CHAPTER 3: ALLOSTATIC LOAD AND BIOLOGICAL AGING IN MIDLIFE ADULTS – TWO SIDES OF THE SAME COIN?

Introduction

Aging, in a broad sense, describes “the time-dependent functional decline that affects most living organisms” (López-Otín et al., 2013, p. 1194). The dependency of aging processes on time is subject to robust interindividual variation, with individuals of identical chronological age presenting a variety of endophenotypes. It is a spectrum, on one end-- progeroid syndromes characterized by accelerated aging and early mortality (Dreesen & Stewart, 2011), and on the other end-- supercentenarians resilient to the throes of aging (Willcox et al., 2008). 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 biological aging have been implemented at the clinical (Kim, Myers, Wyckoff, Cherry, & Jazwinski, 2017), physiological (Cohen et al., 2013; Levine, 2013), and molecular levels (Hannum et al., 2013; Horvath, 2013; von Zglinicki & Martin-Ruiz, 2005). The validity of these indices is judged with respect to The American Federation for Aging Research criteria for biomarkers of aging. Namely, a biomarker of aging must (i) predict remaining life expectancy better than chronological age, (ii) monitor a mechanism underlying the aging process and not a specific disease, (iii) be subject to repeated tests without harming the individual, and (iv) be testable in both humans and laboratory animals.

Composite measures integrating a 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 (Jylhava et al., 2017), show variation by young adulthood (Belsky et al., 2015), and appear responsive to intervention (Belsky, Huffman, et al., 2017). These systemic biomarker composites are predicated on the rationale that molecular changes underlying aging processes within individual cells manifest as organ-level dysregulation.

Allostatic load (AL) is a related concept used to describe “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” (McEwen, 1998, p. 37). Similar to composite measures of biological aging, AL indices combine systemic biomarkers across multiple organ systems to form a measure of cumulative dysregulation resulting from life-course exposures. AL indices show (i) individual differences in longitudinal trajectory (Karlamangla et al., 2006), (ii) are predictive of mortality above and beyond individual organ systems (Castagné et al., 2018), and, (iii) are responsive to differences in life-course exposures known to increase risk for morbidity and mortality (Danese & McEwen, 2012). Despite significant conceptual overlap between the two processes, explicit application of AL measures as biological aging indices is limited.

Allostatic load is a concept rooted in evolutionary biology used to describe the cumulative impact of individual episodes of adaptation to external stimuli, with emphasis on neuroendocrine and immune pathways involved in stress physiology. For example, a review of 26 studies implementing allostatic load identified over 50% as having included proxies for the functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Johnson et al., 2017). Stress is a known to accelerate the progression of several aging hallmarks including mitochondrial dysfunction, telomere shortening, and cellular senescence (Shalev & Hastings, 2017). Further, CpG sites located within glucocorticoid response elements make up as much as 24% of the CpG sites included in epigenetic clock algorithms (Zannas et al., 2015). Thus, allostatic load’s emphasis on stress-related domains may strengthen its ability to approximate biological aging.

Even so, quantitative differences in the types of biomarkers employed and approach toward scale construction distinguish systemic biomarker composites approximating biological age from those approximating AL. We conducted an independent review for studies employing systemic biomarker composites of biological age, identifying 9 studies utilizing Klemera-Doubal Method (KDM) Biological Age, homeostatic dysregulation, or Levine Method Biological Age. Study details are provided in **Table C-15**. Excepting exploratory studies developing and validating homeostatic dysregulation (i.e., Cohen et al., 2013; Li et al., 2015), only 16 different biomarkers were utilized across the other 7 studies. The total number of biomarkers employed ranged from 6 to 12 and those most commonly employed were measures of renal (creatinine), hepatic (alkaline phosphatase), and immune functioning (complete blood count data). Lipoproteins and blood sugar indicators also tended to be included. By contrast, a systematic review of studies investigating associations between AL and socioeconomic status identified 59 different biomarkers across 26 studies (Johnson et al., 2017). The number of biomarkers employed ranged from 6 to 25 and those most commonly included were measures of blood pressure, lipoproteins, blood sugar (HBA1c or glucose), and C-reactive protein (CRP). The most common biomarkers utilized in systemic biomarker composites of biological aging (i.e., creatinine, alkaline phosphatase, and complete blood count data) are rarely observed in AL indices.

Wide variation in biomarkers comprising systemic biomarker composites approximating AL could result from differences in analytical approach. The most common selection criteria for inclusion of a biomarker in a biological age composite is correlation with age, specifically *r* ≥ 0.1 (Levine, 2013). After this initial stage, interrelations among biomarkers are investigated to identify the most parsimonious panel. If any two biomarkers are highly correlated (*r* ≥ 0.4), one is removed from consideration. Generally, the marker less strongly associated with age and/or with a smaller sample size would be removed from the calculation of the final composite. By contrast, AL indices tend to use every biomarker available within the given sample.

Systemic biomarker composites approximating biological aging and those approximating AL also take different approaches toward scale construction. Measures of biological aging are defined by different mathematical operations which impact their interpretation even when they are composed using the same biomarker panel. KDM Biological Age utilizes multiple regression (Klemera & Doubal, 2006), LM Biological Age utilizes Gompertz regression and mortality hazards (Liu et al., 2018), and homeostatic dysregulation is a measure of multidimensional distance (Cohen et al., 2013). A common practice across all three approaches is the use of a reference population to define model parameters prior to implementation in the analytical sample. Mathematical operations used to construct AL indices are often less nuanced (see Howard & Sparks, 2016 for a review). A common theme, however, is to identify individuals at risk based on the distribution of biomarker values *within the analytical sample*. Variation exists on whether risk is partitioned to distinct physiological domains (i.e., cardiovascular, immune, etc.) or assessed collectively across all biomarkers, but the identification of sample-specific cutoffs for risk is a common feature.

One study investigating the comparability of AL indices and epigenetic clock measures of biological aging found little evidence that the two reflect similar processes (McCrory et al., 2020). Parallel findings were observed between epigenetic clocks and systemic biomarker composites of biological aging in the Dunedin birth cohort and Framingham Offspring Study (Belsky, Moffitt, et al., 2017; Murabito et al., 2018). Despite their conceptual similarities, it remains unclear whether systemic biomarker composites approximating AL and those measuring biological aging are quantitatively similar.

To investigate this possibility, we utilized data from The National Survey of Midlife Development in the United States (MIDUS), which has been extensively studied in relation to AL. We analyzed two systemic biomarker composites of biological aging that could be quantified with MIDUS data (i.e., KDM Biological Age and homeostatic dysregulation) as well as three approaches toward the calculation of AL, including a common literature standard and two alternative approaches informed by lessons learned from our experiences with biological aging measures. Our analyses proceeded in four steps. First, we tested associations among chronological age and the different systemic biomarker composites. Second, we tested associations between systemic biomarker composites and tests of functional capacities mediating age-related decline, hereafter referred to as healthspan-related characteristics. Third, we tested the extent to which systemic biomarker composites were associated with 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 systemic biomarker composites were predictive of mortality.

Methods

## Sample

Data were from the MIDUS II and MIDUS Refresher Biomarker Projects, which are subsamples of The MIDUS National Survey: a longitudinal survey of more than 7,000 midlife adults in the United States first established in 1995 by the John D. and Catherine T. MacArther Foundation Research Network on Successful Midlife Development. The MIDUS II Biomarker Project was a longitudinal follow-up of a subsample (*n* = 1,255) of the baseline 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 three sites (Dienberg Love et al., 2010). 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 datasets and documentation are available at the ICPSR website (<http://www.icpsr.umuch.edu/>).

We conducted analyses to test hypotheses about measures of AL and biological aging using data from adults aged 26-86 participating in MIDUS II or MIDUS Refresher Biomarker Projects and for whom all measures could be estimated (*N* = 2,064, 45% male).

## Allostatic load measures

We analyzed three different implementations of allostatic load. The first, ALSTANDARD, was computed using the most common implementation of allostatic load in the literature, and utilizes 24 biomarkers to quantify cumulative risk across seven physiological domains (Brooks et al., 2014; Friedman et al., 2015; Gruenewald et al., 2012; Karlamangla et al., 2014; Mori et al., 2014; Rodriguez et al., 2019; Seeman et al., 2014; Wiley et al., 2016). The seven 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 0-1, representing the proportion of biomarkers for that system that reach high risk quartile values. Risk was defined as the highest or lowest quartile depending on which conferred greater risk for morbidity and mortality. Risk was defined independently for men and women. Scores across the seven domains were summed to produce an AL score ranging from 0-7. Descriptive statistics for the biomarkers included in the calculation of ALSTANDARD are detailed in **Table A-11**. Risk cutoffs are detailed by sex in **Table A-12**.

The second and third AL scores were alternative formulations which sequentially integrated practices commonly employed within systemic biomarker composites of biological aging, namely application of stringent selection criteria for biomarkers employed in the algorithm and use of a reference population. For the second allostatic load score, ALMIDUS, we applied down-selection criteria to decide which biomarkers were utilized in the allostatic load score. Following previous work (Levine, 2013), Pearson correlations were used to assess the association among biomarkers and chronological age. Biomarkers were selected based on their correlation with chronological age (*r* > 0.1) and interdependence from other items in the panel (*r* < 0.4). A final panel of eight biomarkers was selected, which collectively assess the integrity of cardiovascular, renal, immune, and antioxidant systems: creatinine, CRP, glycated hemoglobin (HbA1c), lutein/zeaxanthin, lycopene, retinol, systolic blood pressure, and urinary creatinine. ALMIDUS scores were calculated as the proportion of biomarkers that fell within high risk quartiles, where risk quartiles were defined according to biomarker distributions within the analytical sample (i.e., MIDUS II & MIDUS Refresher). Descriptive statistics for the biomarkers included in the calculation of ALMIDUS are provided in **Table A-13**. Risk cutoffs for biomarkers used in the calculation of ALMIDUS are provided in **Table A-15**.

The final allostatic load score, ALNHANES, utilized the same panel of eight biomarkers as the ALMIDUS, 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 non-pregnant participants aged 26-84 in NHANES III and continuous NHANES panels spanning 1999-2016 (*N* = 56,615, 49% male; **Table A-14**). Risk cutoffs for biomarkers used in the calculation of ALNHANES are provided in **Table A-15**. Analyses to construct allostatic load scores are described in detail in **Appendix A**. A brief summary of the three AL measures is provided in **Table 3-1**.

Table 3-1: Overview of allostatic load measures utilized in MIDUS analysis sample

|  |  |  |  |
| --- | --- | --- | --- |
| **Measure Notation** | **Number of Biomarkers** | **Risk Expressed As** | **Sample Defining Risk** |
| ALSTANDARD | 24 | Proportion of biomarkers at risk within each of 7 independent systems is summed across systems for a range of 0-7 | MIDUS analytical sample |
| ALMIDUS | 8 | Proportion of biomarkers at risk across a total of 8 biomarkers for a range of 0-1 | MIDUS analytical sample |
| ALNHANES | 8 | Proportion of biomarkers at risk across a total of 8 biomarkers for a range of 0-1 | NHANES reference sample |

## Biological aging measures

We analyzed two biological aging measures that could be quantified with MIDUS data: KDM Biological Age and homeostatic dysregulation. Both measures are algorithm-based indices that combine information from multiple organ systems in the body (Cohen et al., 2013; Klemera & Doubal, 2006; Levine, 2013). Biological aging estimates made with these algorithms are predictive of morbidity, mortality, functioning, and exposure in both young and old populations (Belsky, Moffitt, et al., 2017; Hastings, Shalev, & Belsky, 2019; Levine, 2013; Li et al., 2015).

KDM Biological Age is computed from an algorithm derived from a series of regressions of individual biomarkers onto chronological age in a reference population. Following previous work (Belsky, Moffitt, et al., 2017), we formed this reference populations from participants in NHANES III and continuous NHANES panels 1999-2016 aged 30-75 who were non-pregnant at the time of biomarker data collection (N=46,038, 49% male; **Table A-3**). 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.

Homeostatic Dysregulation is computed from an algorithm based on Mahalanobis distance (Mahalanobis, 1936) for a panel of biomarkers computed relative to a reference population. Following previous work (Belsky, Moffitt, et al., 2017), we formed this reference populations from participants in NHANES III and continuous NHANES panels 1999-2016 aged 20-30 who were not obese, non-pregnant, and for whom all biomarkers fell within clinically normal ranges (*N* = 350, 57% male; **Table A-8**). An individual’s homeostatic dysregulation score quantifies how different their physiology is from a young, healthy norm.

We calculated KDM Biological Age and homeostatic dysregulation using the same panel of eight biomarkers used to calculated ALMIDUS and ALNHANES, namely creatinine, CRP, HbA1c, lutein/zeaxanthin, lycopene, retinol, systolic blood pressure, and urinary creatinine. Details on biomarker measurements for the reference population are available from the NHANES website (<https://www.cdc.gov/nchs/nhanes/>).

## Healthspan related characteristics

We tested associations of systemic biomarker composites estimating biological aging and AL 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), lower body strength (chair stand), gait speed, respiratory rate, peak flow, and visual acuity all assessed at the same time as biomarker data collection. We also tested associations with the MIDUS composite variable (B4/RA4)HSYMN, an item constructed as a cumulative count of chronic symptoms and conditions. Associations with measures of self-reported physical and emotional health assessed during baseline visits prior to biomarker collection were also tested. Healthspan-related characteristics, including variable names of all items used in their construction, are described in detail in Appendix B. Advanced chronological age was associated with worse performance on nearly all healthspan-related characteristics (**Table B-2**).

## Life-course risk factors for shorter healthspan

We tested associations of systemic biomarker composites estimating biological aging and AL with risk factors known to predict shorter healthspan: early life adversity, low educational attainment, resource deficits, and mental health problems. We assessed early adversity using the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). We measured educational attainment using a five-level categorical variable describing the highest level of education completed by the participant (less than high school, high school/GED, some college/associate’s degree, bachelor’s degree, & post-graduate or professional degree). We assessed material resources using household-adjusted poverty to income ratio. We assessed mental health problems using the Mood and Anxiety Symptom Questionnaire (MASQ) (Watson et al., 1995), Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), and the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983). Life-course risk factors are described in Appendix B and **Table B-6**.

## Mortality data

Mortality data was drawn from the third wave of The MIDUS National Survey (i.e., MIDUS III). Information on vital status of participants in MIDUS II was obtained from data available at the ICPSR website. This dataset includes all known MIDUS decedents as of March 2018 but does not include data on MIDUS Refresher participants. Associations with mortality were tested via Cox proportional hazard models using the *coxph* function from the ‘survival’ package in R (Therneau, 2020).

## Statistical analyses

Our analyses involved the subset of MIDUS participants participating in the MIDUS II or MIDUS Refresher Biomarker projects with sufficient biomarker data to calculate all five systemic biomarker composites (*N* = 2,064, 45% male; **Table C-16**). Women who were pregnant or breast-feeding at the time of biomarker collection were excluded from analyses.

We tested associations among biomarker composites and chronological age using Pearson correlations. For analyses of healthspan related characteristics and life-course risk factors, we tested associations using linear regression (lm function in R) to compute standardized effect sizes (interpretable as Pearson’s r). For models testing associations between biomarker composites and healthspan-related characteristics, biomarker composites were specified as independent variables and healthspan-related characteristics were specified as dependent variables. For models testing associations between risk factors and biomarker composites, risk factors were specified as independent variables and biomarker composites were specified as dependent variables. Models included participants with available data on all five biomarker composites and the healthspan related characteristic or risk factor under analysis. To allow for comparability across biomarker composites and outcomes, we standardized each composite, healthspan characteristic, and risk factor by sex within each subsample to compute standardized effect sizes in all models. All models included covariate adjustment for chronological age and sex.

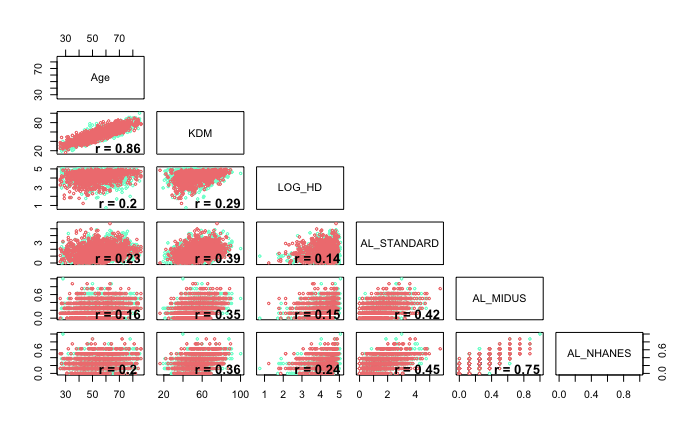
Results

## Associations among measures of allostatic load, biological aging, and chronological age

Chronologically older MIDUS participants had older biological ages and increased AL (**Figure 3.1, Table C-17 Panel A**). MIDUS participants’ chronological ages were most strongly associated with their KDM Biological Ages (*r* = 0.86). Participants’ chronological ages were similarly associated with their homeostatic dysregulation scores (*r* = 0.20) and all three implementations of allostatic load (ALSTANDARD *r* = 0.23, ALMIDUS *r* = 0.16, ALNHANES *r* = 0.20).

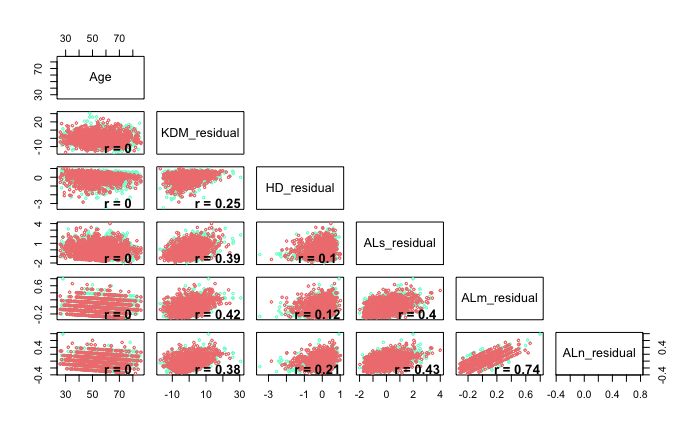
To investigate similarity across biomarker composites, we computed correlations among these five measures. To focus on their ability to index biological aging, we computed associations controlling for variation due to chronological age using residuals of each measures regressed onto chronological age independently by sex. Each adjusted measure was not correlated with chronological age. After adjusting for chronological age, correlations among measures were largely unchanged relative to raw versions. Age-adjusted associations among measures are shown in **Figure 3.2** and **Table C-17 Panel B**.

# Figure 3.1: Associations among biomarker composites hypothesized to index biological age and allostatic load



Associations between chronological age systemic biomarker composites indexing biological aging and allostatic load. Pearson’s correlation coefficient shown at bottom-right was computed after adjustment for sex. Men and women indicated by coral and blue circles respectively.

# Figure 3.2: Associations among age-adjusted biomarker composites hypothesized to index biological age and allostatic load



Associations between chronological age systemic biomarker composites indexing biological aging and allostatic load after adjustment for chronological age. Pearson’s correlation coefficient shown at bottom-right was computed after adjustment for sex. Men and women indicated by coral and blue circles respectively.

## Associations with healthspan related characteristics

We tested associations between systemic biomarker composites and healthspan-related characteristics across three domains: physical functioning, perceptual functioning, and subjective functioning. To control for associations between systemic biomarker composites and chronological age, we conducted these and all subsequent analyses using age-adjusted versions of each measure.

MIDUS participants with more advanced AL performed more poorly on tests of physical functioning, with the exception of peak flow, which was only associated with ALMIDUS and in the opposite direction than expected. Participants’ KDM Biological Ages were also not associated with peak flow, and were associated with grip strength in the opposite direction than expected, replicating previous associations seen between KDM Biological Age and muscle strength in NHANES (Hastings et al., 2019). Participants’ homeostatic dysregulation scores were not associated with any assessments of physical functioning. Participants’ visual acuity was not associated with any measures of biological aging or AL. Effect-sizes are shown in **Figure 3.3** and reported in **Table C-18**.

MIDUS participants with more advanced biological aging and AL also tended to report increased chronic symptoms and conditions, as well worse physical and emotional health. Effect sizes for homeostatic dysregulation scores were smaller and not significant for any measures of subjective functioning and disability. Effect-sizes are shown in **Figure 3.3** and reported in **Table C-18**.

# Figure 3.3: Associations between healthspan-related characteristics and systemic biomarker composites hypothesized to index biological age and allostatic load



Effect-sizes for associations between systemic biomarker composites and healthspan related characteristics. All characteristics were coded such that higher values indicate worse performance. Thus, the expected direction for all associations is positive. Error bars reflect 95% confidence interval of effect-size estimate.

## Associations with life-course risk factors

We next tested whether participants with life-course risk factors for shorter healthspan exhibited more advanced biological aging 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. MIDUS participants with lower educational attainment were also measured as having advanced biological aging as indicated by KDM Biological Age. Material resource deficits were not associated with either biological age index. Participants’ reporting increased early life adversity also exhibited increased AL and biological aging as measured by KDM Biological Age. For perceived stress, individuals reporting increased perceived stress tended to exhibit advanced AL. Perceived stress was also associated with participants’ KDM Biological Ages, but effect sizes were smaller relative to AL indices.

MIDUS participants’ anxiety and depressive symptoms were both associated with their AL scores, as well as their KDM Biological Ages, although to a lesser degree. Participants’ homeostatic dysregulation scores were not associated with any indices of life-course risk exposure. Effect-sizes for associations with life-course risk factors are shown in **Figure 3.4** and reported in **Table C-19**.

# Figure 3.4: Associations between life course risk factors and systemic biomarker composites hypothesized to index biological age and allostatic load



Effect-sizes for associations between systemic biomarker composites and life course risk exposures. All exposures 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.

## 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 tended to exhibit increased risk for all-cause mortality across the follow-up period, and all three AL implementations were significantly associated with all-cause mortality. MIDUS participants’ KDM Biological Ages and homeostatic dysregulation scores were not associated with risk for all-cause mortality. Results for associations between systemic biomarker composites and all-cause mortality are shown in **Table 3-2**.

Table 3-2: Associations between mortality and systemic biomarker composites hypothesized to index biological age and allostatic load

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| --- | --- | --- |
| **Predictor** | **Coefficient (95% CI)** | **p-value** |
| KDM | 0.02 (-0.01, 0.04) | 0.18 |
| HD | 0.35 (-0.06, 0.76) | 0.09 |
| ALSTANDARD | 0.45 (0.24, 0.67) | 4.57E-05 |
| ALMIDUS | 2.75 (1.74, 3.76) | 9.44E-08 |
| ALNHANES | 2.83 (1.73, 3.93) | 4.91E-07 |

Discussion

We studied five systemic biomarker composites proposed to index biological age and AL in a cohort of 2,064 individuals of mixed chronological age drawn from the MIDUS 2 and MIDUS Refresher Biomarker projects. Specifically, we quantified KDM Biological age and homeostatic dysregulation, two patient-level measures hypothesized to index biological age by quantifying decline in system integrity, as well as three different implementations of AL, a process reflecting the cumulative wear and tear on biological systems from repeated exposures to psychosocial stressors. The first implementation, ALSTANDARD, was constructed per literature norms (Gruenewald et al., 2012), while the second two, ALMIDUS and ALNHANES, are novel applications which integrate statistical norms seen in the construction of systemic biomarker composites approximating biological age.

All five measures were correlated with chronological age: chronologically older MIDUS participants exhibiting older KDM Biological Ages, higher levels of homeostatic dysregulation, and higher AL (**Figure 3.1**). Participants’ exhibiting more advanced AL and KDM Biological Age performed worse on tests of physical functioning, reported worse physical and emotional health, and exhibited greater numbers of chronic symptoms and conditions (**Figure 3.3**). In parallel, participants with risk factors for shorter healthy lifespan exhibited more advanced AL scores and KDM Biological Ages as compared to peers of the same chronological age with decreased life-course risk exposure (**Figure 3.4**), excepting that exposure to poverty was not associated with KDM Biological Age.

Our findings suggest hitherto unobserved similarities and differences between systemic biomarker composites approximating biological age and those approximating AL. Associations between AL measures and measures of biological age remained relatively unchanged following adjustment for chronological age, implying a shared quantification of age-independent decline in system integrity (**Figure 2.2**). This observation is contrary to differences observed between epigenetic measures of biological age and AL indices (McCrory et al., 2020). Notably, similar differences were observed between biomarker composites indexing biological age and cellular-level measures like epigenetic age and telomere length (Belsky, Moffitt, et al., 2017; Hastings et al., 2019; Murabito et al., 2018). Taken together, these findings suggest patient-level processes of ‘biological aging’ and ‘allostatic load’ may exhibit significant quantitative similarity.

Allostatic load indices and KDM Biological Ages tended to exhibit similar associations with healthspan related characteristics, although effect sizes observed for AL indices tended to be very slightly larger on average (**Table C-18**). By contrast, AL indices tended to be more responsive to life course risk exposures than KDM Biological Ages. Although effect-sizes for most associations were small, effect sizes for KDM Biological Age were consistently smaller and about half the magnitude as those observed for ALSTANDARD and ALMIDUS, with effect-sizes for ALNHANES lying in between (**Table C-19**). Moreover, AL composites were the only measures associated with risk for mortality (**Table 3-2**).

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 (Beckie, 2012; Suvarna et al., 2020). By contrast, systemic biomarker composites indexing biological age are often judged with respect to their ability to *predict* physical and cognitive functioning as a proxy for healthspan (Justice et al., 2016). 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 biological aging metrics. At the same time, a lack of consistency in analytical approach has limited the generalizability of AL-related findings (Johnson et al., 2017). 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 biological age composites. While effect sizes for ALMIDUS were similar to those observed for ALSTANDARD, both tended to be larger in magnitude than associations observed for ALNHANES. Even so, that similar variability was captured by ALMIDUS using one third of the biomarkers as ALSTANDARD supports the use of biomarker down-selection prior to model building.

The use of a reference population is both a strength and weakness of systemic biomarker composites indexing biological age. On one hand, an external reference reduces cohort-specific biases that may limit the reliability and reproducibility of associations. Indeed, the MIDUS cohort tends to be healthier and wealthier than U.S. population averages (Radler & Ryff, 2010). At the same time, using historical cohorts to define model parameters introduces biases related to cohort effects and mortality selection (Finch & Crimmins, 2004; Moffitt et al., 2017; Yashin et al., 1985). As demonstrated in a recent simulation study, these biases may influence biomarker selection in a manner favoring those with little to no mechanistic links to aging processes (Nelson et al., 2020). Using a reference population to define AL cutoffs in the current work provided no additional benefit beyond that observed by restricting the biomarker panel. Selecting biomarkers based on processes of change in longitudinal cohorts and/or associations with mortality may enhance current approaches. The MIDUS cohort offers a unique opportunity to test this tactic as waves of data collection continue.

The lack of association between any composite and visual acuity is notable. Visual acuity has been linked to mortality (Lee et al., 2002), and is moderately correlated with chronological age (Salthouse et al., 1996). A previous study using data from NHANES observed significant associations between a categorical visual acuity variable and a ten biomarker AL index (Zheng et al., 2014). We also observed significant associations between visual acuity and composites of biological age constructed using a different panel of twelve biomarkers (Hastings, Shalev, & Belsky, 2019). The correlation between visual acuity and chronological age in this study was moderate (*r* = 0.42), and inclusion of retinol (Vitamin A) in our panel would seem to strengthen the likelihood of observing an association. Given each systemic biomarker composite was associated with visual acuity in models run without chronological age (data not shown), it seems likely that any remaining variance independently attributable to biological aging and/or AL was negligible.

We acknowledge limitations in the current work. First, our analysis did not include cellular-level measures of biological aging, which are becoming increasingly robust. Genomic data necessary to calculate these items are not yet available in MIDUS. In a previous analyses, epigenetic clocks were not correlated measures of AL (McCrory et al., 2020). Future studies investigating the affiliation between cellular-level measures of biological aging and systemic, patient-level measures of biological aging would benefit from inclusion of AL based measures, which remain largely neglected in this literature. Second, key biomarkers commonly present in biological aging 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. Third, MIDUS participants tend to be more highly educated, wealthier, and are more likely to be White than the general U.S. population, limiting the generalizability of findings reported here (Radler & Ryff, 2010).

We tested associations of five systemic biomarker composites indexing biological age and AL with healthspan-related characteristics, life-course risk exposures, and mortality. Findings highlight conceptual similarities between these two classes, with interitem associations seemingly reflecting variability not related to chronological age (**Figure 3.1 & 3.2**). 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. Future gerontological work would benefit by considering these dimensions when approaching sampling design and statistical analyses in relation to systemic biomarker composites.

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