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A Comparison of Methods for Assessing Mortality Risk

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Abstract

Objectives—Concepts such as Allostatic Load, Framingham Risk Score, and Biological Age were developed to combine information from multiple measures into a single latent variable that can be used to quantify a person's biological state. Given these varying approaches, the goal of this paper is to compare how well these three measures predict subsequent all-cause and disease-specific mortality within a large nationally representative U.S. sample.

Methods—Our study population consisted of 9,942 adults, ages 30 and above from National Health and Nutrition Examination Survey III. Receiver Operating Characteristic curves and Cox Proportional Hazard models for the whole sample and for stratified age groups were used to compare how well Allostatic Load, Framingham Risk Score, and Biological Age predict ten-year all-cause and disease-specific mortality in the sample, for whom there were 1,076 deaths over 96,420 person years of exposure.

Results—Overall, Biological Age predicted 10-year mortality more accurately than other measures for the full age range, as well as for participants ages 50–69 and 70+. Additionally, out of the three measures, Biological Age had the strongest association with all-cause and cancer mortality, while the Framingham Risk Score had the strongest association with CVD mortality.

Conclusions—Methods for quantifying biological risk provide important approaches to improving our understanding of the causes and consequences of changes in physiological function and dysregulation. Biological Age offers an alternative and, in some cases more accurate summary approach to the traditionally used methods, such as Allostatic Load and Framingham Risk Score.

Keywords

Biological Risk; Aging; Mortality; Biomarkers; Allostatic Load; Biological Age

Introduction

As age increases, the body undergoes progressive deterioration of various physiological systems, and as a result, the risk of disability, mortality and morbidity increase steadily over the adult lifespan (Harman, 2003). Nevertheless, there is significant variation across individuals in the timing of such events. For instance, when comparing persons of a given chronological age, the likelihood of being diagnosed with a disease or dying significantly varies across individuals. These differences in morbidity and mortality risk are likely due to

individual variation in the pace of biological aging. It is hypothesized that the rate at which we age is reliant upon complex interactions between our genes and our environment, in addition to some level of stochasticity (Hayflick, 2007; Finch & Kirkwood, 2000). However, our ability to gain further understanding of mechanisms that govern that rate of physiological decline with age relies on our ability to measure this process. Accordingly, there is a focus on combining measures of physiological functioning using a multi-system approach to explain differences in levels of risk and the pace of aging within populations (Harris et al., 2008).

With the goal of examining the causes of human aging, significant work has gone into identifying biomarkers which precede changes in health status, such as disability, morbidity, and mortality (Butler et al., 2004). The search for reliable “biomarkers of aging” began in the late 1980s and continues today (Sprott, 2010). However, the aging process is exceedingly complex, encompassing a large number of coordinated biological changes across multiple physiological systems. Thus, in recent years there has been an emphasis on incorporating multivariate combinations of biomarkers to better capture this complexity (Crimmins & Seeman, 2004; Goldman et al., 2006; Karlamangla et al., 2002; Wang et al., 2007). Such techniques are desirable given that they rely on the input of information from a number of biological systems and thus may do a better job of measuring the overall decline of the organism than markers of single systems. It is unlikely that any single biomarker will fully encompass the changes associated with the aging process (Sprott, 2010)—especially across individuals or populations, who may be “experiencing” aging differently. In response, concepts such as Allostatic Load, Framingham Risk Score, and Biological Age were developed to combine information from multiple measures into a single latent variable that could be used to quantify a person's biological state.

Allostatic Load was developed as a multi-systems approach to measure cumulative physiological dysregulation and has been found to be a reliable predictor of mortality, cardiovascular disease, and declines in physical and cognitive functioning (Karlamangla et al., 2002; Seeman et al., 1997; Seeman et al., 2002). The maintenance of healthy functioning requires physiological systems to make continuous small adjustments in order to adapt to environmental challenges. This adaptability is referred to as allostasis (Sterling & Eyer, 1998). However, with long-term repeated exposure to life's demands, systems under constant stress tend to breakdown and become dysregulated, leading to declines in overall health (Seeman et al., 2001). Accordingly, the measurement of Allostatic Load is based on the idea that deterioration accumulates over time across multiple physiological systems (McEwan & Stellar, 1993). It combines results from individual indicators to create a cumulative risk score, with the idea that the more individual risk factors (dysregulated systems) a person has, the higher his or her overall risk. The assumption is that moderate dysregulation across systems may be more important for health than the dysregulation of a single system, and this has been supported in empirical studies showing that even when individual components were not found to be “risk factors”, the cumulative biological risk score was able to significantly predict the onset of chronic conditions, disability, cognitive decline, and mortality (Seeman et al., 2002). Typically, the indicators that are used in the calculation measure functioning in metabolic, cardiovascular, inflammatory, hypothalamic-pituitary axis (HPA), and sympathetic nervous systems (SNS) (Seeman et al., 1997).

Although these are usually combined as a count of binary measures, signifying whether an individual is “high risk”, recent evidence is coming out to support the use of continuous measures, or allowing for nonlinear relationships between biomarkers and risk when calculating Allostatic Load. A recent paper by Seplaki et al. (2005) showed that using a two-tailed z-score measure of Allostatic Load significantly improved its predictive ability.

Another early, but recently revised, attempt to use multiple measures to create an overall index of risk was the Framingham Risk Score. The Framingham Risk Score was first developed using longitudinal data from the Framingham Heart Study to predict incidence of coronary heart disease (CHD) or stroke (Anderson et al., 1991; Wilson et al., 1998). Similar to Allostatic Load, FRS is comprised of multiple risk factors that are combined to create an overall risk score. However, unlike Allostatic Load, which relies solely on physiological measures, the Framingham Risk Score also incorporates information about sociodemographic characteristics such as age, sex, and one key behavioral indicator—smoking status. The inclusion of age potentially improves the predictive power of the score, given that aging is believed to be the strongest risk factor for most chronic conditions, as well as mortality.

In keeping with this mindset, Biological Age measures were created to quantify an individual's aging on a physiological level—an idea first proposed by Alex Comfort in 1969 (Comfort, 1969). The measurement combines information on multiple biological systems to quantify where an individual is on the aging trajectory. While similar to Allostatic Load in its use of biomarkers to quantify risk, Biological Age differs in that it incorporates the various age trajectories of measures, rather than high-risk cut-points, into the overall score. The trajectory typically comes from information regarding how different physiological measures change with age within the given population (Levine, 2013). As a result, an individual's Biological Age can be interpreted as how the physiological profile (deregulation and degradation) of the individual compares to that of the average person of that given chronological age within the population from which the equation was generated. For instance, if a person is 50 years old chronologically, but 60 years old biologically, then he or she has a physiological profile—as well as the health risks—of someone who is, on average, ten years older. Overall, it is assumed that persons who appear to be aging faster (older biologically than they are chronologically) are at greater risk for experiencing negative health events like death and disease earlier in their lives. Accordingly, Biological Age is thought to be a more precise estimate of an individual's true remaining life expectancy and in previous studies has been found to predict mortality better than chronological age (Levine, 2013).

Given the various approaches available for estimating biological risk, the goal of this paper is to compare how well Allostatic Load, the Framingham Risk Score, and Biological Age predict subsequent all-cause and disease-specific mortality within a large nationally representative U.S. population. While the selection of biomarkers may be particularly important for a given measure's predictive ability—a question that requires further investigation, taking into account differences between populations—this study aims to compare these composite measures using biomarkers that tend to be readily available in many large population databases.

Materials and Methods

Study Population

Our sample was made up of participants from the third National Health and Nutrition Examination Survey (NHANES III), a nationally representative, cross-sectional study conducted by the National Center for Health Statistics between 1988 and 1994. Survey, laboratory and clinical exam data for NHANES III were collected on a representative sample of the U.S. noninstitutionalized population of all ages. For this study our sample was limited to adults aged 30+ years, to focus on mortality that was age-related and ensure that participants were old enough to be experiencing age-related physiological changes. Of the 15,042 age eligible participants, our final analytic sample was comprised of 9,942 individuals. Excluded participants were those with missing biomarker ($n=5,024$) or demographic data ($n=66$). Compared to our analytic sample, subjects with missing data were on average 6 years older, had about one year less education, were 40% more likely to be black, and were twice as likely to die.

Mortality

Follow-up data on mortality status and person-months of exposure were available from linked records from the National Death Index. Although information was available through 2006 for all subjects, potential follow-up time ranged from approximately 12 to 18 years, because participants took part in NHANES III at different dates between 1988 and 1994. As a result, in addition to the full mortality data, we also created a binary variable for ten-year all-cause mortality. Information was provided for 113 potential underlying causes of death (UCOD-113) and was used to code for all-cause, cardiovascular (CVD), and cancer mortality. Finally, given that our study is concerned with age-related mortality, deaths from violent, accidental or HIV related causes were censored during analysis.

Biological Age

Our Biological Age measurement utilized information on ten factors, covering multiple physiological systems: glycated hemoglobin, total cholesterol, systolic blood pressure, forced expiratory volume at 1 second (FEV1), serum creatinine, serum urea nitrogen, serum alkaline phosphatase, serum albumin, C-reactive protein (CRP), and cytomegalovirus (CMV) optical density. Twenty-one biomarkers were initially considered and those found to be significantly associated with chronological age at $r > 0.10$ were selected. The full list of biomarkers is available in our previous paper (Levine, 2013). While, as mentioned previously, more work can be done to establish an ideal set of biomarkers for the construction of biological age measures, we were limited to those available in the NHANES dataset, and that, at least in a westernized population—such as the U.S.—show changes with age using cross-sectional data.

Biological Age was estimated using the algorithm proposed by Klemmera and Doubal (2006). This measure has been validated using both real and simulated data and has been shown to predict death more accurately than chronological age. Biological Age estimates combine information from equations of chronological age regressed on biomarkers (Table 1). Similar to the Framingham Risk Score, the equation for Biological Age also incorporates

chronological age. Klemmera and Doubal argue that chronological age contributes important information to the calculation of Biological Age, given that they should be linearly related, with a slope of 1, intercept of 0, and residual deviation. As a result, when calculating it for a population, mean Biological Age should equal mean chronological age.

The equation for calculating Biological Age is:

$$BA_{EC} = \frac{\sum_{j=1}^m [(x_j - q_j)] \frac{k_j}{s_j} + \frac{CA}{s_{BA}^2}}{\sum_{j=1}^m \left(\frac{k_j}{s_j} \right)^2 + \frac{1}{s_{BA}^2}}$$

Where, k_j and q_j are the slope and intercept, respectively, for chronological age on each biomarker, x_j is the measured biomarker value, s_j is the root mean squared error of chronological age regressed on a biomarker, and CA is chronological age. Additionally, (the variance of the random variable, R_{BA}) takes into account the variability in the first half of the equation, the mean variance of the biomarkers that is explained by chronological age, and the range of chronological age. Given that differences may be small earlier in the lifespan, but tend to diverge as people age, this value was further transformed, as suggested by Klemmera and Doubal, so that its mean remained the same, but values increase linearly with age, with a difference of five between the oldest and the youngest subject (Klemmera & Doubal, 2006).

Allostatic Load

Although traditionally Allostatic Load incorporates information from neuroendocrine markers such as epinephrine, norepinephrine, and dehydroepiandrosterone sulfate (DHEA-S) (Seeman et al., 1997), these markers are not available in a number of datasets operationalizing allostatic load, including NHANES, resulting in formulations of allostatic load without these components. In accordance with previous estimations of Allostatic Load using NHANES data (Seeman et al., 2008; Crimmins et al., 2009), nine indicators of physiological functioning were used: albumin, C-reactive protein (CRP), waist-to-hip ratio, total cholesterol, high-density lipoprotein cholesterol (HDL), glycated hemoglobin (HbA1c), pulse, systolic blood pressure, and diastolic blood pressure. Clinical or previously defined cut-points (Table 2) were used to categorize subjects as high risk on each biomarker. From this, an index of overall biological risk was estimated as the number of biomarkers on which a subject is classified as high risk, and this value is then used to represent Allostatic Load. Index scores for subjects who were missing on a single biomarker were imputed by taking their percentage of high risk measures and standardizing it to the 0-9 point scale.

To ensure that the limited biomarker list was not altering the ability of Allostatic Load to predict mortality, another Allostatic Load measure was calculated that included five additional markers that had been selected for our Biological Age calculation—FEV1, serum creatinine, serum urea nitrogen, serum alkaline phosphatase, and CMV. For each of these measures, being in the top quintile (creatinine, urea nitrogen, alkaline phosphatase, CMV) or bottom quintile (FEV1) was utilized to signify “high risk”.

Finally, a continuous, z-score measure of Allostatic Load was calculated in accordance with the method outlined by Seplaki et al. (2005). Participants' z-scores were calculated for each of the fourteen biomarkers, as well as for age, and then summed. For all the measures, aside from age, two-tailed z-score measures were used, where a participants' z-score represents the absolute value of the standardized distance between their level of a given biomarker and the population mean of that biomarker—a score of 1 suggests that the participant is either 1 standard deviation above or below the mean. On the other hand, z-scores for age were calculated with the one-tail approach, where a score of 1 signifies that a participant is 1 standard deviation older than the mean age of the population, whereas a score of -1 signifies that a participant is 1 standard deviation younger than the mean age of the population.

Framingham Risk Score

Risk scores were computed in accordance with the updated 2001 Framingham point system guidelines set forth by the National Cholesterol Education Program (NCEP, 2001). As outlined, six markers were used—age, sex, smoking, total cholesterol, HDL, and systolic blood pressure. For every marker, specific levels are associated with a score; often taking into account the subject's sex, age, or medication use. The levels and scores used for the individual markers are shown in Table 3 (males) and Table 4 (females). Next scores for each maker are summed to calculate subjects' total points, which then determine his or her 10-year risk score. For example a 53 year-old male, smoker, with 220 mg/dL total cholesterol, 42 mg/dL HDL, and non-treated systolic blood pressure of 135 mm Hg will have a total of 14 points, which, for males, is associated with a risk score of 16%—or a 16% chance of having a CVD event over the next 10-years.

Statistical Analysis

Pearson's correlations were used to examine the consistency between the five measures—Allostatic Load, Expanded Allostatic Load (with additional biomarkers), Continuous Allostatic Load, Framingham Risk Score, and Biological Age to determine whether participants were being differentially ranked as high or low risk according to which method was used. Next, Receiver Operating Characteristic (ROC) curves were utilized to estimate and compare the ability of each measure, and chronological age, to predict 10-year mortality. ROC curves were run for the full age range and using age-stratified models, to determine whether the four measures of biological risk performed differently for various age groups (ages 30-49, ages 50-69, and ages 70+). Finally, we created quintiles for each risk measure and used age and sex-adjusted Cox proportional hazard models to determine what the strength of the association was between quintiles of risk and all-cause, CVD and cancer mortality.

Results

Sample Description

As shown in Table 5, mean chronological age for the sample is 49.64 years, which is equivalent to mean Biological Age. Mean Allostatic Load is 2.12, while mean Allostatic Load with the addition of the five biomarkers was 3.20, and mean continuous Allostatic Load was 9.25. Framingham Risk Score had a mean of 6.10, signifying that on average, the

population had just over a 6% chance of myocardial infarction or stroke incidence over the next ten years. Overall, the majority of the sample was Non-Hispanic white (82.5%), followed by Non-Hispanic black (9.5%), and finally Hispanic (8.0%). Just over half of the subjects were female (52.9%), and on average they had 12.4 years of education. Finally, between baseline and follow-up (12-18 years), 18.5% of participants died of all-cause mortality, 4.7% died of CVD mortality, and 3.1% died of cancer mortality—contributing to a total of 95,301 person-years for the total sample ($n=9,942$). However when limiting the follow-up time to 10-years—which was needed for the ROC curve analysis given the unequal follow-up times—we found that 10.7% of participants died overall.

Biological Risk Correlations

Correlation coefficients between risk measures are shown in Table 6. Justifiably, the two cutoff-based Allostatic Load measures are highly correlated ($r=.876$). However overall, subjects were ranked somewhat differently depending on which method was used. For instance, the remaining correlations ranged from $r=.401$ for Framingham Risk Score and continuous Allostatic Load to $r=.658$ for Framingham Risk Score and Biological Age, with most of the correlations just over from $r=.50$.

Accuracy in Predicting Mortality

ROC curves measure both the sensitivity (true positive rate) and one minus the specificity (false positive rate) of a model in predicting a dichotomous outcome, in this case ten-year mortality. From this, the area under the curve (AUC) is calculated, signifying the accuracy of the predictions. Results from our ROC curve comparisons of Biological Age, Allostatic Load, expanded Allostatic Load (additional biomarkers), continuous Allostatic Load, and Framingham Risk Score are listed in Figure 1. Biological Age predicted mortality significantly better than the other measures. According to the AUC, Biological Age could be used to classify participants into those who will survive and those who will die over the following ten years with 87.5% accuracy ($AUC=0.8751$). On the other hand, Allostatic Load only predicted with 67.9% ($AUC=0.6791$) accuracy—an almost 23% reduction from Biological Age (Bonferroni $P<.0001$). Furthermore, while expanded Allostatic Load, continuous Allostatic Load, Framingham Risk Score, and chronological age appear to perform somewhat better than the basic Allostatic Load measurement—with AUCs of 0.7703, 0.8156, 0.8102, and 0.8614, respectively—they were still found to be significantly less accurate than Biological Age (Bonferroni $P<.0001$).

When comparing the ROC curves within age ranges, Allostatic Load, expanded Allostatic Load, continuous Allostatic Load and the Framingham Risk Score had similar levels of accuracy to Biological Age for predicting mortality among younger subjects (ages 30-49), while chronological age was significantly less accurate (Bonferroni $P<.0001$). On the other hand, differences between the measures did exist for older age groups. Among subjects ages 50-69, Biological Age and continuous Allostatic Load predicted mortality with about 72% accuracy ($AUC=0.7273$ and 0.7244 , respectively), which was significantly better than the other two Allostatic Load measures, Framingham Risk Score, and chronological age. Finally, for subjects ages 70 and above, Biological Age predicted 10-year mortality significantly better than all three Allostatic Load measures and Framingham Risk Score

(Bonferroni $P < .0001$), but was not significantly different than chronological age (Bonferroni $P = .324$).

Strength of Associations with Mortality

Nevertheless, it has been suggested that ROC curves may not always be the best method for assessing a measure's association with future risk (Cook, 2007), and as a result, Cox proportional hazard models were used to compare the strength of the associations between the five measurements and mortality. Quintiles of Allostatic Load, expanded Allostatic Load, continuous Allostatic Load, Framingham Risk Score, and Biological Age were created and included in adjusted cox proportional hazard models of all-cause, CVD and cancer mortality (Table 7). Models were adjusted for chronological age and sex. Overall, Biological Age appeared to have the strongest association with all-cause mortality. Those in the highest quintile of Biological Age were over 12.5 times as likely to die as those in the lowest quintile (HR: 12.76, $P < .001$). Alternatively, those in the highest quintiles of Allostatic Load, expanded Allostatic Load, continuous Allostatic Load, and Framingham Risk Score had overall mortality risks that were between 2.5-7 times as high as those in the lowest quintile of each measure (HR_{Allostatic Load}: 2.75; HR_{Expanded Allostatic Load}: 3.62; HR_{Continuous Allostatic Load}: 6.97; HR_{Framingham Risk Score}: 4.76).

All five measures were associated with CVD mortality. As expected, the Framingham Risk Score had the strongest association with CVD mortality, with those in the fifth quintile having an over 27-fold increase in risk over those in the first quintile. Participants who were in the highest risk quintile of Biological Age had over a 11-fold increase in the risk of CVD mortality compared to those in the first (lowest) quintile. Participants within the highest quintile for Allostatic Load, expanded Allostatic Load, and Continuous Allostatic Load had a 3.1, 3.8, and 6.6-fold increase in CVD mortality, respectively.

Finally, being in the highest quintile of any of the five measures, compared to the lowest quintile, was associated with an increased risk in cancer mortality. When Biological Age was used, participants with the highest scores were 13.5 times as likely to die of cancer as those with the lowest scores. However, the increase in risk dropped to 3.6 when the Framingham Risk Score was used, 2.1 when the expanded Allostatic Load measure was used, 3.7 when the continuous Allostatic Load measure was used, and 2.0 when the normal Allostatic Load measure was used.

Discussion

Our study showed that while Allostatic Load and the Framingham Risk Score are useful measures for predicting all-cause, CVD and cancer mortality risk, Biological Age may perform just as well, if not slightly better. Compared to the other measures, Biological Age was found to be significantly more accurate in its prediction of ten-year mortality across the full age range and among adults ages 50-69, and age 70 and above. Additionally, Biological Age predicted mortality more accurately than chronological age for the full age range, as well as for those ages 30-49 and 50-69, and Biological Age had the strongest association with all-cause and cancer mortality, and was strongly associated with CVD mortality—although, not as strongly as the Framingham Risk Score. We believe that the level of

predictive ability that was gained by using Biological Age over more traditional measures, such as Allostatic Load, supports the use of more complex models of aging when estimating multisystem physiological state.

One explanation for part of the improvement in mortality prediction when using Biological Age is that the aging process is strongly associated with the incidence of death and disease. While Allostatic Load and the Framingham Risk Score may serve as useful proxies for the degree of dysregulation in the body, there appears to be something inherent to the aging process that cannot be measured as well using sums of dichotomous cut points or categories. One explanation is that the age-related decline of a system is a continuous process, rather than the transition from a healthy state to a high risk state, and while the use of cut-points may be clinically meaningful, this approach may be losing potentially valuable information by ignoring the full range of values for each biomarker and limiting the potential range of values in the overall measure. This may explain why Allostatic Load and the Framingham Risk Score don't perform as well in older adults as Biological Age does. Within older age groups, more individuals may have measures that are clinically classified as "high risk". However, more precise information may be needed to compare the degree of dysregulation among such individuals—rather than only comparing between the risk categories. This is partially substantiated given the improvement in predictive power when using a continuous measure of Allostatic Load over more traditional methods. Nevertheless, the continuous measure was still out-performed by Biological Age, suggesting that incorporating information on age-trajectories of biomarkers in quantifying an individual's discrepancy from his/her expected value, may further improve the overall risk measurements. For instance, while the continuous Allostatic Load measure took into account how much an individual varies from the mean of a given biomarker, Biological Age specifies how much that individual varies from the value that is expected for his/her chronological age—thus potentially incorporating information regarding the association between biomarkers and aging.

Although these measures have been proposed to capture similar constructs, we found that participants rank quite differently on the measures, as evidenced by the surprisingly low correlations between the measures. Therefore, given the theoretic differences between Allostatic Load, the Framingham Risk Score, and Biological Age, the ability of these measures to predict may depend on what the outcome of interest is. For instance, Framingham Risk Score was developed to predict 10-year risk of having a cardiovascular event, and therefore, may not be as well equipped for predicting other outcomes. On the other hand, Allostatic Load is based on the idea that systems that are chronically challenged—typically as a result of stressful environments—will lose their ability to function properly and eventually become dysregulated making them unable to appropriately respond and/or adjust to environmental stimuli (Seeman et al., 2001; McEwan & Stellar, 1993). As a result, this measure may be better suited for measuring differences in health that are believed to be brought on by environmental stressors, such as disadvantage, work stress, or psycho-social factors. Finally, Biological Age may be best suited for predictions related to the aging process, such as timing of events, and differences in the rate of change due to either genetic or environmental factors.

The biomarkers used in the calculations of the various biological risk measures may also contribute to differences in their potential for predicting outcomes of health. As expected, the Framingham Risk Score was very strongly associated with CVD mortality, given that the measures included in its calculation were selected based on their association with the incidence of CVD and stroke (Anderson et al., 1991; Wilson et al., 1998). As a result, Framingham Risk Score may not be as successful in predicting either all-cause or cancer mortality. On the other hand, Allostatic Load is typically estimated using neuroendocrine markers, that weren't available in our dataset (Seeman et al., 1997). We recognize the use of a limited set of biomarkers may have impacted the validity of the measure, decreasing its predictive ability, and therefore, may not fully represent the predictive ability of a more traditional Allostatic Load measures. This is evidenced in our results, given that the inclusion of five additional biomarkers (FEV1, serum creatinine, serum urea nitrogen, serum alkaline phosphatase, and CMV), taken from our Biological Age measure, greatly improved the performance of the Allostatic Load measure. Consequently, this may be important to keep in mind when estimating Allostatic Load in datasets that lack any of the traditional hypothalamic-pituitary axis (HPA), and sympathetic nervous systems (SNS) measures. Finally, the usefulness of biomarkers may also be population-dependent, given that populations may age differently depending on their environmental and genetic characteristics. For instance, the biomarkers used to measure aging may be different for indigenous populations with limited nutrition and high inflammatory loads versus a population like the U.S. with over-nutrition and low rates of infectious disease (Crimmins et al., 2013).

Overall, this study has several strengths, including its use of a large nationally representative sample of the US population, and reliable sources for mortality follow-up and physiological data. Nevertheless, there are limitations to this study that should be acknowledged. First, biomarker data for NHANES respondents is only available for a single time point, preventing us from looking at changes or trajectories of Allostatic Load, Framingham Risk Score, and Biological Age. Next, excluded participants, due to missing data (n=5,090), were more likely to be older, black, of lower socioeconomic status, and more likely to die. Although, this may have impacted our results, it would most likely cause the strength of the association between the four measures and mortality to be underestimated, given that the use of a healthier population should produce more conservative estimates. Finally, follow-up data was only available for mortality, preventing us from examining other negative health events, such as disability onset and disease incidence.

Overall, these methods are important and necessary for our understanding of the causes and results of changes in physiological function and dysregulation with aging. While the traditionally used methods, such as Allostatic Load and Framingham Risk Score proved reliable for predicting a negative health event, such as mortality, Biological Age offers an alternative method that in some cases may be more useful. In moving forward it will be important to examine the trajectories or changes in these measures in order to better understand the progression of disease and susceptibility to death, and in doing so, facilitate our ability to extend healthy life expectancy.

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References

- Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. *Am Heart J*. 1991; 1(2):293–8. 121. [PubMed: 1985385]
- Butler RN, Sprott R, Warner H, et al. Biomarkers of aging: from primitive organisms to humans. *J Gerontol A Biol Sci Med Sci*. 2004; 59:B560–B567. [PubMed: 15215265]
- Comfort A. Test-battery to measure ageing-rate in man. *Lancet*. 1969; 2:1411–1415. [PubMed: 4188291]
- Cook NR. Use and misuse of the receiver operation characteristic curve in risk prediction. *Circulation*. 2007; 115(7):928–35. [PubMed: 17309939]
- Crimmins EM, Seeman TE. Integrating biology into the study of health disparities. *Popul Dev Rev*. 2004; 30:89–107.
- Crimmins EM, Kim JK, Seeman TE. Poverty and biological risk: earlier “aging” of the poor. *J Gerontol A Biol Sci Med Sci*. 2009; 64(2):286–92. [PubMed: 19196637]
- Crimmins, E.; Wheaton, F.; Vasunilashorn, S., et al. A Global Perspective on Physiological Change with Age. In: McDaniel, SA.; Zimmer, Z., editors. *Global Ageing in the Twenty-First Century: Challenges, Opportunities and Implications*. Ashgate Publishing Ltd; England: 2013. p. 79–103.
- Finch, CE.; Kirkwood, TBL. *Chance Development Aging*. Oxford, UK: Oxford University Press; 2000.
- Goldman N, Turra CM, Gleib DA, Lin YH, Weinstein M. Physiological dysregulation and changes in health in an older population. *Exp Geront*. 2006; 41:862–870.
- Harman D. The free radical theory of aging. *Antioxid Redox Signal*. 2003; 5(5):557–61. 2003;5. [PubMed: 14580310]
- Harris, JR.; Gruenewald, TL.; Seeman, T. An overview of biomarker research from community and population-based studies on aging. In: Weinstein, M.; Vaupel, JW.; Wachter, KW., editors. *Biosocial surveys*. Washington, DC: National Academies Press; 2008. p. 96–135. Committee on Population, Division of Behavioral and Social Sciences and Education
- Hayflick L. Biological aging is no longer an unsolved problem. *Ann N Y Acad Sci*. 2007; 1100:1–13. 10.1196/annals.1395.001 [PubMed: 17460161]
- Karlamangla AS, Singer BH, McEwen BS, Rowe JW, Seeman TE. Allostatic load as a predictor of functional decline MacArthur studies of successful aging. *J Clin Epidemiol*. 2002; 55:696–710. [PubMed: 12160918]
- Klemera P, Doubal S. A new approach to the concept and computation of Biological Age. *Mech Ageing Dev*. 2006; 127:240–248. [PubMed: 16318865]
- Levine ME. Modeling the rate of senescence: can estimated Biological Age predict mortality more accurately than chronological age. *J Gerontol A Biol Sci Med Sci*. 2013; 68(6):667–74. [PubMed: 23213031]
- McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. *Arch Int Med*. 1993; 153:2093–2101. [PubMed: 8379800]
- NCEP. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association*. 2001; 285(19): 2486–2497. [PubMed: 11368702]
- Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation -Allostatic load and its health consequences - MacArthur studies of successful aging. *Archives of Internal Medicine*. 1997; 157(19):2259–2268. [PubMed: 9343003]
- Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98(8):4770–4775. [PubMed: 11287659]

- Seeman TE, Singer BH, Ryff CD, Love GD, Levy-Storms L. Social relationships, gender, and allostatic load across two age cohorts. *Psychosomatic Medicine*. 2002; 64:395–406. [PubMed: 12021414]
- Seeman T, Merkin SS, Crimmins E, et al. Education, income and ethnic differences in cumulative biological risk profiles in a national sample of US adults: NHANES III (1988-1994). *Soc Sci Med*. 2008; 66(1):72. [PubMed: 17920177]
- Seplaki CL, Goldman N, Gleit D, Weinstein M. A comparative analysis of measurement approaches for physiological dysregulation in an older population. *Experimental Gerontology*. 2005; 40:438–449. [PubMed: 15919596]
- Sprott RL. Biomarkers of aging and disease: introduction and definitions. *Exp Gerontol*. 2010; 45(1): 2–4. [PubMed: 19651201]
- Sterling, P.; Eyer, J. *Handbook of Life Stress, Cognition, and Health*. Fisher, S.; Reason, J., editors. Wiley; New York: 1988. p. 631-651.
- Wang TJ, Gona P, Larson MG, Levy D, Benjamin EJ, Tofler GH, et al. Multiple biomarkers and the risk of incident hypertension. *Hypertension*. 2007; 49:432–438. [PubMed: 17242302]
- Wilson PW, D'Agostino RB, Levy D, Belanger AM, et al. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998; 97(18):1837–47. [PubMed: 9603539]

Figure 1. ROC Curves of Risk Measures' Predictions of 10-Year All-Cause Mortality

For the full age range (30+), Biological Age predicted 10-year mortality with 87.5% accuracy, which was significantly more accurate than chronological age, Framingham Risk Score or any of the Allostatic Load measures. However, among younger adults, ages 30-49, there was no difference between the predictions by Biological Age and those by the other risk measures, although Biological Age did still predict mortality significantly better than chronological age. For participants ages 50-69, Biological Age predicted mortality better than all other measures, except for the continuous Allostatic Load measure, and finally for participants 70 years of age and over, the only measure that was comparable to Biological Age was chronological age.

Table 1
Values included in the calculation of Biological Age

	<i>q</i>	<i>k</i>	<i>s</i>
CRP	.183	.005	.619
Serum Creatinine	.907	.003	.273
Hba1c	4.58	.017	.945
Albumin	4.42	-.005	.334
Total Cholesterol	.776	171.62	40.38
CMV	.710	.003	1.11
Alkaline Phosphatase	60.62	.440	29.68
FEV1	1628.36	-11.64	198.65
Urea Nitrogen	8.43	.127	4.80
Systolic BP	90.95	.678	15.01

$$s_{BA}^2 = 56.56$$

q: Intercept for chronological age regressed on the biomarker

k: Slope of chronological age regressed on the biomarker

s: Root mean squared error of chronological age regressed on the biomarker

s_{BA}^2 : the variance of the random variable, BA

Table 2
Allostatic Load Cut-points

Indicator	High-risk cut point
Serum Albumin	<3.8 g/dL
C-Reactive Protein	>3.0 mg/L
Glycated Hemoglobin	>=6.4%
Waist-to-Hip Ratio	>.90 (males) >.85 (females)
High-density Lipoprotein Cholesterol	<40 mg/dL
Total Cholesterol	>=240 mg/dL
Pulse (at 60 s)	>=90
Systolic Blood Pressure	>=140 mm Hg
Diastolic Blood Pressure	>=90 mm Hg
<i>Additional Variables for Expanded Measure</i>	
Serum Creatinine	>=1.3
CMVOD	>=3
Alkaline Phosphatase	>101
FEV1/Ht	>=39.33
Urea Nitrogen	>=18

Table 3

Framingham Risk Score Points for Males

Risk Factor	Age Interval										
	All Ages	20-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75+
Age		-9	-4	0	3	6	8	10	11	12	13
Current Smoking		8	8	5	5	3	3	1	1	1	1
Total Cholesterol											
<160	0										
160-199		4	4	3	3	2	2	1	1	0	0
200-239		7	7	5	5	3	3	1	1	0	0
240-279		9	9	6	6	4	4	2	2	1	1
280		11	11	8	8	5	5	3	3	1	1
HDL Cholesterol											
<40	2										
40-49	1										
50-59	0										
60	-1										
Systolic Blood Pressure											
<u>Not Treated</u>											
<120	0										
120-129	0										
130-139	1										
140-159	1										
160	2										
<u>Treated</u>											
<120	0										
120-129	1										
130-139	2										
140-159	2										
160	3										

Risk Factor	Age Interval													
	All Ages	20-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75+			
Total Points	<0	0-4	5-6	7	8	9	10	11	12	13	14	15	16	17
10-year risk	<1	1	2	3	4	5	6	8	10	12	16	20	25	30

Table 4

Framingham Risk Score Points for Females

Risk Factor	Age Interval										
	All Ages	20-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75+
Age		-7	-3	0	3	6	8	10	12	14	16
Current Smoking		9	9	7	7	4	4	2	2	1	1
Total Cholesterol											
<160	0										
160-199		4	4	3	3	2	2	1	1	1	1
200-239		8	8	6	6	4	4	2	2	1	1
240-279		11	11	8	8	5	5	3	3	2	2
280		13	13	10	10	7	7	4	4	2	2
HDL Cholesterol											
<40	2										
40-49	1										
50-59	0										
60	-1										
Systolic Blood Pressure											
Not Treated											
<120	0										
120-129	1										
130-139	2										
140-159	3										
160	4										
Treated											
<120	0										
120-129	3										
130-139	4										
140-159	5										
160	6										

Risk Factor	Age Interval													
	All Ages	20-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75+			
Total Points	<9	9-12	13-14	15	16	17	18	19	20	21	22	23	24	25
10-year risk	<1	1	2	3	4	5	6	8	11	14	17	22	27	30

Table 5
Sample Characteristics (N=9,942)

	Value
Chronological Age, Mean (SD)	49.64 (14.83)
Biological Age, Mean (SD)	49.64 (15.37)
Allostatic Load, Mean (SD)	2.12 (1.47)
Allostatic Load (Expanded), Mean (SD)	3.20 (2.08)
Continuous Allostatic Load, Mean (SD)	9.25 (3.36)
10-year Cardiovascular Risk (Based on Framingham Risk Score, Mean (SD))	6.10 (7.53)
Non-Hispanic White, (%)	82.55
Non-Hispanic Black, (%)	9.51
Hispanic, (%)	7.94
Female, (%)	52.86
Years of Education, Mean (SD)	12.41 (3.23)
All-Cause Mortality (10-year), (%)	10.66
All-Cause Mortality (12-18 years), (%)	18.47
CVD Mortality, (%)	4.74
Cancer Mortality, (%)	3.09
Person-Years (Total)	95,301

Table 6

Correlation Coefficients Between Risk Measures

	Allostatic Load	Expanded Allostatic Load	Continuous Allostatic Load	Framingham Risk Score	Biological Age
Allostatic Load	1.00				
Expanded Allostatic Load	0.876	1.00			
Continuous Allostatic Load	0.469	0.876	1.00		
Framingham Risk Score	0.501	0.565	0.401	1.00	
Biological Age	0.485	0.650	0.571	0.658	1.00

Table 7
Hazard Ratios for Mortality by Measurement Quintiles

Biological Risk Measurement Quintiles	Hazard Ratio		
	All-Cause	CVD	Cancer
Biological Age			
2	1.30	1.87	0.94
3	2.48**	2.57	3.30*
4	5.76***	5.41**	8.45***
5	12.76***	11.33***	13.53***
Allostatic Load			
2	1.18	1.26	0.99
3	1.54***	1.49*	1.46
4	1.93***	1.91***	1.47
5	2.75***	3.06***	2.08**
Expanded Allostatic Load			
2	1.01	0.95	0.92
3	1.61**	1.77*	1.29
4	2.13***	2.31**	1.69
5	3.62***	3.77***	2.14*
Continuous Allostatic Load			
2	1.99**	1.76	2.09*
3	2.79***	2.97*	2.28*
4	3.455***	3.70*	2.30*
5	6.97***	6.58***	3.74**
Framingham Risk Score			
2	1.96	3.09	2.19
3	2.76**	13.45***	2.33
4	3.17**	16.69***	2.35
5	4.76***	27.06***	3.64*

 $P < .001$

**
 $P < .01$

*
 $P < .05$

Reference=1st Quintile

All models are adjusted for age and sex