Review Article

Predictors of Biological Age: The Implications for Wellness and Aging Research

Gerontology & Geriatric Medicine
Volume 7: 1–13

DOI: 10.1177/23337214211046419
journals.sagepub.com/home/ggm
SAGE

Trevor Lohman[1], Gurinder Bains[1], Lee Berk[1], and Everett Lohman[1]

Abstract

As healthspan and lifespan research breakthroughs have become more commonplace, the need for valid, practical markers of biological age is becoming increasingly paramount. The accessibility and affordability of biological age predictors that can reveal information about mortality and morbidity risk, as well as remaining years of life, has profound clinical and research implications. In this review, we examine 5 groups of aging biomarkers capable of providing accurate biological age estimations. The unique capabilities of these biomarkers have far reaching implications for the testing of both pharmaceutical and non-pharmaceutical interventions designed to slow or reverse biological aging. Additionally, the enhanced validity and availability of these tools may have increasingly relevant clinical value. The authors of this review explore those implications, with an emphasis on lifestyle modification research, and provide an overview of the current evidence regarding 5 biological age predictor categories: Telomere length, composite biomarkers, DNA methylation "epigenetic clocks," transcriptional predictors of biological age, and functional age predictors.

Keywords

aging, biogerontology, cardiovascular diseases and risk, cellular processes, chronic diseases, frailty, genetics

Final revision received: July 14, 2021; **accepted:** August 18, 2021.

Introduction

Age-related disease is a persistent and increasingly prevalent burden on healthcare systems around the world (Atella et al., 2019; Benjamin et al., 2018; Chang et al., 2019; Hurd et al., 2013; Mariotto et al., 2011). Any affordable and accessible intervention capable of ameliorating this trend would therefore be of significant value. One class of interventions that seems well suited for this challenge is lifestyle modification (Ruiz-Estigarribia et al., 2020; Wu et al., 2020; Zhang et al., 2021). Although lifestyle-based interventions such as diet and exercise are generally known to increase lifespan (Chudasama et al., 2020), experimental evidence is not as abundant as one might expect. Large volumes of research show positive effects from exercise on specific disease processes (Campbell & Turner, 2018; Edwards et al., 2007; Larson & Bruce, 1987; Warburton & Bredin, 2017), and other studies have found association between lifestyle factors and longevity (Quach et al., 2017; Sae-Lee et al., 2018; Zhao et al., 2019). However, fewer studies experimentally validate or quantify the causal effects of non-pharmaceutical lifestyle modification interventions on lifespan. This is likely due in part to the inherent time scale challenge that longevity research entails. Any future studies that examine lifestyle modification interventions would benefit from a practical tool that is capable of measuring change in expected lifespan.

One persistent challenge when studying the efficacy of interventions intended to increase lifespan is identifying an outcome measure that is both valid and feasible to use experimentally. From a validity perspective, change in total years of lifespan between experimental and control groups would be ideal, except for the fact that it would necessitate

[1]School of Allied Health Professions, Loma Linda University, Loma Linda, CA, USA

Corresponding Author:
Trevor Lohman, Loma Linda University School of Allied Health Professions, Nichol Hall Room 1605, Loma Linda, CA 92350-1729, USA.
Email: tlohman@llu.edu

multi-decade longitudinal studies. Not only does this add significant cost and effort, but it also makes controlling for confounding variables exceedingly difficult. The apparent alternative to measuring actual lifespan would be to identify a biomarker or group of biomarkers capable of estimating remaining years of life. This would grant researchers the ability to test the efficacy of interventions designed to increase lifespan without necessitating the use of long-term longitudinal studies.

Generally, metrics designed to predict remaining lifespan, mortality risk, and age-related morbidity risk have come to be known as predictors of biological age or biomarkers of aging. Consensus around these terms' definitions is lacking, as is the definition of aging more generally (Butler et al., 2004). In his review of recent papers attempting to identify biomarkers of aging, Thomas Johnson cites one of the original clarifying statements by Baker and Sprott (Johnson, 2006):

"A Biomarker of Aging is a biological parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age, than will chronological age." (Baker & Sprott, 1988).

Even though it was written in 1988, this statement went a long way towards establishing the current criteria for biomarkers of aging. A potential concern with this definition for a researcher interested in examining interventions capable of biological age reversal is that there is no mention of lifespan. This definition discusses functional capability only. Another potential point of disagreement among researchers may be the "in the absence of disease" criterion. It seems that a useful metric for aging research would include the effects of age-related disease on lifespan.

In the time since this statement was published, there has been much development and discussion regarding the exact meaning of the term, "biomarker of aging". An interdisciplinary workshop cosponsored by the International Longevity Center-USA, The Ellison Medical Foundation, Kronos Longevity Research Institute, the Institute for the Study of Aging, and Canyon Ranch Health Resort proposed the following three parameters for biomarkers of aging:

1. The biomarker should predict the outcome of a wide range of age-sensitive tests in multiple physiological and behavioral domains, in an age-coherent way, and do so better than chronological age.
2. It should predict remaining longevity at an age at which 90% of the population is still alive and do so for most of the specific illnesses that afflict the species under study.
3. Its measurement should not alter life expectancy or the outcome of subsequent tests of other age-sensitive tests.

The American Federation for Aging Research (AFAR) formulated the criteria for aging biomarkers as follows (Butler et al., 2004; Johnson, 2006; Jylhävä et al., 2017)

1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of life span than chronological age.
2. It must monitor a basic process that underlies the aging process, not the effects of disease.
3. It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging technique.
4. It must be something that works in humans and in laboratory animals, such as mice. This is so it can be tested in lab animals before being validated in humans.

Although both clear and thorough lists, the existence of a biomarker that meets all of the criteria above may be unlikely (Johnson, 2006). Perhaps the most challenging criterion for researchers intending to measure the effects of interventions on lifespan and healthspan is the American Federation for Aging Research criterion listed above. This statement outlines the need for an aging biomarker to separate the aging process from disease processes. This may not always be possible, and it is hard to differentiate the effects of the aging process from the effects of age-related disease. That said, this criterion does illustrate the need to create markers that are not influenced by acute illnesses or diseases that have no effect on lifespan. As mentioned earlier, there is no consensus on what the definition of aging is within the aging research community, let alone agreement that there is a specific aging process or aging rate that is separate from disease processes (Butler et al., 2004; Johnson, 2006). What is clear, even to a lay observer, is that if we examine a large group of 70-year-old people, we would find a phenotypically diverse sample, despite all members being the same chronological age. This is described clearly and concisely by Lowsky et al. in their article's introductory sentence: "For a surprisingly large segment of the older population, chronological age is not a relevant marker for understanding, measuring, or experiencing healthy aging." (Lowsky et al., 2014) This may be the most concise way to illustrate the need for a valid and easy to obtain measure of biological age.

For the purposes of this scoping review, we will be focusing on biomarkers of aging that satisfy at least some of the American Federation of Aging Research biomarkers of aging criteria. Given the lack of consensus around terminology and definition, we will seek to view biomarkers in the context of their ability to predict two aspects of biological age: healthspan and lifespan. These criteria best facilitate the selection of a marker that measures the effectiveness of interventions on biological age reversal. Until recently, the possibility of biological age reversal was uncertain, but thanks to recent experimental trials utilizing biological age predictors we now know that biological age as measured by biomarkers of aging can be slowed or even reversed (Fahy et al., 2019; Fitzgerald et al., 2020; Hachmo et al., 2020). With that in mind, our specific aim is to compile the available

evidence related to various readily accessible biological age predictors. In doing so, we hope to provide a basis for selection in future experimental studies that utilizes wellness and lifestyle interventions to slow or reverse biological aging. For example, investigators could choose to examine diet modification, sleep quality, exercise type or quantity, supplementation, implementation of a stress management program, or any number of other wellness interventions' effects on biological age. This has far reaching implications for the wellness and successful aging research communities, as it provides a means to assess the effectiveness of an intervention on biological age in a comparatively short time frame.

This article investigates and summarizes the following predictors of biological age: Telomere length, allostatic load index, DNA methylation clocks, functional age, and transcriptional predictors of biological age. The ability of these tools to estimate mortality risk and biological age, operationally defined as an estimate of remaining healthspan/lifespan, will be highlighted. Various capabilities and weaknesses of each will be examined as well, including criteria such as: ease of use, accessibility, ability to glean underlying mechanisms influencing lifespan/healthspan, and other relevant features.

Search Strategy and Selection Criteria

Using the PubMed database, Medical Subject Headings (MeSH) terms "Aging" and "Humans" and the specific terms for each of the biomarkers of aging categories: 1) Telomere Length, 2) Frailty Index or Deficit Accumulation or Functional age, 3) Epigenetic clock, 4) Transcriptomic age or Transcriptional age, 5) Composite biomarker or Allostatic load index were combined. Cited papers in the selected publications and papers that referenced the selected publications were also considered. The searches were performed between December 2020 and May 2021.

Telomere Length

Telomeres are repeating sequences of nucleoprotein caps located at the ends of chromosomes (Sanders & Newman, 2013). Each time a cell undergoes mitosis, a section of these nucleotides is cleaved, and the telomere shortens incrementally. This is an overly simplistic description given that oxidative stress is also associated with telomere shortening, and multiple mechanisms exist for telomere lengthening as well (Sanders & Newman, 2013). Even with this simple definition, however, an inference can be drawn that telomere length serves in part as a cumulative measure of cellular division and by extension, age. This would be a well-founded inference and one that has received significant attention from the aging research community. As of March 13, 2021, the search phrase "Telomere Length" on the PubMed database yielded 10,245 results, making it the most investigated biomarker of aging discussed in this article.

Multiple meta-analyses exist examining the relationship between telomere length and age (Gardner et al., 2014; Lapham et al., 2015). Additionally, many studies have shown relationships between telomere length and specific disease processes associated with increased chronological age. A 2014 meta-analysis (43,725 individuals) showed an inverse relationship between telomere length and coronary heart disease independent of traditional vascular risk factors (Haycock et al., 2014). Similar results have been obtained when investigating Alzheimer's disease and telomere length. Both observational and Mendelian randomization studies (a method of analyzing single nucleotide polymorphisms to determine causation) have shown that patients diagnosed with Alzheimer's disease have shorter telomere lengths (Forero et al., 2016; Zhan & Hägg, 2018). Despite this prevalence of age-related telomere research, data pertaining to telomere length and mortality risk specifically has been less consistent. Perhaps the most compelling investigation is a meta-analysis performed by Wang et al. (2018) that examined the relationship between telomere length and all-cause mortality. Twenty-five studies were determined to meet eligibility for inclusion (121,749 combined individuals), including 4 Swedish Twin Registry (STR) cohorts (12,083 individuals). Results from the Swedish twin registry studies showed one standard deviation reduction of leukocytic telomere length corresponded to 13% increased all-cause mortality risk (95% confidence interval 7–19%) (Wang et al., 2018). However, a study by Li et al. that examined nine different biomarkers of aging over a 20 year timeframe found that the only marker not associated with mortality risk was in fact, telomere length (Li et al., 2020). Another Swedish study performed by Svensson et al. examined the relationship between telomere length and mortality in 2744 elderly men and also found no association (Svensson et al., 2014). The evidence presented here indicates that telomere length is associated with various disease processes, but that the research pertaining to its use as a predictor of biological age may be contradictory (Table 1).

Composite Biomarkers/Allostatic Load Indices

In 1998, Bruce McEwen described allostasis as "adaptation in the face of potentially stressful challenges [that] involves activation of neural, neuroendocrine, and neuroendocrine-immune mechanisms." (McEwen, 1998) The phrase "constancy through change" is often used as shorthand to describe allostasis, as it so concisely describes the constant changing physiological processes that maintain homeostasis. Fava et al. describes allostatic load as reflecting the cumulative effects of stressful experiences in daily life that may lead to disease over time (Fava et al., 2019). Like telomere length, allostasis and allostatic load have been extensively researched. Most commonly, this research focuses on the relationship between allostatic load and various health outcomes such as cognition (Juster et al., 2010), chronic stress (Juster et al., 2010), sleep

Table 1. Studies selected for review pertaining to telomere length and its role as an aging biomarker.

Telomere Length				
Study Title	BA Predictor Used	Cohort Name (If Applicable)	*N*	Results
Telomere length and all-cause mortality: A meta-analysis	Telomere length	Multiple cohorts	121,749	One standard deviation reduction of leukocytic telomere length corresponded to 13% increased all-cause mortality risk (95% confidence interval 7–19%) (Wang et al., 2018)
Longitudinal trajectories, correlations, and mortality associations of nine biological ages across 20-year follow-up	Telomere length, DNAm age (4 types), physiological age, cognitive function, functional aging index, and frailty index	Swedish population-based cohort	636	No evidence that telomere length associated with mortality risk (Li et al., 2020)
Leukocyte telomere length is not associated with mortality in older men	Telomere length	Prospective population-based MrOS-Sweden study	2744	Using Cox proportional hazards regression, tertile of LTL did not associate with all-cause mortality [tertile 1 (shortest) or 2 (middle) versus tertile 3 (longest); hazard ratio (HR) = 1.05, 95% confidence interval (CI) 0.85–1.28 and HR = 0.97, 95% CI 0.79–1.19, respectively] (Svensson et al., 2014)

quality (McEwen & Karatsoreos, 2015), age-related disease (Danese & McEwen, 2012), cardiovascular disease (Logan & Barksdale, 2008), and addiction (Koob & Schulkin, 2019), among others. A smaller portion of allostasis research is dedicated to evaluating the performance of allostatic load as a predictor of biological age. The study that has perhaps best demonstrated the capability of an allostatic predictor of biological age is part of the MacArthur studies of successful aging series in 2005 that utilized 10 physiological parameters to generate allostatic load scores in 171 70–79-year-old adults(Karlamangla et al., 2006). An allostatic load score or index falls under a broader category of biological age predictors called composite biomarkers of aging. This is due to the combination of multiple blood biomarkers and clinical measures used to make an estimation regarding mortality risk. Other predictors within this category include phenotypic age (Levine et al., 2018) and physiological age (Li et al., 2020).

In the previously mentioned study published by Karlamangla in 2005 (Karlamangla et al., 2006), allostatic load scores were generated first in 1988 and again in 1991. The mortality status of these individuals was determined 4.5 years later in 1995. This study found that individuals with increased allostatic load in 1991 compared to 1988 had increased risk of all-cause mortality (15% vs. 5%, respectively, p =.47). Further analysis revealed that each incremental increase in allostatic load score was associated with a mortality odds ratio of 3.3 (95% confidence interval 1.1–9.8)(Karlamangla et al., 2006).

A study by Castagne et al. (2018), took another significant step towards establishing allostatic load as a predictor of biological age. This study examined the relationship between 14 biomarkers across four physiological systems and their relationship to mortality in a UK birth cohort study of 8113 adults (Castagné et al., 2018). The hazard ratio for participants with a high allostatic load score was found to be 3.56 (2.2-5.3) and was significantly higher than in participants with a low allostatic load score (Castagné et al., 2018). Their data suggests that those with a high allostatic load score at age 44 are approximately 3 times more likely to die by age 55 (Castagné et al., 2018). The authors also analyzed the relative contribution of each of the 14 biomarkers that comprised the allostatic load score. Interestingly, after adjusting for various risk factors and adverse childhood experiences, 5 of the 14 biomarkers stood out as being significantly related to mortality (C-Reactive Protein, fibrinogen, glycated hemoglobin, heart rate, and peak expiratory flow) (Castagné et al., 2018). This highlights one potential challenge and opportunity for the future use of allostatic load indices as BA prediction tools. The challenge is the general lack of consensus regarding the relative contribution of each marker or combination of markers, and the opportunity is the potential to develop even simpler yet more accurate composite age biomarkers. Future validation studies examining a variety of different indices will be helpful in making these determinations. As it stands, allostatic load appears to be significantly correlated with mortality-risk, and allostatic indices will serve as valuable tools for aging research (Table 2).

DNA Methylation "Epigenetic Clocks"

The term epigenetic "clock" refers to tools that analyze DNA methylation levels within a set of Cytosine-Phosphate-

Table 2. Studies selected for review pertaining to allostatic load indices and composite biomarkers of aging.

Allostatic Load/Composite Biomarkers				
Study Title	BA Predictor Used	Cohort name (if applicable)	*n*	Results
Reduction in allostatic load in older adults is associated with lower all-cause mortality risk: MacArthur studies of successful aging.	Allostatic load index		171	Adjusted for age and baseline allostatic load, each unit increment in the allostatic load change score was associated with mortality odds ratio of 3.3 (95% confidence interval, 1.1–9.8) (Karlamangla et al., 2006)
Allostatic load and subsequent all-cause mortality: Which biological markers drive the relationship? Findings from a UK birth cohort	Allostatic load index	1958 British birth cohort	8113	Hazard ratios for participants with a mid (3 ≤ AL < 5) and high AL (≥5) were 1.98 (1.25–3.13) and 3.56 (2.2–5.53), respectively, and were found to be significantly greater than in participants with a low AL (<3) (Castagné et al., 2018)
An epigenetic biomarker of aging for lifespan and healthspan	Phenotypic age estimator	Third and fourth National Health and Nutrition Examination Survey	9926, 6209	A one-year increase in phenotypic age is associated with a 9% increase in the risk of all-cause mortality (HR = 1.09, p = 3.8E-49), a 9% increase in the risk of mortality from aging-related diseases (HR = 1.09, p = 4.5E-34), a 10% increase in the risk of CVD mortality (HR = 1.10, p = 5.1E-17), a 7% increase in the risk of cancer mortality (HR = 1.07, p = 7.9E-10), a 20% increase in the risk of diabetes mortality (HR = 1.20, p = 1.9E-11), and a 9% increase in the risk of chronic lower respiratory disease mortality (HR = 1.09, p = 6.3E-4) (Levine et al., 2018)

Guanine (CpG) sites and are generally acknowledged as accurate measures of biological age (Bell et al., 2019; Fransquet et al., 2019; Jylhävä et al., 2017; Lu et al., 2019; Perna et al., 2016). In fact, one study we examined made the claim that DNA methylation clocks are the current best predictors of mortality (Unnikrishnan et al., 2019). While this may be true, it is important to realize that the term DNA methylation age or epigenetic clock can refer to many different tools. While all of these "clocks" analyze methylation in specific CpG sites, they all do so in different ways. For example, two clocks that were among the first to generate widespread interest are the Horvath clock (Horvath, 2013) and Hannum clock (Hannum et al., 2013). The Horvath clock is based on methylation levels of 353 CpG sites using the Illumina 27k or 450k array (Horvath, 2013), while the Hannum clock uses 71 CpG sites and utilizes data from the Illumina 450k array (Hannum et al., 2013). Epigenetic clocks' ability to predict biological and chronological age can also be tissue dependent. For example, the Horvath clock performs similarly among various tissue types (Horvath, 2013) ("whole blood, peripheral blood mononuclear cells, cerebellar samples, occipital cortex, buccal epithelium, colon, adipose, liver, lung, saliva, uterine cervix as well as in individual cell types such as CD4 T cells and CD14 monocytes, and immortalized B cells"), while the Hannum clock performs best using peripheral whole blood samples (Hannum et al., 2013; Jylhävä et al., 2017). These clocks also vary in terms of their ability to predict biological and chronological age (chronological age r^2 values = 0.96 for Horvath and 0.91 for Hannum) (Jylhävä et al., 2017). Accessibility is also highly variable; as property of the specific inventor or institution that created the algorithm capable of converting array-based methylation data into other useful data (such as biological age estimation in years or mortality risk among others), some of these tools may be commercial. While other clocks such as the Horvath clock or GrimAge marker created by Steve Horvath and Ake Lu are freely available online.

The clocks mentioned so far are just a few examples of DNA methylation biomarkers of aging. This is to illustrate that the term "epigenetic clock" is broad and not a specific marker. With this in mind, we can say generally that one of the most interesting and unique features of epigenetic clocks is their ability to predict mortality risk, also referenced as time-to-death. A 2016 meta-analysis of 13 cohorts representing a combined sample size of 13,089 showed that epigenetic age acceleration (a measure of the difference between chronological age and epigenetic age) was predictive of mortality independent of chronological age ($p \leq$ to 8.2×10^{-9})(Chen

et al., 2016). This was still found to be true after adjusting for additional risk factors, but at a significance of $p < 5.4 \times 10^{-4}$(Chen et al., 2016). When epigenetic age estimates incorporated additional information pertaining to blood cell composition, the resulting time-to-death predictions were highly significant ($p = 7.5 \times 10^{-43}$). (Chen et al., 2016).

In the time since this 2016 meta-analysis, new DNA methylation clocks have emerged that are even more capable in terms of their ability to estimate mortality risk. For example, a 2017 study by Zhang et al. proposes a mortality risk score based on 10 CpG sites that is strongly associated with all-cause mortality (Zhang et al., 2017). Participants with scores of 1 display a hazard ratio (95% confidence intervals) of 2.16 (1.1–4.24), compared to those with scores of 2–5 showing a hazard ratio of 3.42 (1.81–6.46) compared to those with 5+ scores showing a hazard ratio of 7.36 (3.69–14.68) (Zhang et al., 2017). Another marker called DNAm PhenoAge was calculated in a meta-analysis of five large samples (n = 2,016, n = 2,191, n = 2,553, and n = 657). It was found that a 1-year increase in DNAm PhenoAge is associated with a highly significant 4.5% increase in all-cause mortality risk (meta p-value = 7.9×10^{-47}) (Levine et al., 2018).

In addition to measuring mortality risk, some markers have the added capability of predicting the risk of developing specific disease processes. For example, a metric known as GrimAge can strongly predict time-to-death (Cox regression $p = 2.0 \times 10^{-75}$), time-to-coronary heart disease (Cox regression $p = 6.2 \times 10^{-24}$), and time-to-cancer ($p = 1.3 \times 10^{-12}$) (Lu et al., 2019). The study authors used large scale validation data from the Framingham heart study to complete this analysis. By adding a calculation that quantifies the difference between GrimAge and chronological age (AgeAccelGrim), other relevant age-related associations are found to be present. For example, AgeAccelGrim is associated with comorbidity count ($p = 3.45 \times 10^{-17}$), time to congestive heart failure ($p = 4.9 \times 10^{-10}$), time-to-incident coronary heart disease ($p = 6.2 \times 10^{-24}$), hypertension ($p = 5.1 \times 10^{-13}$), and type 2 diabetes ($p = 0.01$) (Lu et al., 2019). All associations were in the expected direction (increased AgeAccelGrim=increased likelihood of poor outcome) with varying odds ratios (Lu et al., 2019) (Table 3).

Transcriptional Predictors of Biological Age

A transcriptional predictor of biological age analyzes genetic expression in genes associated with aging to make some prediction regarding the biological aging process. One example of this tool is the Transcriptomic Age Prediction Tool (TRAP) which is described in the article titled "The transcriptional landscape of age in human peripheral blood" written by Peters et al. in 2015. This study performed a whole-blood gene expression meta-analysis in 14,983 individuals and identified 1497 genes that are differentially expressed with chronological age. This provided the basis for calculating a "transcriptomic age" and associating it with various age-related phenotypes including: blood pressure, fasting glucose, and BMI (Peters et al., 2015). This was the first large scale meta-analysis to examine age-related gene expression profiles and build a predictor of biological age from these data. The correlation between the transcriptomic age predictor and chronological age was significant ($p < 2 \times 10^{-29}$) (Peters et al., 2015), and observed differences between the transcriptomic age predictor (TRAP) and chronological age are thought to reflect altered biological age. This is supported by consistent associations between increased delta age (increased TRAP compared to chronological age) and higher blood pressure, total cholesterol, fasting glucose levels, and BMI (Peters et al., 2015). Peters et al. identified a subset of 1396 individuals from two studies within their meta-analysis [KORA (Holle et al., 2005) and Rotterdam studies (Hofman et al., 2007)] that had both methylation and gene expression data available. The presence of these two datasets allowed the investigators to generate a transcriptomic predictor of biological age, in addition to Horvath (Horvath, 2013) and Hannum (Hannum et al., 2013) clock values. This gave investigators the opportunity to examine correlation between three different biomarkers of aging: TRAP, Horvath Clock, and Hannum Clock. They found TRAP to correlate positively, albeit weakly, with both clocks (r^2=.1 for Hannum and .33 for Horvath).

Other transcriptional predictors of biological age exist, such as the healthy ageing gene score (Sood et al., 2015) and RNAageCalc (Ren & Kuan, 2020). Like the previously discussed epigenetic clocks, these measures' ability to predict disease process, mortality, and association with age-related phenotypes varies. At the time of this writing, the literature seems to indicate that the transcriptome is an age-associated variable indicating its utility in creating biological age predictors, but existing transcriptomic clocks are pending broader validation (Harries et al., 2011; Holly et al., 2013; Jylhävä et al., 2017) (Table 4).

Functional Age Estimators

Although not blood biomarkers, functional age estimators are included here due to their ease of use and relevance to aging research. The term functional age is now commonly found in the literature, but these tools were initially intended to be a method for estimating frailty and the likelihood of care entry, not biological age. More recently, some functional age estimators have been shown to estimate mortality-risk (Burn et al., 2018; Church et al., 2020; Finkel et al., 2019; Kojima et al., 2018; Li et al., 2020) and therefore present as highly practical measures for lifestyle modification research. The large volume of functional age estimators merits a standalone review, but some notable examples will be discussed here. Two of these are the frailty index (FI) and frailty phenotype (FP). Although they are sometimes discussed as being interchangeable, they are two different tools for different purposes. The term frailty index refers to a method of quantifying frailty in older individuals, with the underlying mechanism being a measurement of deficit accumulation (deficits identified/deficits measured). Rather than a specific tool or metric, it is a method in which various

Table 3. Studies selected for review pertaining to "epigenetic clocks" or DNA methylation biomarkers of aging.

DNA Methylation "Clocks"				
Study Title	BA Predictor Used	Cohort Name (If Applicable)	*n*	Results
DNA methylation GrimAge strongly predicts lifespan and healthspan	GrimAge	Framingham Heart Study Offspring Cohort	2356	Predictive ability for time-to-death (Cox regression p=2.0E-75), time-to-coronary heart disease (Cox p=6.2E-24), and time-to-cancer (p= 1.3E-12) (Lu et al., 2019)
DNA methylation age of human tissues and cell types	DNAm age "Horvath Clock"	82 publicly available datasets	7844	The multi-tissue age predictor performs remarkably well in most tissues and cell types. (Age correlation 0.97, error = 2.9 years) (Horvath, 2013)
Genome-wide methylation profiles reveal quantitative views of human aging rates	"Hannum Clock"		656	Correlation between age and predicted age of 96% and an error of 3.9 years (Hannum et al., 2013)
An epigenetic biomarker of aging for lifespan and healthspan	PhenoAge	Women's Health Initiative (WHI), the Framingham Heart Study (FHS), the Normative Aging Study (NAS), and the Jackson Heart Study (JHS)	2016, 2191, 2553, 657, 1747	A one-year increase in DNAm PhenoAge is associated with a 4.5% increase in the risk of all-cause mortality (Meta (FE) = 1.045, meta p=7.9E-47 (Levine et al., 2018)
Longitudinal trajectories, correlations, and mortality associations of nine biological ages across 20-year follow-up	Telomere length, DNAm age (4 types), physiological age, cognitive function, functional aging index, and frailty index	Swedish population-based cohort	845	Individually, all BAs except for telomere length were associated with mortality risk independently of CA. The largest effects were seen for methylation age estimators (GrimAge) and the frailty index (FI) (Li et al., 2020)
DNA methylation-based measures of biological age: meta-analysis predicting time to death	Horvath and Hannum	13 cohorts	13,089	All considered measures of epigenetic age acceleration were predictive of mortality ($p \leq 8.2 \times 10^{-9}$) (Chen et al., 2016)
DNA methylation signatures in peripheral blood strongly predict all-cause mortality	Zhang 10 CpG clock		1900	Demonstrated that a risk score based on DNAm of ten identified CpGs was a very strong predictor for all-cause, CVD, and cancer mortality (Zhang et al., 2017)

measures of frailty and functional capability can be assessed and from which a scoring system can be derived. Frailty phenotype on the other hand is based on the presence or absence of five signs or symptoms (>10 lbs unintentional weight loss in the past 12 mo., self-reported exhaustion, weak grip strength, slow walking speed, and low physical activity) (Cesari et al., 2014; Fried et al., 2001). Although both FP and FI are associated with mortality-risk (Shi et al., 2019), we will focus our discussion on frailty index. This is not necessarily a comment on either's ability to predict biological age, but rather how responsive each may be to lifestyle interventions. Given the relatively broad scope and ordinal nature of the 5-item frailty phenotype, it may be less responsive to intervention and less suited as a research variable compared to the frailty index (Cesari et al., 2014; Clegg et al., 2013). The frailty phenotype may be better implemented as a screening tool, inclusion/exclusion criterion, or stratification mechanism given that it does not require a full geriatric comprehensive assessment like the FI (Clegg et al., 2013).

One of the originally described functional indices, called the Canadian Study of Healthy Aging (CSHA) frailty index is validated by the Canadian Study of Healthy Aging and examines the presence or absence of 70 clinical deficits in order to quantify fitness and frailty in the elderly (Rockwood et al., 2005). This list of deficits was not meant to be a fixed index;

however, in fact it has been reported that indices with as few as 50 clinical deficits can be highly useful, and some indices with as few as 20 items have been explored (Rockwood & Mitnitski, 2012). Other tools related to the frailty index have been developed such as the Edmonton Frailty scale (Clegg et al., 2013; Rolfson et al., 2006) and Clinical Frailty Scale (Rockwood et al., 2005). The Clinical Frailty Scale is a 7-point scale that is highly correlated to the original 70-point index ($r^2 = .90$) (Rockwood et al., 2005). More importantly given an aging research context, each 1 point increase in the scale was found to correspond with a 21.2% increased risk of death in the next 70 months (Rockwood et al., 2005). In a study of 1788 community-dwelling elders, frailty as defined by the FI was associated with a 2.31-fold increased risk of all-cause death compared to those who scored robust on the index (Shi et al., 2019). Another study of 5536 community-dwelling elderly found the relationship between FI and mortality to be significant ($p < .0001$). Interestingly, a meta-analysis examining frailty index scores between men and women found what the authors described as a "male-female health-survival paradox" (Gordon et al., 2017). The paradox was that at all ages females displayed higher FI scores, despite males having higher mortality rates at each level of the frailty index (Gordon et al., 2017). Frailty sex differences extended to diet as well. A study examining older adults found that low meat consumption (less than 2x/wk.) was associated with increased frailty in men only. Increased frailty in women was associated with decreased fish, meat, vegetables, and potatoes (Shibasaki et al., 2019). Perhaps most relevant to the aim of this article, one study comparing nine different biological age predictors, found frailty index [42-item Rockwood (Jiang et al., 2017)] to have one of the strongest associations with mortality risk among the nine markers examined, being exceeded only by GrimAge (Li et al., 2020). Given these results, some frailty indices may serve lifestyle intervention research well alongside other

Table 4. Studies selected pertaining to biomarkers of aging based on genetic expression.

Transcriptomics				
Study Title	BA Predictor Used	Cohort Name (If Applicable)	*n*	Results
The transcriptional landscape of age in human peripheral blood	Transcriptomic age prediction tool	The Rotterdam Study	14,926	The correlation between chronological age and transcriptomic age was significant in all cohorts ($P < 2E{-}29$) A positive delta age, interpreted as reflecting more rapid biological aging, was consistently associated with higher systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, fasting glucose levels, and body mass index (BMI) Transcriptomic age and epigenetic age (both Hannum and Horvath) were positively correlated, with r^2 values varying between 0.10 and 0.33 (Peters et al., 2015)

Table 5. Studies selected pertaining to functional age estimators and their roles as biomarkers of aging.

Functional Age Estimators				
Study Title	BA Predictor Used	Cohort name (if applicable)	*n*	Results
Frailty index as a predictor of all-cause and cause-specific mortality in a Swedish population-based cohort	42-Item Rockwood	Swedish Adoption/ Twin Study of Aging	1477	The categorized FI levels demonstrated a dose-response increase in mortality risk with increased frailty in both men and women (Jiang et al., 2017)
Frailty phenotype, frailty index, and risk of mortality in Chinese elderly population-Rugao longevity and ageing study	Frailty index	Ageing arm of Rugao Longevity and Ageing Study	1788	Frailty defined by the frailty index was associated with a 2.31-fold (95% CI 1.16–4.6) risk of all-cause death compared with robust elderly (Shi et al., 2019).
Frailty index as a predictor of mortality: A systematic review and meta-analysis	Frailty index	18 cohorts		All meta-analyses suggested that higher FI was significantly associated with higher mortality risk (Kojima et al., 2018)

biomarkers, or perhaps even as stand-alone outcome variables (Table 5).

Discussion

No statement in this article is intended to make a recommendation regarding the use of a specific biological age predictor; neither is this review an exhaustive list. In addition to less investigated biological age predictors like proteomics and metabolomics, there are multitudes of individual markers associated with accelerated biological aging such as glycated hemoglobin, triglycerides, blood pressure, resting heart rate, waist-to-hip ratio, fibrinogen, albumin, crp, interleukin-6, and many others (Jylhävä et al., 2017; Kane & Sinclair, 2019). Our aim is to compile relevant information pertaining to various promising predictors of biological age validated in large cohorts to assist future researchers interested in using them as outcome measures. There is also no implication that all biomarkers of aging are equally valid. A compelling comparison of nine biological age estimators that examined longitudinal trajectories, correlations, and mortality associations across 20 years was performed by Li et al. (2020) Their study examined data from a Swedish-based cohort of 845 men and women aged 63.6 (8.6) at baseline and compared the validity of four different DNA methylation age estimators, Horvath (Horvath, 2013), Hannum (Hannum et al., 2013), PhenoAge (Levine et al., 2018), and GrimAge (Lu et al., 2019), three different functional age estimators [functional aging index (Finkel et al., 2019), frailty index (Jiang et al., 2017), and cognitive function (Reynolds et al., 2005)], telomere length (Berglund et al., 2016), and a composite biomarker called physiological age that included various biomarkers and measures of body composition. All four DNA methylation age estimators, physiological age, and all three functional age estimators were associated with mortality risk independent of chronological age, while telomere length was not. Of the nine biomarkers of aging examined, GrimAge and the frailty index stood out as being most associated with mortality risk.

The information presented here sheds light on the large variety of biomarkers of aging available, each with its own specific capabilities. Even still, the markers discussed are just a small portion of the available biomarkers of aging in existence. Like any other biomarker, the predictor used in future experimental studies should be based on the specific aims and needs of those studies. A study that aims to assess the effects of a vegan diet on coronary heart disease risk may benefit from utilizing the GrimAge marker since it has been shown to predict time-to-coronary heart disease (Lu et al., 2019). Investigators could obtain a baseline GrimAge value, implement an intervention protocol, and obtain a GrimAge value at the conclusion of the trial. When compared to a control group, the difference in GrimAge values could be analyzed to determine if biological age was slowed or reversed. An example of this methodology was implemented in the 2019 Fahy et al. study, Reversal of Epigenetic Aging and Immunosenescent Trends in Humans, in which investigators reported a 2.5 year reversal in mean epigenetic age following a 1-year Human growth hormone and metformin treatment protocol (Fahy et al., 2019). A study that aims to determine the transcriptional basis for any observed changes in biological age resulting from lifestyle modification may find a transcriptomic predictor most appropriate due to the ability to obtain a biological age estimation and gene expression profile from a single blood sample. If an investigator is limited in terms of their capability to analyze gene expression profiles, DNA methylation of CpG sites, or blood biomarkers, perhaps a functional age estimator such as a frailty index could provide relevant data on biological aging changes in an intervention group. If feasibility allows it, the combination of various predictors of biological age could yield even more robust results. Various factors will dictate the most appropriate selection for future lifestyle modification research, not the least of which being accessibility, cost, applicability to multiple tissue types, and conversely, specificity to a study's specific tissue of interest. A possible limitation to this review may be that only articles written in English were included. Additionally, this is an emerging field with many potential biological age predictors to consider. We selected five of the most investigated biological age predictors with large-scale cohort validation; and therefore, there may be promising new predictors that were not included in this review.

Conclusion

This article highlights an inherent challenge in searching for the "best" biomarker(s) of aging. Any researcher seeking to utilize one of these biomarkers must first clearly define their aims. They must also seek to understand and explain how they are using the term biological age or biomarker(s) of aging. It may be preferable to instead use more descriptive terminology such as DNA methylation age/Epigenetic age (BA as measured by an epigenetic clock), transcriptomic age (BA as measured by a transcriptomic age predictor), or functional age (BA as measured by a deficit accumulation index such as a frailty index). These terms go further to explain the nature of the data, how it is obtained, and how it may be best interpreted. They also help to add some clarity given the array of emergent terminology used in biological age prediction research.

Our aim at the outset of this article was to view these markers in the context of their ability to predict healthspan and lifespan. Telomere length is certainly the most extensively studied biomarker of age-related disease. Consequentially, many conclusions have been made regarding the association between telomere length, age, disease, stress, and multiple other health outcomes. While no study that we know of has sought to produce an easy-to-use telomere length biological age prediction tool, TL has been used to predict mortality risk, albeit with mixed results. Epigenetic clocks appear to have the upper hand in terms of accessibility (many are freely accessed online), and they also appear to best

predict time-to-death, time-to-cancer, and other age-related processes (Li et al., 2020; Lu et al., 2019; McCrory et al., 2020). It also seems that they may have the greatest degree of large-scale cohort validation. Perhaps the only area where epigenetic clocks are not the apparent "leader" of the biological age prediction discussion is in their ability to identify the mechanism behind differences in chronological and biological aging, although discovery is taking place rapidly (Zhang et al., 2020). It is in this domain that transcriptional predictors of biological aging may add value as they rely on gene expression data to estimate biological age. A researcher could potentially examine changes in both biological age and genetic expression to make an inference regarding the mechanism behind the observed biological age acceleration/deceleration from a single blood sample. A "best of both worlds" scenario may involve the inclusion of a more validated DNA methylation marker like GrimAge, alongside a genetic expression profile of relevant genetic pathways. This would allow an investigator to report an intervention's effect on biological age, as well as an analysis of the specific changes in gene expression that may have contributed to that change.

Each of these tools has unique capabilities and limitations. For this reason, the most robust option for a future researcher is likely the inclusion of multiple biomarkers of aging based on those unique features.

A central goal of lifestyle modification is to reduce disease risk and promote healthy, successful aging. The ability of biological age predictors to assess an intervention's contribution to mortality/morbidity risk makes them highly relevant measures for studies examining the effects of lifestyle modification on age-related disease. Future studies examining the effects of diet, supplementation, exercise, stress-reduction techniques, sleep quality/quantity, or any number of other lifestyle modification interventions could benefit greatly from the inclusion of a biological age predictor.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Trevor Lohman https://orcid.org/0000-0002-9634-702X

References

Atella, V., Piano Mortari, A., Kopinska, J., Belotti, F., Lapi, F., Cricelli, C., & Fontana, L. (2019). Trends in age-related disease burden and healthcare utilization. *Aging Cell*, *18*(1), e12861. https://doi.org/10.1111/acel.12861.

Baker, GT, 3rd, & Sprott, RL (1988). Biomarkers of aging. *Experimental Gerontology*, *23*(4-5), 223-239. https://doi.org/10.1016/0531-5565(88)90025-3.

Bell, C. G., Lowe, R., Adams, P. D., Baccarelli, A. A., Beck, S., Bell, J. T., Christensen, B. C., Gladyshev, V. N., Heijmans, B. T., Horvath, S., Ideker, T., Issa, J.-P. J., Kelsey, K. T., Marioni, R. E., Reik, W., Relton, C. L., Schalkwyk, L. C., Teschendorff, A. E., Wagner, W., Zhang, K., & Rakyan, V. K. (2019). DNA methylation aging clocks: Challenges and recommendations. *Genome Biology*, *20*(1), 249. https://doi.org/10.1186/s13059-019-1824-y.

Benjamin, EJ, Virani, SS, Callaway, CW, Chamberlain, AM, Chang, AR, Cheng, S, Chiuve, SE, Cushman, M, Delling, FN, Deo, R, de Ferranti, SD, Ferguson, JF, Fornage, M, Gillespie, C, Isasi, CR, Jiménez, MC, Jordan, LC, Judd, SE, Lackland, D, Lichtman, JH, & Muntner, P (2018). Heart disease and stroke statistics-2018 update: A report from the American Heart Association. *Circulation*, *137*(12), e67-e492. https://doi.org/10.1161/cir.0000000000000558.

Berglund, K., Reynolds, C. A., Ploner, A., Gerritsen, L., Hovatta, I., Pedersen, N. L., & Hägg, S. (2016). Longitudinal decline of leukocyte telomere length in old age and the association with sex and genetic risk. *Aging*, *8*(7), 1398-1415. https://doi.org/10.18632/aging.100995.

Burn, R., Hubbard, R. E., Scrase, R. J., Abey-Nesbit, R. K., Peel, N. M., Schluter, P. J., & Jamieson, H. A. (2018). A frailty index derived from a standardized comprehensive geriatric assessment predicts mortality and aged residential care admission. *BMC Geriatrics*, *18*(1), 319. https://doi.org/10.1186/s12877-018-1016-8.

Butler, R. N., Sprott, R., Warner, H., Bland, J., Feuers, R., Forster, M., Fillit, H., Harman, S. M., Hewitt, M., Hyman, M., Johnson, K., Kligman, E., McClearn, G., Nelson, J., Richardson, A., Sonntag, W., Weindruch, R., & Wolf, N. (2004). Aging: The reality: Biomarkers of aging: From primitive organisms to humans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *59*(6), B560-B567. https://doi.org/10.1093/gerona/59.6.B560.

Campbell, J. P., & Turner, J. E. (2018). Debunking the myth of exercise-induced immune suppression: Redefining the impact of exercise on immunological health across the lifespan. *Frontiers in Immunology*, *9*, 648. https://doi.org/10.3389/fimmu.2018.00648.

Castagné, R., Garès, V., Garès, V., Karimi, M., Chadeau-Hyam, M., Vineis, P., Delpierre, C., & Kelly-Irving, M. (2018). Allostatic load and subsequent all-cause mortality: Which biological markers drive the relationship? Findings from a UK birth cohort. *European Journal of Epidemiology*, *33*(5), 441-458. https://doi.org/10.1007/s10654-018-0364-1.

Cesari, M., Gambassi, G., Abellan van Kan, G., & Vellas, B. (2014). The frailty phenotype and the frailty index: Different instruments for different purposes. *Age and Ageing*, *43*(1), 10-12. https://doi.org/10.1093/ageing/aft160.

Chang, A. Y., Skirbekk, V. F., Tyrovolas, S., Kassebaum, N. J., & Dieleman, J. L. (2019). Measuring population ageing: An

analysis of the Global Burden of Disease Study 2017. *The Lancet Public Health*, *4*(3), e159-e167. https://doi.org/10.1016/s2468-2667(19)30019-2.

Chen, B. H., Marioni, R. E., Colicino, E., Peters, M. J., Ward-Caviness, C. K., Tsai, P.-C., ..., Roetker, N. S., Just, A. C., Demerath, E. W., Guan, W., Bressler, J., Fornage, M., Studenski, S., Vandiver, A. R., Moore, A. Z., Tanaka, T., Kiel, D. P., Liang, L., Vokonas, P., Schwartz, J., & Horvath, S. (2016). DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging*, *8*(9), 1844-1865. https://doi.org/10.18632/aging.101020.

Chudasama, Y. V., Khunti, K., Gillies, C. L., Dhalwani, N. N., Davies, M. J., Yates, T., & Zaccardi, F. (2020). Healthy lifestyle and life expectancy in people with multimorbidity in the UK Biobank: A longitudinal cohort study. *PLOS Medicine*, *17*(9), e1003332. https://doi.org/10.1371/journal.pmed.1003332.

Church, S., Rogers, E., Rockwood, K., & Theou, O. (2020). A scoping review of the clinical frailty scale. *BMC Geriatrics*, *20*(1), 393. https://doi.org/10.1186/s12877-020-01801-7.

Clegg, A., Young, J., Iliffe, S., Rikkert, M. O., & Rockwood, K. (2013). Frailty in elderly people. *The Lancet*, *381*(9868), 752-762. https://doi.org/10.1016/S0140-6736(12)62167-9.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiology & Behavior*, *106*(1), 29-39. https://doi.org/10.1016/j.physbeh.2011.08.019.

Edwards, K. M., Burns, V. E., Allen, L. M., McPhee, J. S., Bosch, J. A., Carroll, D., Drayson, M., & Ring, C. (2007). Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain, Behavior, and Immunity*, *21*(2), 209-217. https://doi.org/https://doi.org/10.1016/j.bbi.2006.04.158.

Fahy, GM, Brooke, RT, Watson, JP, Good, Z, Vasanawala, SS, Maecker, H, Leipold, MD, Lin, DTS, Kobor, MS, & Horvath, S (2019). Reversal of epigenetic aging and immunosenescent trends in humans. *Aging Cell*, *18*(6), e13028. https://doi.org/10.1111/acel.13028.

Fava, G. A., McEwen, B. S., Guidi, J., Gostoli, S., Offidani, E., & Sonino, N. (2019). Clinical characterization of allostatic overload. *Psychoneuroendocrinology*, *108*, 94-101. https://doi.org/https://doi.org/10.1016/j.psyneuen.2019.05.028.

Finkel, D., Sternäng, O., Jylhävä, J., Bai, G., & Pedersen, N. L. (2019). Functional aging index complements frailty in prediction of entry into care and mortality. *The Journals of Gerontology: Series A*, *74*(12), 1980-1986. https://doi.org/10.1093/gerona/glz155.

Fitzgerald, K., Hodges, R., Hanes, D., Stack, E., Cheishvili, D., Szyf, M., & Bradley, R. (2020) Reversal of epigenetic age with diet and lifestyle in a pilot randomized clinical trial. *MedRxiv 20*, 2007. https://doi.org/10.1101/2020.07.07.20148098.

Forero, D. A., González-Giraldo, Y., López-Quintero, C., Castro-Vega, L. J., Barreto, G. E., & Perry, G. (2016). Meta-analysis of telomere length in Alzheimer's disease. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *71*(8), 1069-1073. https://doi.org/10.1093/gerona/glw053.

Fransquet, P. D., Wrigglesworth, J., Woods, R. L., Ernst, M. E., & Ryan, J. (2019). The epigenetic clock as a predictor of disease and mortality risk: A systematic review and meta-analysis. *Clinical Epigenetics*, *11*(1), 62. https://doi.org/10.1186/s13148-019-0656-7.

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W. J., Burke, G., & McBurnie, M. A. (2001). Frailty in older adults: Evidence for a phenotype. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(3), M146-M157. https://doi.org/10.1093/gerona/56.3.m146.

Gardner, M., Bann, D., Wiley, L., Cooper, R., Hardy, R., Nitsch, D., _..., Martin-Ruiz, C., Shiels, P., Sayer, A. A., Barbieri, M., Bekaert, S., Bischoff, C., Brooks-Wilson, A., Chen, W., Cooper, C., Christensen, K., De Meyer, T., Deary, I., Der, G., & Ben-Shlomo, Y. (2014). Gender and telomere length: Systematic review and meta-analysis. *Experimental Gerontology*, *51*, 15-27. https://doi.org/10.1016/j.exger.2013.12.004.

Gordon, E. H., Peel, N. M., Samanta, M., Theou, O., Howlett, S. E., & Hubbard, R. E. (2017). Sex differences in frailty: A systematic review and meta-analysis. *Experimental Gerontology*, *89*, 30-40. https://doi.org/10.1016/j.exger.2016.12.021.

Hachmo, Y, Hadanny, A, Abu Hamed, R, Daniel-Kotovsky, M, Catalogna, M, Fishlev, G., ..., Lang, E, Polak, N, Doenyas, K, Friedman, M, Zemel, Y, Bechor, Y, & Efrati, S (2020). Hyperbaric oxygen therapy increases telomere length and decreases immunosenescence in isolated blood cells: A prospective trial. *Aging*, *12*(22), 22445-22456. https://doi.org/10.18632/aging.202188.

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.-B., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., & Zhang, K. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular Cell*, *49*(2), 359-367. https://doi.org/10.1016/j.molcel.2012.10.016.

Harries, L. W., Hernandez, D., Henley, W., Wood, A. R., Holly, A. C., Bradley-Smith, R. M., Yaghootkar, H., Dutta, A., Murray, A., Frayling, T. M., Guralnik, J. M., Bandinelli, S., Singleton, A., Ferrucci, L., & Melzer, D. (2011). Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell*, (*10*(5), 868-878). https://doi.org/10.1111/j.1474-9726.2011.00726.x.

Haycock, P. C., Heydon, E. E., Kaptoge, S., Butterworth, A. S., Thompson, A., & Willeit, P. (2014). Leucocyte telomere length and risk of cardiovascular disease: Systematic review and meta-analysis. *BMJ*, *349*, g4227. https://doi.org/10.1136/bmj.g4227.

Hofman, A., Breteler, M. M. B., van Duijn, C. M., Krestin, G. P., Pols, H. A., Stricker, B. H. C., ..., Tiemeier, H., Uitterlinden, A. G., Vingerling, J. R., & Witteman, J. C. M. (2007). The Rotterdam study: Objectives and design update. *European Journal of Epidemiology*, *22*(11), 819-829. https://doi.org/10.1007/s10654-007-9199-x.

Holle, R, Happich, M, Löwel, H, & Wichmann, HE (2005). KORA–a research platform for population based health research.

Gesundheitswesen, *67*(1), S19-S25. https://doi.org/10.1055/s-2005-858235.

Holly, A. C., Melzer, D., Pilling, L. C., Henley, W., Hernandez, D. G., Singleton, A. B., Bandinelli, S., Guralnik, J. M., Ferrucci, L., & Harries, L. W. (2013). Towards a gene expression biomarker set for human biological age. *Aging Cell*, *12*(2), 324-326. https://doi.org/10.1111/acel.12044.

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

Hurd, M. D., Martorell, P., Delavande, A., Mullen, K. J., & Langa, K. M. (2013). Monetary costs of dementia in the United States. *New England Journal of Medicine*, *368*(14), 1326-1334. https://doi.org/10.1056/NEJMsa1204629.

Jiang, M., Foebel, A. D., Kuja-Halkola, R., Karlsson, I., Pedersen, N. L., Hägg, S., & Jylhävä, J. (2017). Frailty index as a predictor of all-cause and cause-specific mortality in a Swedish population-based cohort. *Aging*, *9*(12), 2629-2646. https://doi.org/10.18632/aging.101352.

Johnson, T. E. (2006). Recent results: Biomarkers of aging. *Experimental Gerontology*, *41*(12), 1243-1246. https://doi.org/10.1016/j.exger.2006.09.006.

Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioral Reviews*, *35*(1), 2-16. https://doi.org/10.1016/j.neubiorev.2009.10.002.

Jylhävä, J., Pedersen, N. L., & Hägg, S. (2017). Biological age predictors. *EBioMedicine*, *21*, 29-36. https://doi.org/10.1016/j.ebiom.2017.03.046.

Kane, A. E., & Sinclair, D. A. (2019). Frailty biomarkers in humans and rodents: Current approaches and future advances. *Mechanisms of Ageing and Development*, *180*, 117-128. https://doi.org/10.1016/j.mad.2019.03.007.

Karlamangla, A. S., Singer, B. H., & Seeman, T. E. (2006). Reduction in allostatic load in older adults is associated with lower all-cause mortality risk: MacArthur studies of successful aging. *Psychosomatic Medicine*, *68*(3), 500-507. https://doi.org/10.1097/01.psy.0000221270.93985.82.

Kojima, G., Iliffe, S., & Walters, K. (2018). Frailty index as a predictor of mortality: a systematic review and meta-analysis. *Age and Ageing*, *47*(2), 193-200. https://doi.org/10.1093/ageing/afx162.

Koob, G. F., & Schulkin, J. (2019). Addiction and stress: An allostatic view. *Neuroscience and Biobehavioral Reviews*, *106*, 245-262. https://doi.org/10.1016/j.neubiorev.2018.09.008.

Lapham, K., Kvale, M. N., Lin, J., Connell, S., Croen, L. A., Dispensa, B. P., Fang, L., Hesselson, S., Hoffmann, T. J., Iribarren, C., Jorgenson, E., Kushi, L. H., Ludwig, D., Matsuguchi, T., McGuire, W. B., Miles, S., Quesenberry, C. P., Rowell, S., Sadler, M., Sakoda, L. C., & Blackburn, E. H. (2015). Automated assay of telomere length measurement and informatics for 100,000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. *Genetics*, *200*(4), 1061-1072. https://doi.org/10.1534/genetics.115.178624.

Larson, E. B., & Bruce, R. A. (1987). Health benefits of exercise in an aging society. *Archives of Internal Medicine*, *147*(2), 353-356. https://doi.org/10.1001/archinte.1987.00370020171058.

Levine, M. E., Lu, A. T., Quach, A., Chen, B. H., Assimes, T. L., Bandinelli, S., Hou, L., Baccarelli, A. A., Stewart, J. D., Li, Y., Whitsel, E. A., Wilson, J. G., Reiner, A. P., Aviv, A., Lohman, K., Liu, Y., Ferrucci, L., & Horvath, S. (2018). An epigenetic biomarker of aging for lifespan and healthspan. *Aging*, *10*(4), 573-591. https://doi.org/10.18632/aging.101414.

Li, X, Ploner, A, Wang, Y, Magnusson, PK, Reynolds, C, Finkel, D, Pedersen, NL, Jylhävä, J, & Hägg, S (2020). Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. *eLife*, *9*, e51507. https://doi.org/10.7554/eLife.51507.

Logan, J. G., & Barksdale, D. J. (2008). Allostasis and allostatic load: Expanding the discourse on stress and cardiovascular disease. *Journal of Clinical Nursing*, *17*(7b), 201-208. https://doi.org/10.1111/j.1365-2702.2008.02347.x.

Lowsky, D. J., Olshansky, S. J., Bhattacharya, J., & Goldman, D. P. (2014). Heterogeneity in healthy aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *69*(6), 640-649. https://doi.org/10.1093/gerona/glt162.

Lu, A. T., Quach, A., Wilson, J. G., Reiner, A. P., Aviv, A., Raj, K., Hou, L., Baccarelli, A. A., Li, Y., Stewart, J. D., Whitsel, E. A., Assimes, T. L., Ferrucci, L., & Horvath, S. (2019). DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging*, *11*(2), 303-327. https://doi.org/10.18632/aging.101684.

Mariotto, A. B., Robin Yabroff, K., Shao, Y., Feuer, E. J., & Brown, M. L. (2011). Projections of the cost of cancer care in the United States: 2010-2020. *Journal of the National Cancer Institute*, *103*(2), 117-128. https://doi.org/10.1093/jnci/djq495.

McCrory, C., Fiorito, G., Hernandez, B., Polidoro, S., O'Halloran, A. M., Hever, A., & Kenny, R. A. (2020). Grimage outperforms other epigenetic clocks in the prediction of age-related clinical phenotypes and all-cause mortality. *The Journals of Gerontology: Series A*, *76*(5), 741-749. https://doi.org/10.1093/gerona/glaa286.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*, 33-44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x.

McEwen, B. S., & Karatsoreos, I. N. (2015). Sleep deprivation and circadian disruption. *Sleep Medicine Clinics*, *10*(1), 1-10. https://doi.org/10.1016/j.jsmc.2014.11.007.

Perna, L., Zhang, Y., Mons, U., Holleczek, B., Saum, K.-U., & Brenner, H. (2016). Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clinical Epigenetics*, *8*, 64. https://doi.org/10.1186/s13148-016-0228-z.

Peters, MJ, Joehanes, R, Pilling, LC, Schurmann, C, Conneely, KN, Powell, J, Reinmaa, E, Sutphin, GL, Zhernakova, A, Schramm, K, Wilson, YA, Kobes, S, Tukiainen, T, Ramos, YF, Göring, HH, Fornage, M, Liu, Y, Gharib, SA, Stranger, BE, De Jager, PL, & Johnson, AD (2015). The transcriptional landscape of

age in human peripheral blood. *Nature Communications*, *6*(1), 8570. https://doi.org/10.1038/ncomms9570.

Quach, A., Levine, M. E., Tanaka, T., Lu, A. T., Chen, B. H., Ferrucci, L., Ritz, B., Bandinelli, S., Neuhouser, M. L., Beasley, J. M., Snetselaar, L., Wallace, R. B., Tsao, P. S., Absher, D., Assimes, T. L., Stewart, J. D., Li, Y., Hou, L., Baccarelli, A. A., Whitsel, E. A., & Horvath, S. (2017). Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging*, *9*(2), 419-446. https://doi.org/10.18632/aging.101168.

Ren, X., & Kuan, P. F. (2020). RNAAgeCalc: A multi-tissue transcriptional age calculator. *Plos One*, *15*(8), e0237006. https://doi.org/10.1371/journal.pone.0237006.

Reynolds, C. A., Finkel, D., McArdle, J. J., Gatz, M., Berg, S., & Pedersen, N. L. (2005). Quantitative genetic analysis of latent growth curve models of cognitive abilities in adulthood. *Developmental Psychology*, *41*(1), 3-16. https://doi.org/10.1037/0012-1649.41.1.3.

Rockwood, K, & Mitnitski, A (2012). How might deficit accumulation give rise to frailty?. *The Journal of Frailty & Aging*, *1*(1), 8-12. https://doi.org/10.14283/jfa.2012.2.

Rockwood, K., Song, X., MacKnight, C., Bergman, H., Hogan, D. B., McDowell, I., & Mitnitski, A. (2005). A global clinical measure of fitness and frailty in elderly people. *Canadian Medical Association Journal*, *173*(5), 489-495. https://doi.org/10.1503/cmaj.050051.

Rolfson, D. B., Majumdar, S. R., Tsuyuki, R. T., Tahir, A., & Rockwood, K. (2006). Validity and reliability of the Edmonton Frail Scale. *Age and Ageing*, *35*(5), 526-529. https://doi.org/10.1093/ageing/afl041.

Ruiz-Estigarribia, L., Martínez-González, M. Á., Díaz-Gutiérrez, J., Gea, A., Rico-Campà, A., & Bes-Rastrollo, M. (2020). Lifestyle-related factors and total mortality in a Mediterranean prospective cohort. *American Journal of Preventive Medicine*, *59*(2), e59-e67. https://doi.org/10.1016/j.amepre.2020.01.032.

Sae-Lee, C, Corsi, S, Barrow, TM, Kuhnle, GGC, Bollati, V, Mathers, JC, & Byun, HM (2018). Dietary intervention modifies DNA methylation age assessed by the epigenetic clock. *Molecular Nutrition & Food Research*, *62*(23), e1800092. https://doi.org/10.1002/mnfr.201800092.

Sanders, J. L., & Newman, A. B. (2013). Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither?. *Epidemiologic Reviews*, *35*(1), 112-131. https://doi.org/10.1093/epirev/mxs008.

Shibasaki, K., Kin, S. K., Yamada, S., Akishita, M., & Ogawa, S. (2019). Sex-related differences in the association between frailty and dietary consumption in Japanese older people: a cross-sectional study. *BMC Geriatrics*, *19*(1), 211. https://doi.org/10.1186/s12877-019-1229-5.

Shi, G.-P., Ma, T., Zhu, Y.-S., Wang, Z.-D., Chu, X.-F., Wang, Y., Chen, Z.-K., Xu, W.-D., Wang, X.-F., Guo, J.-H., & Jiang, X.-Y. (2019). Frailty phenotype, frailty index and risk of mortality in Chinese elderly population- Rugao longevity and ageing study. *Archives of Gerontology and Geriatrics*, *80*, 115-119. https://doi.org/10.1016/j.archger.2018.11.001.

Sood, S., Gallagher, I. J., Lunnon, K., Rullman, E., Keohane, A., Crossland, H., Phillips, B. E., Cederholm, T., Jensen, T., van Loon, L. J., Lannfelt, L., Kraus, W. E., Atherton, P. J., Howard, R., Gustafsson, T., Hodges, A., & Timmons, J. A. (2015). A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status. *Genome Biology*, *16*(1), 185. https://doi.org/10.1186/s13059-015-0750-x.

Svensson, J., Karlsson, M. K., Ljunggren, Ö., Tivesten, Å., Mellström, D., & Movérare-Skrtic, S. (2014). Leukocyte telomere length is not associated with mortality in older men. *Experimental Gerontology*, *57*, 6-12. https://doi.org/10.1016/j.exger.2014.04.013.

Unnikrishnan, A., Freeman, W. M., Jackson, J., Wren, J. D., Porter, H., & Richardson, A. (2019). The role of DNA methylation in epigenetics of aging. *Pharmacology & Therapeutics*, *195*, 172-185. https://doi.org/10.1016/j.pharmthera.2018.11.001.

Wang, Q., Zhan, Y., Pedersen, N. L., Fang, F., & Hägg, S. (2018). Telomere Length and All-Cause Mortality: A Meta-analysis. *Ageing Research Reviews*, *48*, 11-20. https://doi.org/10.1016/j.arr.2018.09.002.

Warburton, D. E. R., & Bredin, S. S. D. (2017). Health benefits of physical activity. *Current Opinion in Cardiology*, *32*(5), 541-556. https://doi.org/10.1097/hco.0000000000000437.

Wu, M.-y., Wang, J.-b., Zhu, Y., Lu, J.-m., Li, D., Yu, Z.-b., Shen, P., Jiang, D.-j., Lin, H.-b., & Chen, K. (2020). Impact of individual and combined lifestyle factors on mortality in China: A cohort study. *American Journal of Preventive Medicine*, *59*(3), 461-468. https://doi.org/10.1016/j.amepre.2020.01.029.

Zhang, YB, Pan, XF, Chen, J, Cao, A, Xia, L, Zhang, Y, Wang, J, Li, H, Liu, G, & Pan, A (2021). Combined lifestyle factors, all-cause mortality and cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies. *Journal of Epidemiology and Community Health*, *75*(1), 92-99. https://doi.org/10.1136/jech-2020-214050.

Zhang, W., Qu, J., Liu, G.-H., & Belmonte, J. C. I. (2020). The ageing epigenome and its rejuvenation. *Nature Reviews Molecular Cell Biology*, *21*(3), 137-150. https://doi.org/10.1038/s41580-019-0204-5.

Zhang, Y., Wilson, R., Heiss, J., Breitling, L. P., Saum, K.-U., Schöttker, B., Holleczek, B., Waldenberger, M., Peters, A., & Brenner, H. (2017). DNA methylation signatures in peripheral blood strongly predict all-cause mortality. *Nature Communications*, *8*, 14617. https://doi.org/10.1038/ncomms14617.

Zhan, Y., & Hägg, S. (2018). Telomere length shortening in Alzheimer's disease: Procedures for a causal investigation using single nucleotide polymorphisms in a Mendelian randomization study. *Biomarkers for Alzheimer's Disease Drug Development*, *1750*, 293-306. https://doi.org/10.1007/978-1-4939-7704-8_20.

Zhao, W, Ammous, F, Ratliff, S, Liu, J, Yu, M, Mosley, TH, Kardia, SLR, & Smith, JA (2019). Education and lifestyle factors are associated with DNA methylation clocks in older African Americans. *International Journal of Environmental Research and Public Health*, *16*(17), 3141. https://doi.org/10.3390/ijerph16173141.