceived stress also exhibited increased AL and BA. MIDUS participants' anxiety and depressive symptoms were both associated with their AL scores, as well as with BA indices, although with smaller effect sizes. Effect sizes for associations with life-course risk factors in primary models are shown in Figure 3 and reported in Supplementary Table C5.

We conducted analyses using fully-adjusted models to evaluate the robustness of findings to covariate adjustment for BMI and race/ethnicity. Covariate adjustment for BMI and race/ethnicity attenuated associations of most life-course risk factors and biomarker indices, with observed effect sizes decreasing by 17%–78% depending upon the exposure and composite tested. Across exposures, the greatest degree of attenuation was observed for early-life adversity, for which effect sizes decreased by 52% on average across the different biomarker indices and below an $\alpha = 0.05$ for all metrics except $AL_{STANDARD}$. By contrast, the smallest degree of attenuation was observed for associations with poverty, which decreased by only 23% on average. Across biomarker indices, covariate adjustment for BMI and race/ethnicity had the largest impact on associations with KDM Biological Age, for which associations with early-life adversity, perceived stress, and depression were attenuated below an $\alpha = 0.05$ level. Although AL_{MIDUS} exhibited the smallest degree of attenuation at 25%, only $AL_{STANDARD}$ remained significantly associated with all life-course risk factors following adjustment for BMI and race/ethnicity. Effect sizes for fully-adjusted models testing associations between life-course risk exposures and biomarker indices are reported in Supplementary Table C6.

To further explore the AL_{MIDUS} measure constructed using a limited biomarker panel, we conducted a leave-one-out analysis for associations with life-course risk factors as described above. Overall, no single biomarker significantly accounted for the utility of AL_{MIDUS} as an indicator of life-course risk exposure. The most consistent trends were observed for the AL_{MIDUS} index constructed without lycopene or without creatinine, for which effect sizes decreased by an average of 7% and 3.5%, respectively.

Associations With Mortality

A total of 130 participants in the analytical sample died between MIDUS II biomarker collection and March 2018 (most recent death recorded), with an average duration of 10.80 years between these events. MIDUS participants with higher AL scores tended to exhibit increased risk for all-cause mortality across the follow-up period, and all 3 AL indices, as well as HD scores, were significantly associated with all-cause mortality. MIDUS participants' KDM Biological Ages were not significantly associated with risk for all-cause mortality. Results for associations between AL scores, BA indices, and all-cause mortality are given in Table 2.

Discussion

We studied 5 biomarker indices proposed to index BA and AL in a cohort of 2064 MIDUS participants of mixed chronological age.

Specifically, we quantified KDM Biological Age and HD, 2 blood-chemistry indices hypothesized to index BA by quantifying decline in system integrity. We compared these 2 indices with 3 different implementations of AL, a process reflecting the cumulative wear and tear on biological systems from repeated exposures to biopsychosocial stressors. The first implementation, $AL_{STANDARD}$, was constructed per literature norms, while the other two, AL_{MIDUS} and AL_{NHANES}, are novel applications that integrate methodological practices seen in the construction of composites approximating BA.

All 5 indices were correlated with chronological age (Figure 1A). Participants' exhibiting advanced AL and BA performed worse on tests of physical functioning, reported worse physical and emotional health, and exhibited greater numbers of chronic symptoms and conditions (Figure 2). In parallel, participants with life-course risk factors for shorter healthy lifespan exhibited advanced AL and BA as compared to peers of the same chronological age with decreased life-course risk exposure (Figure 3). Higher AL and HD scores were also associated with increased risk for mortality across a 10-year follow-up period.

Our findings suggest hitherto unobserved similarities and differences between biomarker indices approximating BA and those approximating AL. Associations between AL indices and measures of systemic BA remained relatively unchanged following adjustment for chronological age, implying a shared quantification dysregulation above and beyond those changes explicitly related to chronological age (Figure 1B). This observation is contrary to associations between epigenetic-based measures of BA and AL indices, which significantly diminished following adjustment for chronological age (47). Similar differences were observed between biomarker composites indexing systemic BA and cellular-level measures like epigenetic age and telomere length (37,46,48). Taken together, these findings suggest patient-level processes of "biological aging" and "allostatic load" may exhibit significant quantitative similarities, and may both be distinct from cellular-level processes of BA.

Literature in the social and behavioral sciences has emphasized AL as an *outcome* responsive to risk factors such as poverty, life stress, and negative health behaviors (49,50). By contrast, biomarker indices approximating BA are often judged with respect to their ability to *predict* physical and cognitive functioning and serve as proxies for healthspan (51). Our current findings support a role for AL in both domains, and give preliminary evidence for superior mortality prediction: a pillar for the validity of BA metrics. Nonetheless, a lack of analytical consistency has limited the generalizability of AL-related findings (52). Our results support the strength of AL measures in these associations but were equivocal on whether they can be improved by adopting more rigorous approaches employed in the construction of BA composites. For example, although previous studies have found value in using an external referent to define model parameters, in our analyses using a reference population to define risk thresholds for AL_{NHANES} provided no additional benefit beyond that observed by restricting the biomarker panel. Further, while effect sizes for AL_{MIDUS} were similar to those observed for $AL_{STANDARD}$, both tended to be larger in magnitude than associations observed for AL_{NHANES}. Importantly, that AL_{MIDUS} exhibited similar utility to $AL_{STANDARD}$, and was more robust to covariate adjustment, despite using one third of the biomarkers, supports the use of biomarker down-selection prior to model building. Doing so may reduce the variability of biomarkers employed across studies, as was observed for measures of KDM Biological Age and PhenoAge in our literature review. Ultimately, we believe the best approach will be to identify an initial panel of theory-driven biomarkers to assay, followed by quantitative screening to ensure parsimony and predictive value before combining into a composite index. Screening biomarkers based on processes of change in longitudinal cohorts and/or associations with mortality may further enhance current approaches (53). The MIDUS cohort offers a unique opportunity to test this approach as waves of data collection continue.

Our results are also unclear on whether or not there is a "best" approach for quantifying aging. Differences in the analytical approach used to generate each measure are indicative of differences in how each method conceptualizes BA. KDM Biological Age measures are constructed using multivariate regression, and biological age is the average change in physiology occurring with increased chronological age. By contrast, HD measures are constructed using multidimensional distances that conceptualize BA as the deviation from an ideal physiological state attained in young adulthood. Finally, AL is reflected as the accumulation of physiological changes that manifest only when they reach a critical threshold. Based on the current analyses, it seems apparent that a reliance upon chronological age during parameterization can weaken the ultimate product, as exemplified by diminished effect sizes for our KDM Biological Age measure and null association with mortality. It is important to note that BA may not occur at a linear rate. In support of this idea, nonlinear changes in the plasma proteome across the lifespan have been reported, instead occurring at distinct phases in the fourth, seventh, and eighth decades of life (54). A common feature of HD and AL measures is the use of a static reference point, whether that point is an ideal state or a critical threshold. However, they are agnostic to the patterns of change between individual values and that referent. This may perhaps allow them to account for nonlinear changes in biomarker values more easily than the regression-based KDM measure.

We acknowledge limitations of this research. First, our analysis did not include cellular-level measures of BA, which are becoming increasingly robust. Genomic data necessary to calculate these items are not yet available in MIDUS. This is an important avenue for future research, as previous work has demonstrated the potential for BA measures implemented at different levels of analysis to capture distinct aspects of the aging process. For example, analyses from the Dunedin birth cohort and Framingham Offspring Study demonstrated minimal overlap between epigenetic clocks and biomarker-based indices of BA (37,48). Similar weak correlations (r = .26–0.38) were observed between epigenetic clocks and AL within The Irish Longitudinal Study on Aging cohort (47). Notably, the strongest correlations were observed between AL and the Levine epigenetic clock, which was trained to predict a biomarker-based BA score (PhenoAge) rather than chronological age (55). Thus, future studies investigating the relationship between cellular-level measures and measures of systemic BA would benefit from inclusion of AL-based measures, which remain largely neglected in this literature. Second, key biomarkers commonly present in BA composites were not available in MIDUS. For example, the lack of complete blood count data and alkaline phosphatase limited our ability to assess functioning of the immune and hepatic systems, respectively. Our panel did include creatinine, a theory-driven biomarker of renal integrity, which demonstrated a marginal but consistently positive impact on the utility of the AL_{MIDUS} measure in leave-one-out analyses. Further, we supplemented our measures using carotenoids indexing antioxidant capacity, including lycopene, which had a marginal but consistently positive impact on the utility of the AL_{MIDUS} measure as an indicator of life-course risk exposure. We also included the carotenoids retinol and lutein, both of which have been shown to modulate stress reactivity in animal models (56,57). That effect sizes observed here

are similar to those in our previous work using a more traditional panel (46), combined with the influence of stress-related processes on aging biology (19), highlights the potential of carotenoids for use in future biomarker indices. Third, MIDUS participants tended to be more educated, wealthier, and are more likely to be White than the general U.S. population, thereby limiting the generalizability of findings presented here (26).

Overall, our findings highlight similarities between indices approximating AL and systemic BA, with inter-item associations seemingly reflecting variability not related to chronological age (Figure 1). Our results also demonstrate the robustness of existing approaches toward the measurement of systemic BA, which predicted functioning, exposure, and mortality despite being constructed with a nontraditional biomarker panel highlighted by indicators of antioxidant capacity and/or the HPA axis. Moreover, comparing between the different AL implementations demonstrates the benefit of implementing a rigorous down-selection process to determine which biomarkers are included in the panel, as well as urge caution when comparing biomarker distributions across cohorts. Finally, we highlight a potential shortcoming of parameterization approaches which rely on the assumption that BA is a linear process. Future gerontological work would benefit by considering these dimensions when approaching sampling design and statistical analyses in relation to systemic biomarker indices.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Author Contributions

W.J.H., D.M.A., and I.S. conceived the original study. W.J.H. conceived analytical approach, conducted analyses, and drafted manuscript under the supervision of D.M.A. and I.S. All authors approved the final manuscript.

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Conflict of Interest

None declared.

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