Longevity biomarkers: applications and limitations

Bow T. Shrike[1], Gustav Nilsonne[2], Vincent Weisser[1], Rakhan Aimbetov[1,*]

[1] VitaDAO

[2] Karolinska Institutet, Stockholm, Sweden

* To whom correspondence should be addressed; r.aimbetov@gmail.com

Abstract

One major barrier to longevity research is evaluating the impact of interventions that improve human health and longevity because they are complex processes that occur over long time scales. Instead, measurable phenotypic traits or proxies of longevity, termed longevity biomarkers, may be used to assess the effectiveness of longevity interventions, or prognosticate clinical outcomes. Longevity biomarkers are critical tools for predicting lifespan and susceptibility to age-related diseases, but there exists a dizzying array of options, with at times contradictory readouts. Strengths of longevity biomarkers include providing insight into an individual's biological age, as opposed to chronological age, which is pivotal in evaluating targeted interventions that address aging and age-related conditions. However, most longevity biomarkers also exhibit notable weaknesses, such as a lack of specificity and lack of standardization across different studies and applications. These limitations underscore the need for more research to enhance their accuracy and reliability in long-term longitudinal studies. In the present review, we discuss key features of popular clinical biomarkers used to predict morbidity and mortality associated with advanced age, identify existing bottlenecks, and integrate the field consensus on further directions for robust life- and healthspan estimation.

Keywords: aging, biomarker, longevity

Introduction

The longevity research community confronts significant obstacles in evaluating the impact of health and longevity interventions. Presently, the most precise and dependable method for gauging an individual's health and longevity is through direct, long-term observation, which is both expensive and time-intensive. An alternative approach involves using biomarkers (Moqri et al., 2023). Biomarkers are measurable phenotypic traits that correlate with specific biological functions or their dysfunctions: “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions” (FDA-NIH Biomarker Working Group, 2016). Biomarkers offer a means to assess the effects of interventions more rapidly and accurately, providing insights into the mechanisms of aging and disease without the need to wait for a human generation to pass.

Historically, biomarkers were used for diagnostics and prophylaxis of morbidity. Some individual biomarkers correlate with all-cause and cause-specific mortality and burden of disease (GBD 2019 Risk Factors Collaborators, 2020). However, biomarkers in longevity research are limited due to inherent methodological constraints imposed by the aging process. For example, any biomarker exists and changes in several dimensions:

1. **Temporal.** Some biomarkers are useful on short timescales (less than one day), while others are useful across decades.

2. **Tissue-, organ-, organ system-specific.** Some biomarkers reflect changes in one organ system or one tissue instead of measuring organismal aging as a whole.

3. **Pathophysiological.** Some biomarkers measure pathology prevalent in old age instead of measuring aging in the absence of pathology.

4. **Demographic.** The predictive utility of a biomarker can vary by sex due to differences in physiology, an individual’s ethnic background, or environmental contributors.

5. **Axiological.** Not all biomarkers have the same value in terms of their predictive power. This underscores a crucial distinction between the total organismal phenotype, or the **phenome**, and the subset of clinically applicable traits, the **clinome**.

6. **Epistemological.** Although some biomarkers may robustly change with age, there is no clear idea of what these changes mean biologically.

7. **Practical.** Some biomarkers cannot be put to routine clinical use despite their theoretical significance due either to cost, ease of implementation, or ethics.

Although the field of longevity biomarker research has not yet matured, vital inroads toward an integrative framework for biomarker discovery and implementation have been made. To circumvent the outlined complexity, biomarker panels and combined scores in combination with ML/AI algorithms and dimensionality reduction techniques, such as **principal component analysis** (PCA), have been proposed to capture the aging biomarker dynamics in its entirety, extracting non-linear features of the aging phenotype from multimodal datasets (Pyrkov and Fedichev, 2019; Bafei and Shen, 2023). The concept of biological age and the development of

associated aging clocks have significantly advanced our understanding of the biology of aging as a measurable quantitative trait, amenable to interventions (Ferrucci et al., 2020; Moqri et al., 2023).

Still, the longevity community is faced with systemic deficiencies. A recent survey of 395 professionals across various sectors of longevity identified major bottlenecks that hinder the translation of fundamental geroscience to the clinic (Florea et al., 2023). The absence of validated biomarkers was the primary barrier to progressing geroprotective interventions into clinical trials (tied with overall insufficient funding) (Florea et al., 2023). Most peer-reviewed studies on longevity biomarkers are small and exploratory, and most proposed biomarkers have never been tested in a replication study. The survey's authors duly note that "to accelerate progress across the field, it may be more effective for investigators working on biomarkers to focus on the clinical validation of existing biomarkers rather than the development of new biomarkers" (Florea et al., 2023).

The same survey pinpointed the most impactful solutions to the identified bottlenecks (Florea et al., 2023). Across all participants, the availability of large-scale public datasets was mentioned most frequently (Florea et al., 2023). Consequently, the field needs consortia to come together and pool data in order to obtain large cohorts with sufficiently long follow-up times. Further, putative biomarkers need to be tested in combination so that the incremental value of one over another can be estimated.

The conceptual framework for biomarker discovery adopted by the **American Federation for Aging Research** is instrumental in the identification of useful candidates, setting three essential criteria for biomarker selection (Baker and Sprott, 1988; Butler et al., 2004; Sprott, 2010). First, a candidate biomarker should predict the outcome of a wide range of age-sensitive tests in multiple physiological and behavioral facets of aging better than chronological age. Second, it should predict remaining longevity at an age when 90% of the population is still alive, and account for most of the specific illnesses that afflict the species under study. Third, its measurement should be minimally invasive and not alter life expectancy or the outcome of subsequent tests of other age-sensitive tests (adapted from (Baker and Sprott, 1988; Butler et al., 2004; Sprott, 2010)).

Alongside the paramount importance of developing reliable biomarkers for longevity research, challenges hinder their implementation. One major challenge is the difficulty in comparing results across different studies. Variations in measurement protocols and biomarker value ranges can impede the comparison of individual results and the formulation of broad conclusions. A second challenge is that the response to interventions measured by biomarkers can vary greatly between individuals, complicating the task of identifying and quantifying intervention impacts. Finally, the lack of standardized protocols for validating biomarker results further complicates cross-study comparisons. This underscores the urgent need for both reliable biomarkers and standardized validation protocols to accurately evaluate the effects of interventions on health and longevity.

The core list of published research on the selected biomarkers used in this review was assembled by querying the Web of Science citation database (www.webofscience.com) with the

((“biomarker name”) AND (longevity OR ag*ing)) query, followed by sorting the output by descending citation number. Highly cited research papers, presenting population-level study results, were manually curated and used to outline the general narrative. Complementary publications were retrieved from PubMed (pubmed.ncbi.nlm.nih.gov) on an ad hoc basis to provide context and additional information where necessary.

In the present review, we first discuss what features biomarkers that forecast morbidity and mortality, or **prospective biomarkers**, should possess (Califf, 2018). Next, we overview the current longevity biomarker landscape, focusing exclusively on research done in humans, assess the biomarkers’ applied utility for health- and lifespan prediction, and interrogate their potential limitations.

Features of useful longevity biomarkers

Not all biomarkers are created equal. Here, we consider “good” biomarkers to be those with the following characteristics: 1) Discriminative ability, 2) Strong predictive power, 3) Strong correlation with the biological function, 4) Ease of measurement in humans, and 5) Low cost to measure.

A **discriminative ability** means there is a sufficiently large difference, with minimal overlap, between the values of interest. Consider two different biomarkers that measure the same phenotype, X and Y, calculated for three n = 1000-sized samples from three distinct populations (e.g. current smokers, former smokers, and never smokers) (**Fig 1A**). The means of both biomarkers, X and Y, are the same—50, 70, and 125—for these respective populations. Depending on how the values spread around the mean, there is a small (X) or a large (Y) degree of overlap between the biomarker values in the three populations, despite their means being the same – e.g. see sample 1 vs. sample 2 or sample 2 vs. sample 3.

However, the differences between populations for biomarkers X and Y are statistically significant in each case, with ***p*-values** approaching 0 due to sample size. *P*-values estimate the likelihood that the samples are derived from the same population, i.e. any observed difference is due to the sampling chance alone. At $p = 0.05$, which is a common statistical significance cut-off used in biomedical research, there is a 5% probability that we would have gotten the observed difference, or a more extreme difference, had the values on average not been different between the groups. While statistical significance (or *p*-value) is necessary, it is insufficient for establishing the biomarker utility. By calculating the effect sizes using the pooled measures of variance for two samples, we can see that the differences in biomarker X values are tighter than those for biomarker Y, which makes X a better estimator of the phenotype. Dynamic range is one key aspect of discriminative ability. Along with the dynamic range, a second aspect of discriminative ability is the age range over which the biomarker is useful. Some biomarkers may only be useful in the >50 age range, or not work in children.

A **strong predictive power** means that knowing the levels of the biomarker will let one accurately predict the phenotype in question. This implies that the biomarker needs to be hard to manipulate without also altering the phenotype. For example, consider grip strength: if grip strength is used as a proxy for general sarcopenia, training only grip strength could improve the biomarker without affecting overall muscle mass or improving lifespan.

Most quantitative biomarkers exist as a continuous variable. For example, assume a biomarker Z follows a normal distribution in the population, ranging from 0 to 125 arbitrary units (**Fig 1B**). The strength of predicting a certain outcome, measured by biomarker Z, would be expressed in terms of relative outcome probabilities associated with defined biomarker values or ranges thereof – e.g. for persons in the 3rd tertile according to the biomarker value vs. persons in the 1st tertile. Terms such as **relative risk ratio**, **odds ratio**, and **hazard ratio** are different epidemiological measures of effect size (**Fig 1C**). As discussed above, a large effect size is preferable. Importantly, the effect sizes calculated for sampled populations are point estimates (not true values) of the population parameter studied. To account for variability and sampling bias, point estimates are expressed together with confidence intervals – e.g. the true population parameter value lies somewhere within the specified range with 95% confidence (the 95% confidence interval, 95% CI). If the CI for a risk ratio includes 1, there might not be any difference between biomarker values in the parent population despite the significant estimated effect size in the sample.

A **strong correlation with biological function** means the biomarker is a good indication of the biology of what it is measuring. This can be measured by its correlation to other biological markers and its sensitivity and specificity to that phenotype. For example, if you measured blood pressure, you would want to see how it correlates with other cardiovascular markers like total cholesterol or pulse wave velocity. You would also compare performance between different ways of measuring blood pressure (e.g. electronic vs. manual sphygmomanometer).

Another key feature of a “good” biomarker is that it is easy to measure. **Ease of measurement** means biomarker detection uses assays that are quantifiable, have minimal operator bias, and can be performed with minimal training. For example, drawing blood is easy, whereas spinal taps are less so.

Finally, the **cost** includes the dollar amount per test, cost/availability of any specialized equipment needed, time to perform, and time to analyze. If a blood test performs equally well with an MRI scan, the blood test is superior. While costs for new technologies generally decline overall with time, lower costs allow for wider implementation. Implementing tests on a larger scale enables standardization and common interpretation of the test.

A large number of biomarkers exist to assess physiological parameters, predict morbidity and mortality, and forecast the effectiveness of therapeutic interventions. Here, we evaluate the most widely implemented biomarkers and their combinations—classical and novel—in clinical practice and evaluate their utility for biogerontology research. Since age-targeted longitudinal studies need reliable biomarkers reflective of a pro-longevity phenotype, a critical analysis of

present approaches to risk and health estimation and the framework for appraisal of their practicality are of utmost importance. We first consider biomarkers by broad categories—physiological, neurological, laboratory, genetic, and epigenetic—discussing them on their own merit, and then evaluate the usefulness of combinations of biomarkers and combined clinical scores for lifespan and/or disease prediction.

Longevity biomarker landscape

Physiological

Herein, physiological biomarkers refer to non-molecular measurements of human morphology, body composition, and physical function. Although physiological biomarkers do not directly measure basic molecular processes underlying aging (Xia et al., 2017), they may be suitable for routine clinical use due to their key strengths: minimal or non-invasive nature, low cost, low technological requirement, and ability to collect longitudinal measurements. For example, physiologic biomarkers like **grip strength**, **VO_2max**, **gait speed**, **30-second chair stand test**, body mass normalized to height (**body mass index** (BMI)) or the composite **Fried's frailty index** (discussed below), are widely applied in medical practice (**Table 1**). Seemingly unsophisticated, some phenotypic biomarkers outperformed early versions of DNA methylation-based biological aging clocks (see **Aging clocks**) in assessing health status of the individual (Belsky et al., 2018), or performed on par with the complex blood-based aging clock, **PhenoAge** (Liu et al., 2018; Pyrkov et al., 2021).

Evaluating physiological parameters of vigor becomes more important during age-related deterioration. Total and regional body fat (adiposity), and lean muscle mass, when coupled with functional parameters, i.e. various means to measure physical performance, serve as robust predictors of morbidity and mortality.

Aging is associated with an increase in adiposity and a redistribution of body fat to the abdominal or visceral regions (Kuk et al., 2009). Excess **body weight** alone increased the risk of death within 26 years of follow-up by 1% for each extra pound (0.45 kg) in the 30-42 years-old cohort and by 2% between the ages of 50 and 62 years (Kopelman, 2000). Furthermore, in middle-aged men, even more important than overall obesity as an independent risk factor for coronary heart disease (CHD) is **abdominal obesity** (Lakka et al., 2002). Abdominal obesity comprises both the **subcutaneous fat** that can obscure the abdominal muscles, and **visceral fat** underneath the abdominals. While subcutaneous abdominal fat and visceral fat are both determinants of central obesity, they have different profiles in terms of predicting morbidity and mortality. Visceral adipose tissue is a unique pathogenic fat depot (Fox et al., 2007). Visceral fat is a significant predictor of mortality in men after adjustment for age, follow-up time, subcutaneous fat, and liver fat (Kuk et al., 2006). Moreover, visceral fat correlated with markers of inflammation

and oxidative stress (Pou et al., 2007), underscoring the relationship between visceral adiposity and metabolic health risks (Goodpaster et al., 2003, 2005).

In clinical practice, body fat is often estimated using a formula that attempts to correct weight for height: the **body mass index** (BMI). The BMI assumes that most variation in weight for people with the same height is due to fat mass (Kopelman, 2000). BMI is a strong predictor of overall mortality above and below 22.5–25 kg/m² (Prospective Studies Collaboration et al., 2009; Flegal et al., 2013). The progressive excess mortality above this range is attributed to vascular disease and may be causal. For example, at 30–35 kg/m², median survival is reduced by 2–4 years, whereas at 40–45 kg/m², survival is reduced by 8–10 years, which is comparable with the effects of smoking (Prospective Studies Collaboration et al., 2009). Thus, BMI is a valuable, albeit crude, population-level estimate of overweight and overall obesity (Lakka et al., 2002).

Since BMI fails to distinguish between the weight of fat and muscles or body frame, **waist-to-hip ratio** (WHR) and **waist circumference** (WC) are used to gain additional information on the nature of obesity (Williams et al., 1997). On average, each 1 standard deviation (SD) increase in WHR (0.06), WC (9.8 cm), and BMI (3.5 kg/m^2) increased the mortality risk by >20% (Lakka et al., 2002). WC is a significant predictor of type 2 diabetes and cardiovascular disease after control for BMI, providing a reasonable approximation of visceral fat in clinical settings (Janssen et al., 2002; Kuk et al., 2006). Both general and abdominal obesity throughout adulthood is associated with an increased risk of pre-frailty/frailty in later years (Uchai et al., 2023). Thus, physical measurements serve as a first screen for factors that may limit human longevity.

These physical measurements can be refined in a laboratory setting. There, body composition can be measured using **bioelectrical impedance analysis** (BIA) (Kyle et al., 2004). This analysis assumes that the fat-free mass, which includes visceral organs, contains most of the water and conducting electrolytes in the body (Sergi et al., 2017). Using conversion formulas, BIA allows for the estimation of total body fat, **total skeletal muscle mass**, and muscle mass in the extremities, termed **appendicular skeletal muscle mass** (Sergi et al., 2017). Although these measurements require a lab, they can provide information on both body fat and muscle mass.

Loss of muscle mass is another factor associated with mortality, termed sarcopenia. Sarcopenia is defined as the “age-related loss of skeletal muscle mass plus loss of muscle strength and/or reduced physical performance” (Chen et al., 2020). It is associated with increased likelihood of adverse outcomes including falls, fractures, physical disability, and mortality (Cruz-Jentoft et al., 2019; Cruz-Jentoft and Sayer, 2019). While loss of muscle mass is one measure of adverse outcomes, recent diagnostic guidelines suggest that loss of muscle strength (dynapenia) is superior to loss of muscle mass (sarcopenia) for predicting adverse outcomes (Cruz-Jentoft et al., 2019). Notably, both men and women lose strength at a higher rate than they lose muscle mass (Goodpaster et al., 2006). Additionally, the robust correlation between strength and mortality persisted independently of low muscle mass, indicating that muscle strength, as an indicator of muscle quality, holds greater significance than quantity when estimating mortality risk

(Newman et al., 2006). Due to technological limits, defining sarcopenia using muscle quantity and quality remains problematic (Cruz-Jentoft et al., 2019). Thus, strength might serve as an inherent indicator of mid- and late-life vitality and endurance that correlates with longevity into old age (Rantanen et al., 2000).

Maximal voluntary strength is a measure of the functioning of both the neural and the muscular systems, and thus may be an indicator of a person's overall vigor (Rantanen et al., 2000). Both sarcopenia and dynapenia are assessed by muscle strength evaluations. Popular evaluations include **grip strength** (Roberts et al., 2011; Bohannon, 2019), **5-times sit-to-stand test** (Muñoz-Bermejo et al., 2021), and the **30-second chair stand test** (Jones et al., 1999). Muscle strength evaluations may be coupled with assessment of muscle quantity and quality using BIA or MRI. Mobility performance is appraised by a functional test, like measurement of **gait speed** (Studenski et al., 2011; Guralnik et al., 2000) or the **6-minute walk test** (Enright et al., 2003; Enright, 2003) **(Table 1)**. Except for grip strength, the other tests are more useful for the elderly where loss of function and performance compared to earlier life is seen.

Grip strength, which moderately correlates with overall strength, serves as a reliable surrogate for arm and leg strength. In healthy populations, hand grip strength measured during mid-life predicts risk of mortality from all causes over a follow-up of 25 (Rantanen et al., 1999), 30 (Rantanen et al., 2000), and 40 years (Metter et al., 2002), independent of BMI. The protective effect of muscular strength was evident across a range of causes of mortality and socioeconomic contexts (Leong et al., 2015). However, variations in measuring grip strength, such as dynamometer choice and protocols, may introduce measurement error, and hinder study comparisons (Roberts et al., 2011). Its ease of use makes it advisable for routine hospital and community healthcare practice.

Performance measures are basic integrators of the health of older people (Studenski et al., 2003). The relationship between physical activity volume and health status shows a graded linear pattern, indicating that the most active individuals experience the lowest risk of health issues (Warburton et al., 2006). **Cardiorespiratory fitness** (CRF), or "the capacity of the cardiovascular (heart and blood vessels) and respiratory (lungs) systems to supply oxygen-rich blood to the working skeletal muscles and the capacity of the muscles to use oxygen to produce energy for movement", is a predictor of mortality independent of other factors (Booth et al., 2012; Ross et al., 2016). Exercise capacity is a powerful predictor of mortality: every 1 **metabolic equivalent** (MET; resting metabolic rate of 3.5 ml O_2 kg^{-1} min^{-1} (Jetté et al., 1990; Franklin et al., 2018)) increase in treadmill performance was associated with a 12–15% improvement in survival (Myers et al., 2002; Harber et al., 2017). Further, risk-adjusted all-cause mortality (median follow-up of 8.4 years) was inversely proportional to CRF and was lowest in elite performers (≥97.7th percentile according to peak METs in a treadmill test): e.g. elite vs. low performers (<25th percentile) adjusted HR was 0.20 (95% CI, 0.16-0.24; P < 0.001) (Mandsager et al., 2018). The increase in all-cause mortality associated with reduced CRF, e.g. low vs. elite adjusted HR of 5.04 (95% CI, 4.10-6.20; P <

0.001), was comparable to or greater than traditional clinical risk factors, such as coronary artery disease, smoking, or diabetes (Mandsager et al., 2018). To sum up, CRF 2 SDs above the mean for age and sex displays the greatest survival compared with all other performance groups, with no apparent upper limit (Mandsager et al., 2018). In a clinical setting, the gold standard to determine CRF is **VO_2max**, or maximal oxygen uptake under peak exercise load measured by open-circuit spirometry (Hawkins et al., 2007).

Regular physical activity significantly contributes to both primary and secondary prevention of various chronic diseases (Warburton et al., 2006). Any amount of physical activity is beneficial with a longevity benefit at 3–5 times the recommended leisure-time physical activity minimum (Arem et al., 2015). In addition to lower physical capacity, the overall activity becomes less frequent and intense with age, and with progressive shortening of the activity bouts that can be tolerated without resting, indicative of functional decline (Schrack et al., 2019; Wanigatunga et al., 2019).

Cardiovascular stiffness increases with age (Glasser et al., 1997; Mitchell et al., 2004; Zieman et al., 2005). A sedentary lifestyle during healthy aging is linked to decreased left ventricular compliance and diminished diastolic performance, an effect potentially reversed by sustained endurance training (Arbab-Zadeh et al., 2004). Vascular compliance can be measured using **pulse wave velocity**. The slower the pulse wave velocity, the greater the vessel compliance. Pulse wave velocity increased from 1% in both sexes aged 50 years to 64% in men and 74% in women aged 70 years (Mitchell et al., 2007). Pulse wave velocity significantly predicts all-cause mortality (Laurent et al., 2001). When adjusted for age, sex, and standard risk factors, individuals in the highest (11.8 m/s) aortic pulse wave velocity group had a HR for a major cardiovascular event of 3.4 (95% CI, 1.4–8.3; P=0.008) compared to those in the lowest (7.8 m/s) aortic PWV group (Mitchell et al., 2010).

Another measure of cardiovascular function is **blood pressure**. High blood pressure, or hypertension, is a major modifiable risk factor globally, affecting 34% of men and 32% of women aged 30-79 (NCD Risk Factor Collaboration (NCD-RisC), 2021). In absolute numbers, the prevalence of people aged 30–79 years with hypertension doubled from 317 million men and 331 million women in 1990 to 652 million men and 626 million women in 2019 due to population growth and aging (NCD Risk Factor Collaboration (NCD-RisC), 2021).

Hypertension is a principal contributor to the development of cardiovascular disease, stroke, renal impairment, and is a risk factor for functional and cognitive decline, physical disability, and dementia (Buford, 2016; Fuchs and Whelton, 2020). In a meta-analysis of 61 prospective observational studies, encompassing ~1 million people, a 20 mm Hg lower usual (long-term average) systolic blood pressure or a 10 mm Hg lower usual diastolic blood pressure at baseline was associated with ≥2-fold reduction in age-specific HRs for death from stroke, ischemic heart disease, and other vascular causes in middle age in men and women; the association was less pronounced, albeit still positive, in older age (Lewington et al., 2002). This relationship was evident with no threshold down to at least 115/75 mm Hg (Lewington et al., 2002). Moreover, non-vascular

mortality positively correlates with blood pressure – with each 20 mm Hg lower usual systolic blood pressure corresponding to an age-standardized HR of 0.88 (95% CI, 0.87-0.89) (Lewington et al., 2002). In a study assessing the effect of modifiable risk factors on myocardial infarction incidence, hypertension accounted for OR of 2.48 (99% CI, 2.30-2.68), adjusted for age, sex, and smoking, with population attributable risk of 23.4% (99% CI, 21.7-25.1) (Yusuf et al., 2004).

The last cardiovascular parameter, **heart rate variability**, has predictive power in short-term (~5 min) and long-term (24 h) formats with lower values associated with higher cardiovascular risk (Shaffer and Ginsberg, 2017). Heart rate variability (by all measures) decreases with aging, falling below the threshold associated with an increased risk of mortality at >65 years (Umetani et al., 1998). Additionally, the heart rate profile during exercise and recovery is a strong predictor of sudden death (Cole et al., 1999; Jouven et al., 2005).

Reduced physical ability and overall weakness can be summarized with the term frailty. A working definition of frailty is used clinically to predict adverse outcomes in the elderly: falls, hospitalizations, disability, and death (Fried et al., 2001). Frailty is defined as a “biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes”. The scale of frailty is quantized as having none, one, or more components of the composite phenotype: unintentional weight loss, muscular weakness, poor endurance and energy, slowness, low physical activity level (Fried et al., 2001). Frail people are more vulnerable to the effects of potential stressors and deterioration than others of the same chronological age (Church et al., 2020). Overall, frailty predicts shortened life and healthspan.

Neurological

Along with frailty, the decline of neurological function is one major organism-level sign of aging (Peters, 2006). Neurological phenotypes are most apparent in the waning of cognitive ability and changes in brain anatomy (Oschwald et al., 2019). Measuring these parameters provides feedback on key aging targets, and gives a functional measure of aging. Aging is characterized by a decrease in almost every neurological function tested, including cognition, vision, strength, steadiness, reactions, speed, coordination, fatigue, gait, station, sensations, and tasks of daily living (Potvin et al., 1980). Collectively, cognitive tasks that involve planning, assessing, and executing goals are termed executive function. Executive function also declines with age (Aging Biomarker Consortium et al., 2023b).

According to the “common cause” hypothesis, reduction in both sensory and cognitive function is a manifestation of the aging brain, so both can be used to gauge underlying organ deterioration (Baltes and Lindenberger, 1997). **Sensory acuity** is linked to cognitive performance, and this link becomes more pronounced as a person gets older (Lindenberger and Baltes, 1994; Baltes and Lindenberger, 1997). Scoring in the lower third of a multisensory test of hearing, vision, olfaction, and touch is associated with a 2 times higher risk of dementia and faster cognitive

decline in adults aged 70-79 (Brenowitz et al., 2020). Importantly, **hearing loss** alone independently associates with cognitive decline and incident cognitive impairment in the elderly: there is a 24% increased risk compared to individuals with normal hearing at baseline (Lin et al., 2013). Based on a cross-sectional study, a 25 dB reduction in hearing is equivalent to a 6.8-year age difference as measured by executive function tests (Lin et al., 2011). This suggests that both multisensory loss and cognitive decline provide indirect measures of aging.

Neuroanatomically, aging is marked by a reduction in **total brain volume**, ventricular enlargement, and sulcal widening (Blinkouskaya et al., 2021). This can be partly explained by the reduced brain perfusion due to cardiovascular stiffness (Reeve et al., 2024). In people with atherosclerosis, for example, there may be continuous small hypoxic events which cause loss of brain tissue (Chojdak-Łukasiewicz et al., 2021). Other factors may include dementia, Alzheimer's disease, and other non-vascular etiologies (Peters, 2006).

Total and regional brain volumes can be measured by magnetic resonance imaging (MRI) (Blinkouskaya et al., 2021) **(Table 1)**. Hallmarks of brain aging include alterations in brain anatomy, like an increase in **ventricular volume**, decrease in **temporal lobe volume**, and hippocampal atrophy (especially in individuals >70 years) (Scahill et al., 2003). Volumetric MRI in three age groups (14-34, 35-54, >55 years) with 3.5 years of follow-up revealed differential age-related changes in certain brain regions: longitudinal data showed a significant reduction in **hippocampal volume**, **white matter volume** (WMV), and **total brain volume** in two senior cohorts, suggesting these regions are vulnerable to age effects (Liu et al., 2003). For WMV, the volume difference between scans was at 2% (Liu et al., 2003).

In healthy adults, longitudinal changes in brain volume are not uniform. Regional gray matter, represented by cortical structures and prefrontal cortex in particular, is susceptible to differential aging-related effects (Raz et al., 1997; Lemaitre et al., 2012; Farokhian et al., 2017). This highlights the vulnerability of the most recent neural evolutionary acquisitions, i.e. associative cortices, as opposed to more ancient sensory parts of the brain (Raz et al., 1997).

Notably, in addition to the reduction in WMV with age seen by structural MRI, remarkable alterations have been revealed using diffusion tensor imaging/diffusion MRI (Sullivan and Pfefferbaum, 2006). Parameters like **fractional anisotropy** (FA) and **mean diffusivity** (MD) reflect diffusion directionality of water molecules and, indirectly, white matter integrity (Sullivan and Pfefferbaum, 2006). A decrease in FA, or increase in MD, suggest microstructural abnormalities in the white matter axonal orientation and its myelination. Importantly, these changes are similar to those seen in neurodegenerative diseases and are associated with cognitive performance (Raghavan et al., 2021).

Since brain anatomies reflective of age-related changes can be captured by imaging techniques, analysis and interpretation of the results become the next challenges. Brain MRI scans are combined with machine learning (ML) to construct **“brain age”** clocks (More et al., 2023). Aging clocks estimate a person’s age based on one cohesive set of biomarkers (see **Aging**

clocks). Here, the brain age clocks estimate a person's age based on brain scans. The brain age is then compared to the person's chronological age, to determine a "brain-predicted" age difference. If the brain age is greater than the chronological age, it suggests aging-related brain alterations and implies accelerated degeneration (Franke and Gaser, 2019).

A brain age model based on T1-weighted MRI scans predicts mortality (but not morbidity) in older individuals: having a higher brain-predicted age difference significantly associates with mortality before the age of 80; each extra year of brain-predicted age led to a 6.1% relative increase in the risk of death between age 72 and 80 (HR = 1.061; 95% CI, 1.031, 1.091; P<0.001) (Cole et al., 2018). Furthermore, lower fluid cognitive ability, weaker grip strength, worse lung function, and slower walking speed all strongly correlated with an older-appearing brain, as demonstrated by a higher brain-predicted age difference (Cole et al., 2018).

A brain age estimate based on convolutional neural networks accurately predicted the chronological age of healthy adults using either processed volumetric maps or raw T1-weighted MRI data: the lowest mean absolute error (MAE) of 4.16 years was achieved for a model trained on volumetric gray matter input (Pearson's R = 0.96 for chronological vs. brain-predicted age) (Cole et al., 2017). For raw unprocessed data, MAE was 4.65; R = 0.94 (Cole et al., 2017). On average, this suggests the brain aging clock can predict to within 5 years of chronological age, especially in persons >65 years.

One limitation of this approach is differences between scanners at different facilities. While reliable in a test-retest setting, pre-processing appears to be required to achieve between-scanner reliability for the brain-predicted age be suitable for use in both longitudinal and multicenter research. Refinements to brain age estimates include the blend of structural and functional MRI data, which outperforms either modality alone, with a MAE of 4.29 years (Liem et al., 2017). The multimodal approach, like the majority of the other techniques the authors evaluated, revealed a considerably increased rate of brain aging in participants with objective cognitive impairment (Liem et al., 2017). This supports the hypothesis that brain age and cognitive decline are connected (Elliott et al., 2021).

Laboratory

Serum markers are one subset of biomarkers that can be identified using laboratory tests on biopsies or bodily fluids. These biomarkers are important indicators of the efficacy of pharmacological and lifestyle interventions. Bodily fluids provide accessible and readily measurable markers of health and risk that correlate with life- and healthspan, and are routinely tested for a range of parameters to evaluate organ function and overall health. Some of these parameters correlate with age and age-related comorbidities (Justice et al., 2018). The most important biomarkers for aging gauge biochemical markers of aging, glucose and lipid homeostasis, inflammation, purine catabolism, and kidney function **(Table 1)**.

Serum markers can provide insight into the connections between the brain and other systems of the body and organ aging. For example, the **hypothalamic-pituitary axis**, exerting its neuroendocrine influence on all aspects of human physiology, including energy metabolism, circadian rhythm, and reproductive function, is linked to five of the nine classical hallmarks of aging (Kim and Choe, 2019). The preserved hypothalamic-pituitary-testicular axis in exceptional longevity has been reported (Aleksic et al., 2022). Further, **total testosterone** and **free testosterone** levels are inversely correlated with cognitive impairment in aged males, highlighting the neuroprotective importance of this particular sex hormone—as well as an intact hypothalamus-pituitary axis in general—for healthy aging (Holland et al., 2011). This suggests multiple serum markers can be used to read out neurologic function, along with other systemic functions.

Another example of using serum markers to read out neurology phenotypes is a proteomic approach to assemble a panel of neurology-related serum proteins. A panel of 90 neurology-related protein biomarkers linked plasma proteomics to cognitive function and brain structure (Harris et al., 2020). The study sought to test if any associations between the neurology-related plasma protein levels and general fluid cognitive ability could be accounted for by structural brain variables. Of the 90 proteins, 22 were associated with general fluid cognitive ability in later life; 10 were mediated by total brain volume (Harris et al., 2020). These findings offer a method for the high-throughput and affordable detection of plasma proteins that may aid in the identification of neurologic biological pathways. Thus, both traditional and novel serum markers correlate with neurological function and may be useful for predicting the rate of age-related loss of cognitive function.

Glucose homeostasis is one of the key biomarkers for aging (Chia et al., 2018). The most basic test for the body's ability to utilize glucose is **fasting blood glucose** (FBG) level (Sarwar et al., 2010; Palliyaguru et al., 2021). The weakness of this test is that it captures only a snapshot of glucose level variability throughout the day and fails to account for "spikes" and "dips" in concentration as a result of food intake, physical activity, stress, etc. **Glycated hemoglobin** (HbA1c), on the other hand, reflects the average blood glucose concentration in the few months preceding the test (because red blood cells have a lifespan of around 120 days) and thus serves as an accurate estimator of the glycemic status (Bennett et al., 2007) and a robust predictor of mortality (Currie et al., 2010). However, to achieve and maintain optimal HbA1c, the control of **postprandial glucose** levels is more important than FBG (Woerle et al., 2007). Further, **2-hour post-challenge blood glucose** (2h-BG) is a better predictor for all-cause and cardiovascular mortality than FBG (Borch-Johnsen et al., 2001). Although all aforementioned parameters correlate with markers of cardiovascular health (such as markers of risk for atherosclerosis), the magnitude of postprandial blood glucose swings, rather than the mean values of glucose concentration over time, has the strongest association (Temelkova-Kurktschiev et al., 2000). This finding suggests the primary importance of acute postprandial fluctuation measurements over FBG or HbA1c for longevity biomarker purposes (Monnier et al., 2006).

Better correlates of cardiovascular health and morbidity are blood lipid parameters such as **total triglyceride** and **total cholesterol** levels. Nonfasting triglyceride levels are positively associated with the incidence rates of myocardial infarction, ischemic heart disease, and death (Nordestgaard et al., 2007). Similarly, total cholesterol is one predictor of ischemic heart disease and vascular mortality (Prospective Studies Collaboration et al., 2007). Cholesterol can be subdivided based on the type of particle carrying it. The ratios of total cholesterol or **low-density lipoprotein** (LDL) **cholesterol** to **high-density lipoprotein** (HDL) **cholesterol** demonstrate that HDL cholesterol has a protective effect against ischemic heart disease mortality (Prospective Studies Collaboration et al., 2007). Lowering LDL levels using statins reduces the risk of major vascular events irrespective of age, sex, baseline LDL cholesterol, or previous vascular disease (Mihaylova et al., 2012). Importantly, the association of ischemic heart disease-related hazard ratios with total, non-HDL, and HDL cholesterol becomes less steep with age, meaning these readouts are impractical for risk prediction later in life. Modulating cholesterol levels at late stages of life has a less pronounced effect on mortality (Prospective Studies Collaboration et al., 2007). In addition, longitudinal studies report that total, LDL, and HDL cholesterol decrease with age at about 1% per year, starting at age 65, in both men and women (Ferrara et al., 1997). Thus, cholesterol biomarkers are more useful for predicting untimely demise, rather than increased total lifespan.

Other serum biomarkers report systemic inflammation, which impacts life and health span. For example, **C-reactive protein** (CRP) and **interleukin** (IL)**-6** are used as predictors of myocardial infarction and cardiovascular death (Pai et al., 2004). CRP is a marker for systemic inflammation and metabolic syndrome (Ridker et al., 2003), and is superior to LDL cholesterol in predicting cardiovascular events (Ridker et al., 2002). Depending on the concentration range at which CRP levels are detected, regular and high-sensitivity formats of the test exist, tailored to measure acute and low-grade inflammation, respectively (Wolska and Remaley, 2022). CRP correlates positively with total cholesterol, triglycerides, serum uric acid, body mass index (BMI), and negatively with HDL (Frohlich et al., 2000). Further, after adjustment for lifestyle factors, the ratio of total to HDL cholesterol, postmenopausal hormone therapy, diabetes, hypertension, and covariates such as BMI and lipid levels, the final pooled risk ratio of developing non-fatal myocardial infarction or fatal cardiovascular disease for men and women was 1.68 in the group with high levels of CRP (≥3 mg/L), compared to the group with low levels (<1 mg/L) (95% CI, 1.18-2.38; P = 0.008) (Pai et al., 2004). In a longitudinal study with a 9-year follow-up, higher baseline IL-6 and steeper increases in IL-6 over time associated with elevated multimorbidity, suggesting that this biomarker may serve as an early warning sign of impending health decline (Fabbri et al., 2015). Both IL-6 and CRP correlate with lifespan. In men, each standard deviation increase in either biomarker was associated with a 12-15% decrease in survival time and ~1-year shorter lifespan (Wassel et al., 2010). In women, the 7% reduction in survival time and 1.35-year shorter lifespan was linked only to IL-6 and depended on their estrogen therapy status (Wassel et

al., 2010). These results underscore the sexual dimorphism in life expectancy and associated pathomechanisms, reflected in the biomarker behavior and its prognostic utility.

While inflammatory serum markers may impact life expectancy, they may not predict cognitive decline. Initial studies found these markers might predict age-related neurodegeneration and cognitive dysfunction (as early as 25 years prior to the onset) (Schmidt et al., 2002; Weaver et al., 2002). However, later evidence challenged the applicability of serum inflammation markers to assess a person's cognitive status. In one prospective study aimed at establishing the link between several serum inflammation markers and dementia, IL-6 showed marginal influence, while CRP did not (Engelhart et al., 2004). A different marker for inflammation—**α_1-antichymotrypsin** (ACT)—associated with all types of dementia examined (Engelhart et al., 2004). This was supported by a second study including four inflammation markers and several cognitive tests (Dik et al., 2005). In that study, high ACT strongly associated with worse performance, while elevated CRP, IL-6, and low albumin did not (Dik et al., 2005). These results were independent of other factors tied to cognitive decline, including **apolipoprotein E** (ApoE) ε4 phenotype, vascular diseases, and diabetes mellitus (Dik et al., 2005). However, a fourth study revealed a moderate association of CRP and IL-6, but not ACT, with cognitive function and decline, that was more robust in ApoE ε4 allele carriers (Schram et al., 2007). Thus, while systemic inflammatory factors may predict lifespan, their ability to predict cognitive decline remains limited.

Another serum biomarker that positively correlates with age and cardiovascular risk is serum uric acid. Serum **uric acid** levels increase with age (Kuzuya et al., 2002) and significantly correlate with hypertension (Sundstrom et al., 2005) and cardiovascular events, such as myocardial infarction, ischemic stroke, and all-cause mortality (Bos et al., 2006; Chen et al., 2009). In men, serum uric acid rises at a rate of ~7 μmol/L per decade (Zitt et al., 2020). In women, the level remains relatively constant until the age of 50, then increases sharply by ~20 μmol/L per decade thereafter (Zitt et al., 2020). Serum uric acid levels also associate with markers of sarcopenia, with mixed results. In a cohort of people with high handgrip strength, the hazard ratio of all-cause mortality was linear with serum uric acid levels (Guo et al., 2021). In the low-strength cohort, the relationship curve was J-shaped with higher ratios on either extreme of the serum uric acid concentration range (Guo et al., 2021). In contrast, other investigators observed that serum uric acid levels are positively associated with skeletal muscle index, grip strength, and gait speed (Liu et al., 2022). The reported contrasting effects of serum uric acid on overall mortality risk and functional capacity characteristics confounds the use of this biomarker for lifespan prediction and the assessment of health status.

Oxidative stress accompanies the progression of age-related pathologies. Recently, urinary **8-oxo-7,8-dihydro-2′-deoxyguanosine** (8-oxo-dGsn) and **8-oxo-7,8-dihydroguanosine** (8-oxo-Gsn), products of DNA and RNA oxidation respectively, were proposed as aging biomarkers (Gan et al., 2012). When normalized by urine creatinine level, both metabolites were positively correlated with age (Gan et al., 2018). 8-oxo-Gsn was more linearly age-correlated than

8-oxo-dGsn and was ~2-fold more abundant, suggesting it is more useful for physiological age estimation (Gan et al., 2018). For instance, higher 8-oxo-Gsn was significantly linked to frailty (defined as satisfying three or more Fried's frailty phenotype criteria) (Liang et al., 2020). Age, heart rate, systolic blood pressure, hemoglobin, and Na^+ independently associated with frailty in the same study (Liang et al., 2020). Furthermore, increased urinary levels of 8-oxo-Gsn associated with poor physical performance (grip strength, walking speed, repeated chair stand test) and cognitive function (Montreal cognitive assessment 5-minute protocol (Nasreddine et al., 2005)) (Jiang et al., 2022). One weakness of using 8-oxo-Gsn is the need for liquid chromatography-tandem mass spectrometry (LC-MS/MS). This renders 8-oxo-Gsn unsuitable for fast, straightforward, and operator bias-free implementation in the clinic.

Another group of serum biomarkers measure kidney function. Impairment of renal function correlates with an increase in cardiovascular risk (Schiffrin et al., 2007). One commonly used quantitative measure of kidney filtering capacity is **glomerular filtration rate** (GFR). GFR is estimated by plugging the serum **creatinine** concentration into various equations (Stevens et al., 2006). Notably, as a person gets older, and GFR worsens, the hazard ratios for death from any cause, any cardiovascular event, and any hospitalization progressively increase (Go et al., 2004).

One challenge with GFR is the choice of equation used to estimate it. The equation of choice needs to account for factors such as age, sex, race, and lean muscle mass, all of which affect muscle creatinine production (Knight et al., 2004). In addition, serum creatinine levels positively associate with confounders like smoking status, hyperlipidemia, and hypertension after adjusting for creatinine clearance (Knight et al., 2004). Moreover, in humans over 75 years old, the decline in GFR does not lead to increases in serum creatinine because the diminished filtration rate is compensated by lower lean muscle and creatinine production. This precludes the use of GFR across all ages (Fastbom et al., 1996).

One alternative to GFR is measuring **cystatin C**. Cystatin C, produced by all nucleated cells, may be a superior marker for GFR estimation than creatinine (Dharnidharka et al., 2002). Cystatin C is a strong predictor of all-cause mortality and the cardiovascular composite (death from cardiovascular causes, myocardial infarction, stroke) (Shlipak et al., 2005). However, like creatinine, this protein marker is influenced by non-renal factors. Older age, male sex, greater weight, greater height, current cigarette smoking, and high CRP levels each independently and positively associate with serum cystatin C levels (Stevens et al., 2009). Furthermore, the equation combining both creatinine and cystatin C performed better at GFR estimation than either marker alone (Inker et al., 2012). Additional renal markers may give a better picture of kidney function.

Along with serum markers, kidney function can be measured with urine biomarkers. Excretion of **albumin** in urine—albuminuria—is a sign of renal disease that often precedes age-dependent changes in GFR (Klausen et al., 2004). Urinary albumin predicts cardiovascular morbidity and general mortality in diabetic and non-diabetic, hypertensive and non-hypertensive patient populations (Gerstein et al., 2001; Hillege et al., 2002). Microalbuminuria (urinary

albumin/creatinine ratio 30-300 mg/g) increases the cardiovascular risk 2- to 4-fold (Arnlov et al., 2005). Interestingly, the risk was still evident in patients with albumin to creatinine ratio well below the cut-off for microalbuminuria, possibly evincing underlying low-grade inflammation and endothelial dysfunction (Gerstein et al., 2001).

There are also many other serum molecules and proteins that can be checked to reveal various aspects of health. However, many of these reveal poor health, rather than limitations to lifespan. Furthermore, the complexity of the aging phenotype is reflected in the fact that it is unlikely that any single metabolite or protein is sufficiently robust for appraisal of long-term health, disease susceptibility, and associated risk. Laboratory tests aimed at a single biomarker suffer from both intraday variability and responsiveness to confounding influences such as lifestyle (sedentary or not, smoking status, alcohol consumption, sleep patterns, etc.), diet, fluid intake, body composition, sex, and the effect of other metabolic factors. These make the conventional tests relatively good assessors of health and risk at any given point in time, but poor longitudinal predictors of lifespan. To increase the predictive power of blood plasma biomarkers, panels of biomarkers and combined scores are needed (Murata et al., 2024). These panels may also consider genetic or epigenetic information.

Another approach is to compare differences in serum biomarkers between offspring of long-lived individuals and controls. This approach suggests that FBG (Rozing et al., 2010a), **fasting insulin** (Rozing et al., 2010a), **free triiodothyronine** (Rozing et al., 2010b), **adiponectin** (Atzmon et al., 2008), **total cholesterol-to-HDL cholesterol** ratio (Terry et al., 2007), and **lipoprotein particle size** (Barzilai et al., 2003; Heijmans et al., 2006) are different for long-lived families. While promising, additional validation of these markers is needed.

In addition to serum markers, the cellular blood component can serve as a robust biomarker of physiological decline (Yadav et al., 2024). For example, **red cell distribution width**, derived by dividing the standard deviation of erythrocyte volume by the mean corpuscular volume and expressed in percentages, increases with age (Lippi et al., 2014) and can be used to predict morbidity and mortality in the general population (Patel et al., 2010). Furthermore, our picture of lifespan can be broadened by leukocyte differential tests: a significant positive association between **neutrophil-to-lymphocyte ratio** and overall mortality has been reported (Cataudella et al., 2017).

Genetic and epigenetic

While biomarkers may report on health, the greatest factor governing longevity is an individual's genetics. For example, using twin studies, frailty and falls are estimated to be more heritable than breast cancer (Steves et al., 2012). Furthermore, twin studies suggest genetics have a stronger effect on age of death after 60 compared to prior to 60 (Steves et al., 2012; vB Hjelmborg et al., 2006). If lifestyle was more important than genetics, one would predict a survivorship bias against poor lifestyle in extreme old age. However, in one retrospective analysis of long-lived Ashkenazi Jews (living independently, age >95), no differences were observed in

lifestyle between those living a long time and those who did not (Rajpathak et al., 2011). This suggests that genes and their regulation underlie longevity. Along with measuring the DNA sequences in the human genome, gene expression levels (mRNA), non-coding regulatory RNA profiles, and relative protein abundances all vary with time, so these may provide feedback on current aging processes. Epigenetic markers, or non-genetic modifications to DNA and access to DNA, such as DNA methylation and histone modification patterns, also impact longevity. Finally, non-human genetics may impact longevity due to the profusion of microbes living in the human gut and other organs—the microbiome (**Fig 2**; **Table 1**).

Examining the microbiome, or the genomes of the associated microbes living in our gut, skin or elsewhere represents a new genetic approach to assessing phenotypes (Yatsunenko et al., 2012). One strength of this approach is that sampling is straightforward and non-invasive because fecal samples can be analyzed for the gut microbiome. However, sequencing remains costly and there is large heterogeneity between individuals, complicating interpretation. One approach to solving this challenge is to analyze the metabolites produced by the microbial community instead of the community itself (Zhou et al., 2021). Metabolite analysis could improve interpretation of results because it will tie in to human metabolism directly, and because it focuses on the functional aspects of the microbiome (**Fig 2**). How well they compete with other measurements remains to be seen. The large heterogeneity of microbiomes between humans limits their application at this stage. Thus, microbiome analyses are best interpreted as developing technologies.

Human genetic markers have many strengths in predicting longevity. One strength of genetic markers is that sequence variation can be determined at any stage of the individual's life, so early predictions/interventions could be tailored to an individual's genetic make-up. Genetic markers have a track record of success in predicting risk to diseases that limit lifespan, such as cardiovascular disease and cancer (Ashley et al., 2012). Mutations in some genes have been identified as a major risk factor for the development of certain pathologies, e.g. ***BRCA1*** and ***BRCA2*** in breast and ovarian cancer (Antoniou et al., 2003; Mavaddat et al., 2013), whereas for other genes the relationship is causal, e.g. ***LMNA*** in Hutchinson-Gilford progeria (Eriksson et al., 2003; De Sandre-Giovannoli et al., 2003). Sequencing analysis is straightforward, and multiple targets can be evaluated per run. Marker analysis can vary from checking one or more single genes (e.g. ***IGFIR*** (Suh et al., 2008), ***APOE*** (Liu et al., 2013; Reeve et al., 2024), ***FOXO3*** (Willcox et al., 2008; Gellhaus et al., 2023), ***INK4A*** (Kim and Sharpless, 2006)) to more comprehensive analyses of genomic differences (Sebastiani et al., 2012; Zenin et al., 2019; Deelen et al., 2019; Lin et al., 2021; Ying et al., 2024a).

Limitations of these markers include challenges in fixing the underlying problem, potential contributions of assortative mating, logistics of measurements, and ethical challenges. While genetic predictors that mark specific diseases are known, and genetics are known to have an effect on mortality, there is not yet a list of genes that predict longevity. Compounding this challenge is that we lack the technology to fix poor genetics, limiting the current utility of knowing

the genes. Also, one alternative explanation for the contribution of genetics to longevity is assortative mating, or selection bias. Many heritability estimates assume human mate selection is random. However, if this assumption is false, the heritability estimates would overestimate the contribution of genetics to any given trait, including longevity. It is possible the contribution may be ~20% overestimated (Border et al., 2022; Ruby et al., 2018). Including assortative mating models could improve the estimation of genetic contributions to longevity.

Compounding these problems are the logistic challenges of cost and interpretation of the results. Compared to a skin or chair test, analyzing genetic markers is more expensive and requires additional interpretation. The population-level variability with some of these markers may also limit their predictive value. The ethical concern is discrimination against those with poor genetics for care and insurance coverage. Consequently, legislation may prohibit insurers from using genetic markers in their analyses, which limits the commercial development of these biomarkers. From a research standpoint, this is less of an issue because genetic markers are a starting point instead of an endpoint. Researchers may dive deeper into the mechanism, or use the genetic biomarker to identify a subset of people who would benefit from an intervention. For example, small non-coding RNAs like microRNAs may be used to predict neurodegenerative disease (Sheinerman and Umansky, 2013; Roy et al., 2022), while long non-coding RNAs may be used to assess autophagic function (Chowdhury et al., 2022).

Some limitations of genetic markers can be overcome by epigenetic markers because epigenetic markers take into account environmental factors that may be interpreted as lifestyle. DNA methylation patterns (Moore et al., 2013), histone modifications (Bannister and Kouzarides, 2011), and telomere length (Blackburn et al., 2015) are three common epigenetic markers. These epigenetic markers, especially methylated DNA patterns, serve as the foundation for biological clocks—an attempt to quantify the extent of the aging process independently of time alive (**Fig 3**; see **Aging clocks**). Consequently, epigenetic markers also have a strong ability to predict age. However, similar logistic issues exist compared to genetic markers. Sampling remains more invasive, and cost is higher than desired. While the legal and ethical risks may be reduced, they are not completely eliminated.

Aging clocks

Genetic, epigenetic, and environmental factors affect both a person's susceptibility to disease and lifespan, which limits the use of chronological age (time alive since birth) alone as a biomarker of aging. Chronological age fails to reflect a person's risk of morbidity and mortality on longer timeframes. This occurs because humans age at different rates, with individual biomarker profiles often markedly deviating from populational trends (Ahadi et al., 2020). Therefore, there is a need to estimate a "biological age" (**Fig 3**). Biological age is an attempt to normalize the vagaries of human aging, and enable comparison of how much life a person may have left to live.

The primary tools for estimating biological age are **"aging clocks"**. Aging clocks employ various tools and approaches, depending on clinical endpoints and model data (Jylhävä et al., 2017). Aging clocks use data ranging from genetic and molecular level information to organism level information, though epigenetic DNA methylations were the first set of data used (Hannum et al., 2013; Horvath, 2013). The covalent attachment of a methyl group to the C5 a cytosine base at a CpG dinucleotide site to form 5-methylcytosine (5mC) modulates gene transcription in a tissue- and cell type-specific manner (Moore et al., 2013). The degree of DNA methylation at certain sites can be assessed in blood cells and tissues using microarrays, pyrosequencing, and whole-genome bisulfite sequencing (Ferrucci et al., 2020). DNA methylation patterns at CpG dinucleotide sites throughout the genome correlate with chronological age, so the first aging clocks were constructed by applying machine learning approaches to DNA methylation array outputs (Hannum et al., 2013; Horvath, 2013). The human genome contains many potential CpG methylation sites that can be used to generate non-overlapping epigenetic aging clocks (Porter et al., 2021). Depending on which CpG sites are used, distinct clocks that could reflect different aspects of the biology of aging can be generated (Porter et al., 2021). However, methylation levels at most CpG sites change minimally with age. For example, the average change of CpG methylation between the ages <35 and >55 years is 3.2% (Horvath and Raj, 2018). There is no consensus on what physiological differences these methylation changes signify (Bell et al., 2019). However, these aging clocks provide insights into the biology of aging, highlighting both tissue-/cell type-specific and universal aspects of associated temporal deterioration.

The earliest **epigenetic clocks** relied on modification levels of a few cytosines (Bocklandt et al., 2011; Garagnani et al., 2012). The methylation clock research gained traction after the introduction of elastic net procedure, which used more cytosines to increase predictive accuracy. The first two epigenetic aging clocks to utilize the elastic net were the **Hannum clock** and the **Horvath clock**. The Hannum clock measures 71 CpG sites from blood, and achieves a correlation of 0.96 between chronological age and predicted age with a 3.9-year mean error (Hannum et al., 2013). The Horvath clock used 353 methylation sites from multiple tissues, and had a similar correlation (0.96) to biological age, and error (3.6 years) (Horvath, 2013). Over the next 10 years, epigenetic aging clocks and their iterations based on cross-sectional data correlate with multimorbidity and mortality in diverse populations (Marioni et al., 2015; Chen et al., 2016; Zhang et al., 2017), and reflect the influence of environmental factors (Quach et al., 2017). Large longitudinal studies confirmed the utility of epigenetic marks in quantifying personal aging trajectories and risk prediction (Li et al., 2020; Belsky et al., 2022). Comparing results from these clocks to chronological age provides a measure of "accelerated aging", where a large difference between a person's biological and chronological ages is linked to higher risk and a faster onset of age-related morbidity.

Second generation epigenetic clocks improved on first generation clocks by incorporating physiological data into training sets. These clocks produced solutions tailored for

morbidity/mortality risk estimation at the expense of chronological age prediction accuracy (**Fig 4**). For example, the aging clock **PhenoAge** uses methylation surrogates (epigenetic representations) of nine blood biomarkers plus chronological age to predict 10- and 20-year mortality, along with the aging outcomes of cancer, healthspan, physical function, and Alzheimer's disease (Levine et al., 2018). These predictions were superior to the Hannum and Horvath clocks (Levine et al., 2018). A similar second generation aging clock, **GrimAge**, measures methylation surrogates of smoking pack-years, seven plasma proteins, chronological age, and sex; GrimAge outperforms first generation aging clocks in measuring time to death (Lu et al., 2019). Similarly, **DunedinPACE** aims to measure the rate of aging, and performs similarly to GrimAge (Belsky et al., 2022). This DNA methylation-based panel builds on data from a longitudinal cohort of individuals all born in 1972-1973, and improves on a panel of blood biomarkers (Belsky et al., 2022). This panel is used in the so-called "Rejuvenation Olympics". Overall, DNA methylation clocks have improved since their initial debut.

One reason for the improvement was moving away from training models to match chronological age. Minimization of predicted chronological age estimation error costs the model biologically relevant information like the ability to predict death (Zhang et al., 2019). More accurate models trained on chronological age data lose predictive power in terms of health or mortality risk (Pyrkov et al., 2018b). Instead, training clocks with phenotypical correlates of age-related decline, like changes in blood biochemistry (Levine, 2013; Putin et al., 2016; Mamoshina et al., 2019), daily activity pattern (Pyrkov et al., 2018a), and body constitution (Fermín-Martínez et al., 2023), yielded clocks that were more accurate predictors of morbidity and mortality. Integrating additional measures helped improve the accuracy of aging clocks.

Recent technological advances have provided more accurate information about gene function for assembling aging clocks. Developments in -omics beyond DNA sequencing and methylation arrays, such as transcriptomics and proteomics, allow the use of gene expression profiles for biological age estimates (Solovev et al., 2020; Rutledge et al., 2022). Since the cellular transcript pool and protein makeup represent the realization of genetic information, they provide a more direct and actionable understanding of pathways and networks involved in aging and pathology (**Fig 2**). For example, 1497 genes change expression levels with age (Peters et al., 2015), while 529 proteins change in abundance with age in human plasma (Johnson et al., 2020). This allows the construction of many different **transcriptional** and **proteomic aging clocks**. One such transcriptional aging clock had an average absolute difference between predicted and chronological age of 7.8 years (Peters et al., 2015). One example proteomic aging clock that measured 76 proteins in the blood proteome had an error of 5.7 years (R = 0.94) (Tanaka et al., 2018). Measuring the top eight proteins on this list sacrificed some accuracy, with a difference of 13.1 years (R = 0.92), while the top protein alone (**GDF15**) had a difference of 16.6 years (R = 0.82) (Tanaka et al., 2018). A second blood proteome model of 373 sex-independent proteins had good accuracy in predicting age in discovery, validation, and four independent test cohorts (R =

0.93–0.97) (Lehallier et al., 2019). The explosion of information enables the construction of more complex aging clocks.

Finally, measures of metabolites and microbes are also in development to measure aging. The identification and quantification of fluctuating metabolites as a function of biological age using mass spectrometry or NMR also measure age-associated risk (Panyard et al., 2022; Robinson and Lau, 2023). Attempts to measure aging via changes in the microbiome use "**metagenomic aging clocks**". These clocks rely on the changes in intestinal microflora composition with age, health, diet, and exercise (Yatsunenko et al., 2012; Conlon and Bird, 2014). Distinct adult and elderly microbiome taxonomic profiles exist, with transitions around both 20 and 70 years old (Odamaki et al., 2016). A metagenomic aging clock built using a deep neural network trained on >4000 metagenomic profiles from healthy individuals aged 18-90 had a MAE of 5.91 years (Galkin et al., 2020b). Curiously, the metagenomic clock assigned a greater estimated age to individuals with type 1 diabetes, suggesting an association between microflora and metabolism (Galkin et al., 2020b). More work is needed to develop metabolomic and metagenomic aging clocks.

Despite the success of aging clocks, they inherit shortcomings from their training sets. One challenge is that the publicly available datasets used come in a variety of non-standardized formats, contain missing values, and often have no information on comorbidities. For example, a study on Alzheimer's disease may include a mix of people with a range of comorbidities like diabetes, cancer, cardiovascular disease (Galkin et al., 2020a). Consequently, there is a need to use a multimodal approach when defining biological age. A comprehensive framework, including state-of-the-art -omics technologies, well-established and assessable clinical indicators, and physical and cognitive performance indices (Jansen et al., 2021; Sun et al., 2021; Mavromatis et al., 2023; McGreevy et al., 2023) are all needed. Advances in artificial intelligence (AI)-driven tools, including deep neural networks and convolutional neural networks, grasp non-linear relationships between biological features and accelerate both novel biomarker discovery and the proof-of-concept for newer aging clocks (Zhavoronkov et al., 2019; Galkin et al., 2021). One exciting application of AI to longevity research is the use of **generative adversarial networks** (GAN) for missing value imputation (a common problem with clinical datasets), and training set expansion (Galkin et al., 2020a). Once a patient's biomarker blueprint is made, a GAN can be used to predict future changes in the patient's biomarker profile—move it back and forth in time—and suggest custom geroprotective interventions (Zhavoronkov and Mamoshina, 2019). Overall, aging clocks improve our ability to estimate mortality and morbidity.

Comorbidities

One limitation to longevity is the presence of other diseases that truncate lifespan. Many longevity markers discussed so far measure the development of such diseases. For example, glucose measurements are used to diagnose diabetes; cholesterol and triglyceride levels are markers for cardiovascular disease; urinary creatinine and albumin measure kidney function (see

Laboratory biomarkers). CRP and IL-6 measure acute phase responses to disease, and upregulate clotting factors; consequently, elevated IL-6 levels are associated with thrombosis and stroke (Ding et al., 2023). Hypertension, CRF are also used as longevity readouts (see **Physiological biomarkers**), whereas hearing acuity can signify cognitive decline (see **Neurological biomarkers**). Thus, longevity biomarkers overlap with the largest chronic diseases in the elderly worldwide (ischaemic heart disease, diabetes, stroke, chronic kidney disease, lung cancer, and age-related hearing loss) (GBD 2019 Diseases and Injuries Collaborators, 2020).

Since chronic diseases are tied into our measures of longevity, it becomes challenging to consider interventions expected to increase lifespan in individuals without the disease vs. reducing the proportion of these comorbidities in the population. On one hand, reducing chronic disease is relevant to longevity research, because it will directly impact longevity in affected individuals. On the other hand, biomarkers that measure chronic diseases may be of limited use for individuals without chronic disease. Thus, one challenge is determining the populations in which a given biomarker is most useful.

Two other challenges to measuring longevity are medical emergencies and acute infectious diseases. Minimizing both of these threats will improve lifespan because both increase with age. For example, falls are a leading cause of injury-related death in the elderly (CDC, 2024). Infectious diseases, such as urinary tract infections, lower respiratory tract infections, skin and soft tissue infections, intra-abdominal infections (cholecystitis, diverticulitis, appendicitis, abscesses), infective endocarditis, bacterial meningitis, tuberculosis, and herpes zoster, are prevalent in the elderly and three times as deadly as in young adult patients (Yoshikawa, 2000). On one hand, this reveals a need to track frailty (i.e. susceptibility to physical trauma), and immunity (i.e. susceptibility to infectious disease) with age. On the other hand, behavioral interventions (e.g. avoid stairs, precautions for disease), may reduce vulnerability and extend lifespan. Thus, tracking frailty and immunity may predict longevity in the population, but require adjustment at the individual level for utility.

One common factor in comorbidities that limit longevity is chronic inflammation (Furman et al., 2019). Chronic inflammation is the inability to resolve an inflammatory state; in contrast to acute inflammation, there is continual immune stimulation. In some cases, chronic inflammation occurs due to a failure to deal with pathogens. In other cases, chronic inflammation is due to the production of pro-inflammatory signals in the absence of pathogens, called **“sterile inflammation”** (Chen and Nunez, 2010). Examples of sterile inflammation include inflammation stimulated by uric acid (gout) (Martinon et al., 2006), and responses to proteins and nutrients (e.g. LDL) that get oxidized over time (atherosclerosis) (Libby et al., 2019). The association of chronic inflammation with limiting longevity is strong enough that a separate term was coined to better discuss the connections between aging phenotypes and inflammation: **“inflamm-aging”** (Franceschi et al., 2018). Typically inflamm-aging focuses on low-grade, sterile inflammation, but infectious origins are challenging to rule out.

Inflamm-aging has the potential to account for most deleterious aging phenotypes: pathway enrichment analysis showed that proteins implicated in inflammation and immune function are especially adept at predicting age (Lehallier et al., 2020). Additionally, inflamm-aging is linked to age-related sarcopenia (Liang et al., 2022), Alzheimer's disease (Xia et al., 2016), gut microbiota (Franceschi et al., 2018), metabolic state (López-Otín et al., 2016; Franceschi et al., 2018), and disease resistance (Chambers and Akbar, 2020). All of these conditions associate with longevity. This creates a complex network of feedback loops that are challenging to sort out.

Therapeutic blocking of inflammation to extend longevity may require nuance. Notably, acute inflammation is necessary for fighting pathogens and limiting damage from them. As an extreme example, anti-inflammatory interventions during sepsis are more efficacious in patients with a higher risk of death, whereas in cases where subjects are likely to survive, disrupting well-regulated inflammatory pathways may weaken protective host defense mechanisms and lead to a worse outcome (Eichacker et al., 2002). This was tragically learned when the blockade of **tumor necrosis factor alpha** (TNF-α) caused increased mortality (Eichacker et al., 2002). Another challenge is that certain areas of the body require inflammation for proper function. For instance, low-grade steady-state inflammation has been shown to be essential for the co-existence of the host with the gut commensal microbiota (Varol et al., 2015; Schnell et al., 2023). Postnatal cyclic mammary gland remodeling, i.e. branching morphogenesis, also relies on macrophage recruitment and local homeostatic inflammation (Van Nguyen and Pollard, 2002; Varol et al., 2015).

Over the years, the focus on targeting inflammation has shifted from blocking it to promoting successful resolution (Buckley et al., 2013). Resolution of inflammation restores tissue homeostasis and is associated with pathways and interventions that may enhance longevity (Serhan and Levy, 2018). Thus, measuring both pro-inflammatory and pro-resolving markers may help us understand the balance between inflammation and health. Altogether, accounting for comorbidities remains one challenge when measuring and enhancing longevity.

Biomarker panels

With all of these different indicators of health and mortality, combining them may give a more holistic picture of an individual's overall health. By combining multiple markers, panels can provide a more nuanced picture of an individual's health status and better report changes over time (Shamir, 2015). Finally, using multiple markers can reduce the variability and noise inherent in measurements of individual biomarkers. However, there are also challenges to using biomarker panels. One challenge is that the interpretation of biomarker panels can be complex and may require specialized expertise, especially if different markers contradict each other. Also, not all markers included in a panel may be relevant to all individuals, leading to unnecessary testing and increased costs. Overall, biomarker panels are a valuable tool to evaluate health and disease.

Choosing the optimal biomarker panel remains a complicated question. Several groups of biomarkers that reflect a) biological age, b) rate of aging, and c) risk of various age-related

diseases may not give the same results. Logistic factors unrelated to aging also impact the choice of biomarkers. For example, biomarkers that have long been measured and are widely available (such as with a general blood test), have more years of longitudinal data, and are more affordable. Databases such as **UK Biobank** and **NHANES** provide large datasets suitable for robust analysis of biomarker behavior. However, if the biomarker is new, there may be limited data in human populations on that biomarker. Therefore, updates to new biomarkers may take years to validate. This complicates the development of consistent and wide use of biomarker panels.

The proliferation of wearable devices that measure biometric data like heart rate, sleep pattern, and exercise provides large amounts of longitudinal data. This may provide a simple set of markers to predict and measure longevity, i.e. **"digital biomarkers"** (Vasudevan et al., 2022). Parameters such as daily steps, heart rate variability, resting heart rate, exercise, blood oxygen saturation, and VO_2max are collected throughout the day for millions of users. Many apps now include reminders, badges, and other tools to encourage people to achieve certain levels of activity. One current opportunity is developing AI algorithms to produce meaningful assessments using data from wearables (Acosta et al., 2022). The sheer amount of longitudinal, non-invasive data that can be generated provides an affordable and convenient approach for health-centered decision-making on longevity.

Along with physiological parameters that give overall estimates, some biomarker panels focus on assessing risk for key causes of mortality. Since one global leading cause of disability and death is cardiovascular disease (GBD 2019 Diseases and Injuries Collaborators, 2020; Tsao et al., 2023), biomarker panels that assess cardiovascular disease may also predict longevity. Composite scores based on blood biomarkers include the **Framingham Risk Score** (Wilson et al., 1998), and **QRISK3** (Hippisley-Cox et al., 2017), which combine age, systolic blood pressure, smoking status, **total cholesterol**, and **HDL cholesterol** measurements (**Fig 4**). The Framingham Risk Score is predictive for longevity potential (with AUC = 64.7) (Beekman et al., 2016). When this score is combined with physiological parameters involved in immune responses, glucose, lipid, and energy metabolism, the prediction performance for longevity potential is further improved (up to AUC = 71.4) (Beekman et al., 2016). Thus, cardiovascular biomarker panels give a reasonable start to using biomarker panels to estimate longevity.

A modified approach to biomarker panels intended for both lay people and physicians is the **Life's Simple 7** panel, created by the **American Heart Association** (AHA). These guidelines focus on determining optimal cardiovascular health using seven different biomarkers and habits (Lloyd-Jones et al., 2010). The seven metrics are diet quality, amount of weekly physical activity, exposure to cigarette smoking, body mass index, fasting blood glucose, total cholesterol, and blood pressure (Lloyd-Jones et al., 2010). Each category is given descriptors (ideal, intermediate, or poor) for each metric based on accepted clinical thresholds. In a meta-analysis of 9 prospective cohort studies, having the highest number of ideal cardiovascular health metrics (generally ≥5 versus 0-2) was associated with a risk reduction of 0.20 for CVD (95% CI, 0.11-0.37), 0.31 for

stroke (95% CI, 0.25-0.38), 0.25 for CVD mortality (95% CI, 0.10-0.63), and 0.55 for all-cause mortality (95% CI, 0.37-0.80) (Fang et al., 2016). Life's Simple 7 predicts cardiovascular disease (CVD)-free survival, associates with overall longevity and higher quality of life (Lloyd-Jones et al., 2010). In 2022, this model was updated. These updates added sleep duration as an eighth component, replaced total cholesterol with total non-HDL cholesterol, and revamped the scoring system to a more intuitive range of 1-100. The new aggregate score, now called **Life's Essential 8** (LE8), calculates the unweighted average of all 8 component metric scores (Lloyd-Jones et al., 2022). In the new system, average cardiovascular scores are considered high (80-100), moderate (50-79), or low (0-49). Higher LE8 scores associate with both a lower risk of cardiovascular death and all-cause death (Isiozor et al., 2023; Sun et al., 2023). This framework provides a straightforward biomarker panel that can be used to improve overall health and early lifestyle interventions. Importantly, this biomarker panel is web accessible via the AHA page, and allows multiple measurements.

Along with LE8, other indices and scores may be used to predict and assess specific aspects of aging and risk of death **(Table 1)**. For example, the degree of frailty can be measured by **Fried's frailty index** (Fried et al., 2001), **Charlson comorbidity index** (Charlson et al., 1987), and **Elixhauser comorbidity index** (Elixhauser et al., 1998). Fried's index measures frailty using weight loss, exhaustion, low physical activity, slowness, and weakness to assign people to three categories: not frail, pre-frail, and frail (Fried et al., 2001). The Charlson comorbidity index assigns scores based on 17 potential comorbidities plus age (Charlson et al., 1987), while the Elixhauser comorbidity index measures 30 (Elixhauser et al., 1998). While the Charlson index is easier to calculate, the Elixhauser index is more comprehensive and has greater statistical power (Sharma et al., 2021). While these indices are useful for the general population, they may not be helpful in predicting lifespan for individuals who are not frail and have few to no comorbidities. New panels are needed to measure these features.

Current approaches to biomarker panels need to consider many potential trade-offs. Ideally, such composite scores should be straightforward, easy and cheap to measure, require minimal collection effort, and display minimal operator variability. The age range and overall health status in which they operate are also important considerations. The goal for true longevity biomarkers (vs. markers of comorbidities) is to provide feedback into the aging process early enough to allow intervention, and to provide information on the success of those markers. Complementing bloodwork panels and epigenetic clocks with advances in artificial intelligence, machine learning, big data, statistics, and availability of health datasets will allow new types of "aging clocks" to be developed.

Understanding the myriad potential biomarkers, and their relative value remains an ongoing challenge. The longevity field gained momentum to specify key definitions and formalize the framework for the longevity-tailored biomarker identification and validation as surrogate endpoints in the clinical setting with the advent of the international panel of the **Biomarkers of Aging**

Consortium (Moqri et al., 2023, 2024). Similarly, a different organization, the **Aging Biomarkers Consortium** (Ren et al., 2023), expressed its position on the biomarker landscape in a series of comprehensive reviews: on general biomarkers of aging (Aging Biomarker Consortium et al., 2023a), on specific biomarkers of cardiac aging (Aging Biomarker Consortium et al., 2023e), vascular aging (Aging Biomarker Consortium et al., 2023d), brain aging (Aging Biomarker Consortium et al., 2023b), skeletal aging (Aging Biomarker Consortium et al., 2023c). Thus, concerted worldwide efforts to formulate a roadmap to defining and implementing the most promising biomarkers for translational geriatric research have been initiated. In the wake of the "-omics" era, guidelines for multiomic integration of time-resolved molecular-genetic readouts, processed with the help of AI, have been published (Solovev et al., 2020; Rutledge et al., 2022), underscoring the importance of the data-first approach to biomarker mining and validation.

The computational toolbox at the field's disposal has been augmented with several critical user-friendly solutions for deploying aging clocks in a standardized fashion, allowing the comparison of outputs across research formats and objectives: the **pyaging** (de Lima Camillo, 2024) and **biolearn** (Ying et al., 2024b) Python packages. Further, for no-code analysis of biomarker dynamics in large-cohort longitudinal studies, platforms such as **ClockBase** (Ying et al., 2023) (clockbase.org) and **HALL** (Li et al., 2024) (ngdc.cncb.ac.cn/hall/index), utilizing multidimensional data from publicly available repositories, such as **GEO** and NHANES, now exist.

Conclusion

There remains an outstanding need to identify robust biomarkers for longevity research. Here, we surveyed existing biomarkers that are useful, in our opinion, for assessing personal aging trajectories and predicting life- and healthspan. A generalizable foundation for biomarker evaluation yet to be created will aid researchers and the public to better consider what biomarkers they may choose to measure. We consolidated the current longevity biomarker trends, discussing their prospective functionality for predicting the number of disease-free years and time to death, i.e. **prospective biomarkers**, as compared to **response biomarkers**, which are those that are sensitive to longevity interventions and will be useful in the context of clinical trials (Cummings and Kritchevsky, 2022). As a result, we present a broad narrative on popular longevity biomarkers, their application and limitations, enabling informed choice for biomarker measurement.

Acknowledgments

We thank Alexey Strygin, Evgeni Bikov, Gherardo Baudo, and Susanne Neumann for their help in manuscript preparation; members of VitaDAO and ResearchHub for constructive feedback and helpful criticisms. We apologize to those authors whose work we were unable to cite due to space constraints.

Conflicts of interest

The authors are members of VitaDAO and ResearchHub, which commissioned this work.

Author contributions

Conceptualization: BTS, GN, VW, RA

Supervision: BTS, RA

Writing—original draft: BTS, RA

Writing—review & editing: BTS, GN, VW, RA

Figure preparation: RA

References

Acosta, J.N., G.J. Falcone, P. Rajpurkar, and E.J. Topol. 2022. Multimodal biomedical AI. *Nat. Med.* 28:1773–1784. doi:10.1038/s41591-022-01981-2.

Aging Biomarker Consortium, H. Bao, J. Cao, M. Chen, M. Chen, W. Chen, X. Chen, Y. Chen, Y. Chen, Y. Chen, Z. Chen, J.K. Chhetri, Y. Ding, J. Feng, J. Guo, M. Guo, C. He, Y. Jia, H. Jiang, Y. Jing, D. Li, J. Li, J. Li, Q. Liang, R. Liang, F. Liu, X. Liu, Z. Liu, O.J. Luo, J. Lv, J. Ma, K. Mao, J. Nie, X. Qiao, X. Sun, X. Tang, J. Wang, Q. Wang, S. Wang, X. Wang, Y. Wang, Y. Wang, R. Wu, K. Xia, F.-H. Xiao, L. Xu, Y. Xu, H. Yan, L. Yang, R. Yang, Y. Yang, Y. Ying, L. Zhang, W. Zhang, W. Zhang, X. Zhang, Z. Zhang, M. Zhou, R. Zhou, Q. Zhu, Z. Zhu, F. Cao, Z. Cao, P. Chan, C. Chen, G. Chen, H.-Z. Chen, J. Chen, W. Ci, B.-S. Ding, Q. Ding, F. Gao, J.-D.J. Han, K. Huang, Z. Ju, Q.-P. Kong, J. Li, J. Li, X. Li, B. Liu, F. Liu, L. Liu, Q. Liu, Q. Liu, X. Liu, Y. Liu, X. Luo, S. Ma, X. Ma, Z. Mao, J. Nie, Y. Peng, J. Qu, J. Ren, R. Ren, M. Song, Z. Songyang, Y.E. Sun, et al. 2023a. Biomarkers of aging. *Sci. China Life Sci.* 66:893–1066. doi:10.1007/s11427-023-2305-0.

Aging Biomarker Consortium, Y.-J. Jia, J. Wang, J.-R. Ren, P. Chan, S. Chen, X.-C. Chen, J.K. Chhetri, J. Guo, Q. Guo, L. Jin, Q. Liu, Q. Liu, W. Ma, Z. Mao, M. Song, W. Song, Y. Tang, D. Wang, P. Wang, L. Xiong, K. Ye, J. Zhang, W. Zhang, X. Zhang, Y. Zhang, Z. Zhang, Z. Zhang, J. Zheng, G.-H. Liu, Y. Eve Sun, Y.-J. Wang, and G. Pei. 2023b. A framework of biomarkers for brain aging: a consensus statement by the Aging Biomarker Consortium. *Life Med.* 2:lnad017. doi:10.1093/lifemedi/lnad017.

Aging Biomarker Consortium, J. Suo, Y. Gan, Y. Xie, S. Xu, J. Wang, D. Chen, L. Chen, L. Deng, S. Feng, J.J. Han, Q. Jiang, G. Lei, P. Liu, X. Luo, X. Ma, J. Qu, C. Song, P. Tang, T. Tang, S. Wang, X. Wei, C. Wu, G. Xiao, L. Yang, L. Zhang, W. Zhang, Z. Zhang, G.-H. Liu, C. Zhang, G. Pei, J. Luo, R. Yue, and W. Zou. 2023c. A framework of biomarkers for skeletal aging: a consensus statement by the Aging Biomarker Consortium. *Life Med.* 2:lnad045. doi:10.1093/lifemedi/lnad045.

Aging Biomarker Consortium, L. Zhang, J. Guo, Y. Liu, S. Sun, B. Liu, Q. Yang, J. Tao, X.-L. Tian, J. Pu, H. Hong, M. Wang, H.-Z. Chen, J. Ren, X. Wang, Z. Liang, Y. Wang, K. Huang, W. Zhang, J. Qu, Z. Ju, G.-H. Liu, G. Pei, J. Li, and C. Zhang. 2023d. A framework of biomarkers for vascular aging: a consensus statement by the Aging Biomarker Consortium. *Life Med.* 2:lnad033. doi:10.1093/lifemedi/lnad033.

Aging Biomarker Consortium, W. Zhang, Y. Che, X. Tang, S. Chen, M. Song, L. Wang, A.-J. Sun, H.-Z. Chen, M. Xu, M. Wang, J. Pu, Z. Li, J. Xiao, C.-M. Cao, Y. Zhang, Y. Lu, Y. Zhao, Y.-J. Wang, C. Zhang, T. Shen, W. Zhang, L. Tao, J. Qu, Y.-D. Tang, G.-H. Liu, G. Pei, J. Li, and

F. Cao. 2023e. A biomarker framework for cardiac aging: the Aging Biomarker Consortium consensus statement. *Life Med.* lnad035. doi:10.1093/lifemedi/lnad035.

Ahadi, S., W. Zhou, S.M. Schüssler-Fiorenza Rose, M.R. Sailani, K. Contrepois, M. Avina, M. Ashland, A. Brunet, and M. Snyder. 2020. Personal aging markers and ageotypes revealed by deep longitudinal profiling. *Nat. Med.* 26:83–90. doi:10.1038/s41591-019-0719-5.

Aleksic, S., D. Desai, K. Ye, S. Duran, T. Gao, J. Crandall, G. Atzmon, N. Barzilai, and S. Milman. 2022. Integrity of hypothalamic-pituitary-testicular axis in exceptional longevity. *Aging Cell*. 21:e13656. doi:10.1111/acel.13656.

Antoniou, A., P.D.P. Pharoah, S. Narod, H.A. Risch, J.E. Eyfjord, J.L. Hopper, N. Loman, H. Olsson, O. Johannsson, A. Borg, B. Pasini, P. Radice, S. Manoukian, D.M. Eccles, N. Tang, E. Olah, H. Anton-Culver, E. Warner, J. Lubinski, J. Gronwald, B. Gorski, H. Tulinius, S. Thorlacius, H. Eerola, H. Nevanlinna, K. Syrjäkoski, O.-P. Kallioniemi, D. Thompson, C. Evans, J. Peto, F. Lalloo, D.G. Evans, and D.F. Easton. 2003. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am. J. Hum. Genet.* 72:1117–1130. doi:10.1086/375033.

Arbab-Zadeh, A., E. Dijk, A. Prasad, Q. Fu, P. Torres, R. Zhang, J.D. Thomas, D. Palmer, and B.D. Levine. 2004. Effect of aging and physical activity on left ventricular compliance. *Circulation*. 110:1799–1805. doi:10.1161/01.cir.0000142863.71285.74.

Arem, H., S.C. Moore, A. Patel, P. Hartge, A. Berrington de Gonzalez, K. Visvanathan, P.T. Campbell, M. Freedman, E. Weiderpass, H.O. Adami, M.S. Linet, I.-M. Lee, and C.E. Matthews. 2015. Leisure time physical activity and mortality: a detailed pooled analysis of the dose-response relationship. *JAMA Intern. Med.* 175:959–967. doi:10.1001/jamainternmed.2015.0533.

Arnlov, J., J.C. Evans, J.B. Meigs, T.J. Wang, C.S. Fox, D. Levy, E.J. Benjamin, R.B. D'Agostino, and R.S. Vasan. 2005. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals - The Framingham heart study. *Circulation*. 112:969–975. doi:10.1161/circulationaha.105.538132.

Ashley, E.A., R.E. Hershberger, C. Caleshu, P.T. Ellinor, J.G.N. Garcia, D.M. Herrington, C.Y. Ho, J.A. Johnson, S.J. Kittner, C.A. Macrae, G. Mudd-Martin, D.J. Rader, D.M. Roden, D. Scholes, F.W. Sellke, J.A. Towbin, J. Van Eyk, B.B. Worrall, and American Heart Association Advocacy Coordinating Committee. 2012. Genetics and cardiovascular disease: a policy statement from the American Heart Association. *Circulation*. 126:142–157. doi:10.1161/CIR.0b013e31825b07f8.

Atzmon, G., T.I. Pollin, J. Crandall, K. Tanner, C.B. Schechter, P.E. Scherer, M. Rincon, G. Siegel, M. Katz, R.B. Lipton, A.R. Shuldiner, and N. Barzilai. 2008. Adiponectin levels and genotype: a potential regulator of life span in humans. *J. Gerontol. A. Biol. Sci. Med. Sci.* 63:447–453. doi:10.1093/gerona/63.5.447.

Bafei, S.E.C., and C. Shen. 2023. Biomarkers selection and mathematical modeling in biological age estimation. *Npj Aging*. 9:13. doi:10.1038/s41514-023-00110-8.

Baker, G.T., and R.L. Sprott. 1988. Biomarkers of aging. *Exp. Gerontol.* 23:223–239. doi:10.1016/0531-5565(88)90025-3.

Baltes, P.B., and U. Lindenberger. 1997. Emergence of a powerful connection between sensory and cognitive functions across the adult life span: a new window to the study of cognitive aging? *Psychol. Aging*. doi:10.1037/0882-7974.12.1.12.

Bannister, A.J., and T. Kouzarides. 2011. Regulation of chromatin by histone modifications. *Cell*

Res. 21:381–395. doi:10.1038/cr.2011.22.

Barzilai, N., G. Atzmon, C. Schechter, E.J. Schaefer, A.L. Cupples, R. Lipton, S. Cheng, and A.R. Shuldiner. 2003. Unique lipoprotein phenotype and genotype associated with exceptional longevity. *JAMA*. 290:2030–2040. doi:10.1001/jama.290.15.2030.

Beekman, M., H.-W. Uh, D. van Heemst, M. Wuhrer, L.R. Ruhaak, V. Gonzalez-Covarrubias, T. Hankemeier, J.J. Houwing-Duistermaat, and P.E. Slagboom. 2016. Classification for longevity potential: the use of novel biomarkers. *Front. Public Health*. 4:233. doi:10.3389/fpubh.2016.00233.

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

Belsky, D.W., A. Caspi, D.L. Corcoran, K. Sugden, R. Poulton, L. Arseneault, A. Baccarelli, K. Chamarti, X. Gao, E. Hannon, H.L. Harrington, R. Houts, M. Kothari, D. Kwon, J. Mill, J. Schwartz, P. Vokonas, C. Wang, B.S. Williams, and T.E. Moffitt. 2022. DunedinPACE, a DNA methylation biomarker of the pace of aging. *eLife*. 11:e73420. doi:10.7554/eLife.73420.

Belsky, D.W., T.E. Moffitt, A.A. Cohen, D.L. Corcoran, M.E. Levine, J.A. Prinz, J. Schaefer, K. Sugden, B. Williams, R. Poulton, and A. Caspi. 2018. Eleven telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: do they measure the same thing? *Am. J. Epidemiol.* 187:1220–1230. doi:10.1093/aje/kwx346.

Bennett, C.M., M. Guo, and S.C. Dharmage. 2007. HbA(1c) as a screening tool for detection of type 2 diabetes: a systematic review. *Diabet. Med. J. Br. Diabet. Assoc.* 24:333–343. doi:10.1111/j.1464-5491.2007.02106.x.

Blackburn, E.H., E.S. Epel, and J. Lin. 2015. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. *Science*. 350:1193–1198. doi:10.1126/science.aab3389.

Blinkouskaya, Y., A. Caçoilo, T. Gollamudi, S. Jalalian, and J. Weickenmeier. 2021. Brain aging mechanisms with mechanical manifestations. *Mech. Ageing Dev.* 200:111575. doi:10.1016/j.mad.2021.111575.

Bobrov, E., A. Georgievskaya, K. Kiselev, A. Sevastopolsky, A. Zhavoronkov, S. Gurov, K. Rudakov, M. Del Pilar Bonilla Tobar, S. Jaspers, and S. Clemann. 2018. PhotoAgeClock: deep learning algorithms for development of non-invasive visual biomarkers of aging. *Aging*. 10:3249–3259. doi:10.18632/aging.101629.

Bocklandt, S., W. Lin, M.E. Sehl, F.J. Sánchez, J.S. Sinsheimer, S. Horvath, and E. Vilain. 2011. Epigenetic predictor of age. *PLoS One*. 6:e14821. doi:10.1371/journal.pone.0014821.

Bohannon, R.W. 2019. Grip strength: an indispensable biomarker for older adults. *Clin. Interv. Aging*. 14:1681–1691. doi:10.2147/CIA.S194543.

Booth, F.W., C.K. Roberts, and M.J. Laye. 2012. Lack of exercise is a major cause of chronic diseases. *Compr. Physiol.* 2:1143–1211. doi:10.1002/cphy.c110025.

Borch-Johnsen, K., A. Neil, B. Balkau, S. Larsen, A. Nissinen, J. Pekkanen, J. Tuomilehto, P. Jousilahti, J. Lindstrom, M. Pyorala, K. Pyorala, E. Eschwege, G. Gallus, M.P. Garancini, L.M. Bouter, J.M. Dekker, R.J. Heine, H.G. Nijpels, C.D.A. Stehouwer, E.J.M. Feskens, D. Kromhout, M. Peltonen, A. Pajak, J. Eriksson, and Q. Qiao. 2001. Glucose tolerance and

cardiovascular mortality - comparison of fasting and 2-hour diagnostic criteria. *Arch. Intern. Med.* 161:397–405. doi:10.1001/archinte.161.3.397.

Border, R., S. O'Rourke, T. de Candia, M.E. Goddard, P.M. Visscher, L. Yengo, M. Jones, and M.C. Keller. 2022. Assortative mating biases marker-based heritability estimators. *Nat. Commun.* 13:660. doi:10.1038/s41467-022-28294-9.

Bos, M.J., P.J. Koudstaal, A. Hofman, J.C.M. Witteman, and M.M.B. Breteler. 2006. Uric acid is a risk factor for myocardial infarction and stroke - The Rotterdam Study. *Stroke*. 37:1503–1507. doi:10.1161/01.str.0000221716.55088.d4.

Brenowitz, W.D., A.R. Kaup, and K. Yaffe. 2020. Incident dementia and faster rates of cognitive decline are associated with worse multisensory function summary scores. *Alzheimers Dement. J. Alzheimers Assoc.* 16:1384–1392. doi:10.1002/alz.12134.

Buckley, C.D., D.W. Gilroy, C.N. Serhan, B. Stockinger, and P.P. Tak. 2013. The resolution of inflammation. *Nat. Rev. Immunol.* 13:59–66. doi:10.1038/nri3362.

Buford, T.W. 2016. Hypertension and aging. *Ageing Res. Rev.* 26:96–111. doi:10.1016/j.arr.2016.01.007.

Butler, R.N., R. Sprott, H. Warner, J. Bland, R. Feuers, M. Forster, H. Fillit, S.M. Harman, M. Hewitt, M. Hyman, K. Johnson, E. Kligman, G. McClearn, J. Nelson, A. Richardson, W. Sonntag, R. Weindruch, and N. Wolf. 2004. Biomarkers of aging: from primitive organisms to humans. *J. Gerontol. A. Biol. Sci. Med. Sci.* 59:B560-567. doi:10.1093/gerona/59.6.b560.

Califf, R.M. 2018. Biomarker definitions and their applications. *Exp. Biol. Med. Maywood NJ*. 243:213–221. doi:10.1177/1535370217750088.

Cataudella, E., C.M. Giraffa, S. Di Marca, A. Pulvirenti, S. Alaimo, M. Pisano, V. Terranova, T. Corriere, M.L. Ronsisvalle, R. Di Quattro, B. Stancanelli, M. Giordano, C. Vancheri, and L. Malatino. 2017. Neutrophil-to-lymphocyte ratio: an emerging marker predicting prognosis in elderly adults with community-acquired pneumonia. *J. Am. Geriatr. Soc.* 65:1796–1801. doi:10.1111/jgs.14894.

CDC. 2024. Older adult falls data. *Older Adult Fall Prev.*

Chambers, E.S., and A.N. Akbar. 2020. Can blocking inflammation enhance immunity during aging? *J. Allergy Clin. Immunol.* 145:1323–1331. doi:10.1016/j.jaci.2020.03.016.

Charlson, M.E., P. Pompei, K.L. Ales, and C.R. MacKenzie. 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J. Chronic Dis.* 40:373–383. doi:10.1016/0021-9681(87)90171-8.

Chen, B.H., R.E. Marioni, E. Colicino, M.J. Peters, C.K. Ward-Caviness, P.-C. Tsai, N.S. Roetker, A.C. Just, E.W. Demerath, W. Guan, J. Bressler, M. Fornage, S. Studenski, A.R. Vandiver, A.Z. Moore, T. Tanaka, D.P. Kiel, L. Liang, P. Vokonas, J. Schwartz, K.L. Lunetta, J.M. Murabito, S. Bandinelli, D.G. Hernandez, D. Melzer, M. Nalls, L.C. Pilling, T.R. Price, A.B. Singleton, C. Gieger, R. Holle, A. Kretschmer, F. Kronenberg, S. Kunze, J. Linseisen, C. Meisinger, W. Rathmann, M. Waldenberger, P.M. Visscher, S. Shah, N.R. Wray, A.F. McRae, O.H. Franco, A. Hofman, A.G. Uitterlinden, D. Absher, T. Assimes, M.E. Levine, A.T. Lu, P.S. Tsao, L. Hou, J.E. Manson, C.L. Carty, A.Z. LaCroix, A.P. Reiner, T.D. Spector, A.P. Feinberg, D. Levy, A. Baccarelli, J. van Meurs, J.T. Bell, A. Peters, I.J. Deary, J.S. Pankow, L. Ferrucci, and S. Horvath. 2016. DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging*. 8:1844–1865. doi:10.18632/aging.101020.

Chen, G.Y., and G. Nunez. 2010. Sterile inflammation: sensing and reacting to damage. *Nat. Rev.*

Immunol. 10:826–837. doi:10.1038/nri2873.

Chen, J.-H., S.-Y. Chuang, H.-J. Chen, W.-T. Yeh, and W.-H. Pan. 2009. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. *Arthritis Rheum.-Arthritis Care Res.* 61:225–232. doi:10.1002/art.24164.

Chen, L.-K., J. Woo, P. Assantachai, T.-W. Auyeung, M.-Y. Chou, K. Iijima, H.C. Jang, L. Kang, M. Kim, S. Kim, T. Kojima, M. Kuzuya, J.S.W. Lee, S.Y. Lee, W.-J. Lee, Y. Lee, C.-K. Liang, J.-Y. Lim, W.S. Lim, L.-N. Peng, K. Sugimoto, T. Tanaka, C.W. Won, M. Yamada, T. Zhang, M. Akishita, and H. Arai. 2020. Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J. Am. Med. Dir. Assoc.* 21:300-307.e2. doi:10.1016/j.jamda.2019.12.012.

Chia, C.W., J.M. Egan, and L. Ferrucci. 2018. Age-related changes in glucose metabolism, hyperglycemia, and cardiovascular risk. *Circ. Res.* 123:886–904. doi:10.1161/CIRCRESAHA.118.312806.

Chojdak-Łukasiewicz, J., E. Dziadkowiak, A. Zimny, and B. Paradowski. 2021. Cerebral small vessel disease: a review. *Adv. Clin. Exp. Med. Off. Organ Wroclaw Med. Univ.* 30:349–356. doi:10.17219/acem/131216.

Chowdhury, S.G., D. Bhattacharya, and P. Karmakar. 2022. Exosomal long noncoding RNAs - the lead thespian behind the regulation, cause and cure of autophagy-related diseases. *Mol. Biol. Rep.* 49:7013–7024. doi:10.1007/s11033-022-07514-x.

Church, S., E. Rogers, K. Rockwood, and O. Theou. 2020. A scoping review of the Clinical Frailty Scale. *BMC Geriatr.* 20:393. doi:10.1186/s12877-020-01801-7.

Cole, C.R., E.H. Blackstone, F.J. Pashkow, C.E. Snader, and M.S. Lauer. 1999. Heart-rate recovery immediately after exercise as a predictor of mortality. *N. Engl. J. Med.* 341:1351–1357. doi:10.1056/nejm199910283411804.

Cole, J.H., R.P.K. Poudel, D. Tsagkrasoulis, M.W.A. Caan, C. Steves, T.D. Spector, and G. Montana. 2017. Predicting brain age with deep learning from raw imaging data results in a reliable and heritable biomarker. *NeuroImage*. 163:115–124. doi:10.1016/j.neuroimage.2017.07.059.

Cole, J.H., S.J. Ritchie, M.E. Bastin, M.E. Bastin, M.C.V. Hernández, S.M. Maniega, S.M. Maniega, N.A. Royle, J. Corley, J. Corley, A. Pattie, S.E. Harris, Q. Zhang, Q. Zhang, Q. Zhang, Q. Zhang, N.R. Wray, P. Redmond, R.E. Marioni, J.M. Starr, S.R. Cox, J.M. Wardlaw, D.J. Sharp, D.J. Sharp, D.J. Sharp, D.J. Sharp, and I.J. Deary. 2018. Brain age predicts mortality. *Mol. Psychiatry*. doi:10.1038/mp.2017.62.

Conlon, M.A., and A.R. Bird. 2014. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*. 7:17–44. doi:10.3390/nu7010017.

Coulter, T., C. Hill, and A.J. McKnight. 2024. Insights into the length and breadth of methodologies harnessed to study human telomeres. *Biomark. Res.* 12:127. doi:10.1186/s40364-024-00668-9.

Cruz-Jentoft, A.J., G. Bahat, J. Bauer, Y. Boirie, O. Bruyère, T. Cederholm, C. Cooper, F. Landi, Y. Rolland, A.A. Sayer, S.M. Schneider, C.C. Sieber, E. Topinkova, M. Vandewoude, M. Visser, M. Zamboni, and Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. 2019. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. 48:16–31. doi:10.1093/ageing/afy169.

Cruz-Jentoft, A.J., and A.A. Sayer. 2019. Sarcopenia. *Lancet Lond. Engl.* 393:2636–2646. doi:10.1016/s0140-6736(19)31138-9.

Cummings, S.R., and S.B. Kritchevsky. 2022. Endpoints for geroscience clinical trials: health outcomes, biomarkers, and biologic age. *GeroScience*. 44:2925–2931. doi:10.1007/s11357-022-00671-8.

Currie, C.J., J.R. Peters, A. Tynan, M. Evans, R.J. Heine, O.L. Bracco, T. Zagar, and C.D. Poole. 2010. Survival as a function of HbA(1c) in people with type 2 diabetes: a retrospective cohort study. *Lancet*. 375:481–489. doi:10.1016/s0140-6736(09)61969-3.

De Sandre-Giovannoli, A., R. Bernard, P. Cau, C. Navarro, J. Amiel, I. Boccaccio, S. Lyonnet, C.L. Stewart, A. Munnich, M. Le Merrer, and N. Lévy. 2003. Lamin A truncation in Hutchinson-Gilford progeria. *Science*. 300:2055. doi:10.1126/science.1084125.

Deelen, J., D.S. Evans, D.E. Arking, N. Tesi, M. Nygaard, X. Liu, M.K. Wojczynski, M.L. Biggs, A. van der Spek, G. Atzmon, E.B. Ware, C. Sarnowski, A.V. Smith, I. Seppälä, H.J. Cordell, J. Dose, N. Amin, A.M. Arnold, K.L. Ayers, N. Barzilai, E.J. Becker, M. Beekman, H. Blanché, K. Christensen, L. Christiansen, J.C. Collerton, S. Cubaynes, S.R. Cummings, K. Davies, B. Debrabant, J.-F. Deleuze, R. Duncan, J.D. Faul, C. Franceschi, P. Galan, V. Gudnason, T.B. Harris, M. Huisman, M.A. Hurme, C. Jagger, I. Jansen, M. Jylhä, M. Kähönen, D. Karasik, S.L.R. Kardia, A. Kingston, T.B.L. Kirkwood, L.J. Launer, T. Lehtimäki, W. Lieb, L.-P. Lyytikäinen, C. Martin-Ruiz, J. Min, A. Nebel, A.B. Newman, C. Nie, E.A. Nohr, E.S. Orwoll, T.T. Perls, M.A. Province, B.M. Psaty, O.T. Raitakari, M.J.T. Reinders, J.-M. Robine, J.I. Rotter, P. Sebastiani, J. Smith, T.I.A. Sørensen, K.D. Taylor, A.G. Uitterlinden, W. van der Flier, S.J. van der Lee, C.M. van Duijn, D. van Heemst, J.W. Vaupel, D. Weir, K. Ye, Y. Zeng, W. Zheng, H. Holstege, D.P. Kiel, K.L. Lunetta, P.E. Slagboom, and J.M. Murabito. 2019. A meta-analysis of genome-wide association studies identifies multiple longevity genes. *Nat. Commun.* 10:3669. doi:10.1038/s41467-019-11558-2.

Dharnidharka, V.R., C. Kwon, and G. Stevens. 2002. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am. J. Kidney Dis.* 40:221–226. doi:10.1053/ajkd.2002.34487.

Dik, M.G., M.G. Dik, C. Jonker, C. Jonker, C.M. Jonker, C.E. (Erik) Hack, C.E. (Erik) Hack, C.E. Hack, C.E. Hack, J. Smit, H.C. Comijs, H.C. Comijs, P. Eikelenboom, and P. Eikelenboom. 2005. Serum inflammatory proteins and cognitive decline in older persons. *Neurology*. doi:10.1212/01.wnl.0000158281.08946.68.

Ding, J., L. Pan, D. Lan, Z. Chen, Z. Wang, M. Zou, and R. Meng. 2023. Inflammatory markers differentiate cerebral venous sinus thrombosis from mimics. *Thromb. Haemost.* 123:326–335. doi:10.1055/a-1951-3402.

Eichacker, P.Q., C. Parent, A. Kalil, C. Esposito, X. Cui, S.M. Banks, E.P. Gerstenberger, Y. Fitz, R.L. Danner, and C. Natanson. 2002. Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. *Am. J. Respir. Crit. Care Med.* 166:1197–1205. doi:10.1164/rccm.200204-302OC.

Elixhauser, A., C. Steiner, D.R. Harris, and R.M. Coffey. 1998. Comorbidity measures for use with administrative data. *Med. Care*. 36:8–27. doi:10.1097/00005650-199801000-00004.

Elliott, M.L., D.W. Belsky, A.R. Knodt, D. Ireland, T.R. Melzer, R. Poulton, S. Ramrakha, A. Caspi, T.E. Moffitt, and A.R. Hariri. 2021. Brain-age in midlife is associated with accelerated biological aging and cognitive decline in a longitudinal birth cohort. *Mol. Psychiatry*. 26:3829–3838. doi:10.1038/s41380-019-0626-7.

Engelhart, M.J., M.I. Geerlings, J. Meijer, A. Kiliaan, A. Ruitenberg, J.C. van Swieten, T. Stijnen, A. Hofman, J.C.M. Witteman, and M.M.B. Breteler. 2004. Inflammatory proteins in plasma and

the risk of dementia: the Rotterdam study. *Arch. Neurol.* 61:668–672. doi:10.1001/archneur.61.5.668.

Enright, P.L. 2003. The six-minute walk test. *Respir. Care*. 48:783–785.

Enright, P.L., M.A. McBurnie, V. Bittner, R.P. Tracy, R. McNamara, A. Arnold, A.B. Newman, and Cardiovascular Health Study. 2003. The 6-min walk test: a quick measure of functional status in elderly adults. *Chest*. 123:387–398. doi:10.1378/chest.123.2.387.

Eriksson, M., W.T. Brown, L.B. Gordon, M.W. Glynn, J. Singer, L. Scott, M.R. Erdos, C.M. Robbins, T.Y. Moses, P. Berglund, A. Dutra, E. Pak, S. Durkin, A.B. Csoka, M. Boehnke, T.W. Glover, and F.S. Collins. 2003. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*. 423:293–298. doi:10.1038/nature01629.

Fabbri, E., Y. An, M. Zoli, E.M. Simonsick, J.M. Guralnik, S. Bandinelli, C.M. Boyd, and L. Ferrucci. 2015. Aging and the burden of multimorbidity: associations with inflammatory and anabolic hormonal biomarkers. *J. Gerontol. A. Biol. Sci. Med. Sci.* 70:63–70. doi:10.1093/gerona/glu127.

Fadri, M.T.M., J.B. Lee, and A.J. Keung. 2024. Summary of ChIP-seq methods and description of an optimized ChIP-seq protocol. *Methods Mol. Biol. Clifton NJ*. 2842:419–447. doi:10.1007/978-1-0716-4051-7_22.

Fang, N., M. Jiang, and Y. Fan. 2016. Ideal cardiovascular health metrics and risk of cardiovascular disease or mortality: a meta-analysis. *Int. J. Cardiol.* 214:279–283. doi:10.1016/j.ijcard.2016.03.210.

Farokhian, F., C. Yang, I. Beheshti, H. Matsuda, and S. Wu. 2017. Age-related gray and white matter changes in normal adult brains. *Aging Dis.* 8:899–909. doi:10.14336/AD.2017.0502.

Fastbom, J., P. Wills, C. Cornelius, M. Viitanen, and B. Winblad. 1996. Levels of serum creatinine and estimated creatinine clearance over the age of 75: a study of an elderly Swedish population. *Arch. Gerontol. Geriatr.* 23:179–188. doi:10.1016/0167-4943(96)00719-4.

FDA-NIH Biomarker Working Group. 2016. BEST (Biomarkers, EndpointS, and other Tools) resource. Silver Spring (MD): Food and Drug Administration (US); Co-published by National Institutes of Health (US), Bethesda (MD).

Fermín-Martínez, C.A., A. Márquez-Salinas, E.C. Guerra, L. Zavala-Romero, N.E. Antonio-Villa, L. Fernández-Chirino, E. Sandoval-Colin, D.A. Barquera-Guevara, A. Campos Muñoz, A. Vargas-Vázquez, C.D. Paz-Cabrera, D. Ramírez-García, L.M. Gutiérrez-Robledo, and O.Y. Bello-Chavolla. 2023. AnthropoAge, a novel approach to integrate body composition into the estimation of biological age. *Aging Cell*. 22:e13756. doi:10.1111/acel.13756.

Ferrara, A., E. Barrett-Connor, and J. Shan. 1997. Total, LDL, and HDL cholesterol decrease with age in older men and women. The Rancho Bernardo Study 1984-1994. *Circulation*. 96:37–43. doi:10.1161/01.cir.96.1.37.

Ferrucci, L., M. Gonzalez-Freire, E. Fabbri, E. Simonsick, T. Tanaka, Z. Moore, S. Salimi, F. Sierra, and R. de Cabo. 2020. Measuring biological aging in humans: a quest. *Aging Cell*. 19:e13080. doi:10.1111/acel.13080.

Flegal, K.M., B.K. Kit, H. Orpana, and B.I. Graubard. 2013. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA*. 309:71–82. doi:10.1001/jama.2012.113905.

Florea, M., M. Hamalainen, P. Seebold, N. Cheng, P. Murray, A.J. Colville, S. Zheng, D. Ingham, R. Kantelal, R.D. Sicilia, and (Longevity Biotech Fellowship Consortium). 2023. Longevity

bottlenecks. doi:10.1101/2023.08.18.553936.

Fox, C.S., J.M. Massaro, U. Hoffmann, K.M. Pou, P. Maurovich-Horvat, C.-Y. Liu, R.S. Vasan, J.M. Murabito, J.B. Meigs, L.A. Cupples, R.B. D'Agostino, and C.J. O'Donnell. 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 116:39–48. doi:10.1161/circulationaha.106.675355.

Franceschi, C., P. Garagnani, P. Parini, C. Giuliani, and A. Santoro. 2018. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14:576–590. doi:10.1038/s41574-018-0059-4.

Franke, K., and C. Gaser. 2019. Ten years of BrainAGE as a neuroimaging biomarker of brain aging: what insights have we gained? *Front. Neurol.* 10:789. doi:10.3389/fneur.2019.00789.

Franklin, B.A., J. Brinks, K. Berra, C.J. Lavie, N.F. Gordon, and L.S. Sperling. 2018. Using metabolic equivalents in clinical practice. *Am. J. Cardiol.* 121:382–387. doi:10.1016/j.amjcard.2017.10.033.

Fried, L.P., C.M. Tangen, J. Walston, A.B. Newman, C. Hirsch, J. Gottdiener, T. Seeman, R. Tracy, W.J. Kop, G. Burke, M.A. McBurnie, and Cardiovascular Health Study Collaborative Research Group. 2001. Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* 56:M146-156. doi:10.1093/gerona/56.3.m146.

Frohlich, M., A. Imhof, G. Berg, W.L. Hutchinson, M.B. Pepys, H. Boeing, R. Muche, H. Brenner, and W. Koenig. 2000. Association between C-reactive protein and features of the metabolic syndrome - A population-based study. *Diabetes Care*. 23:1835–1839. doi:10.2337/diacare.23.12.1835.

Fuchs, F.D., and P.K. Whelton. 2020. High blood pressure and cardiovascular disease. *Hypertens. Dallas Tex 1979*. 75:285–292. doi:10.1161/HYPERTENSIONAHA.119.14240.

Furey, T.S. 2012. ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. *Nat. Rev. Genet.* 13:840–852. doi:10.1038/nrg3306.

Furman, D., J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, L. Ferrucci, D.W. Gilroy, A. Fasano, G.W. Miller, A.H. Miller, A. Mantovani, C.M. Weyand, N. Barzilai, J.J. Goronzy, T.A. Rando, R.B. Effros, A. Lucia, N. Kleinstreuer, and G.M. Slavich. 2019. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25:1822–1832. doi:10.1038/s41591-019-0675-0.

Galkin, F., P. Mamoshina, A. Aliper, J.P. de Magalhães, V.N. Gladyshev, and A. Zhavoronkov. 2020a. Biohorology and biomarkers of aging: current state-of-the-art, challenges and opportunities. *Ageing Res. Rev.* 60:101050. doi:10.1016/j.arr.2020.101050.

Galkin, F., P. Mamoshina, A. Aliper, E. Putin, V. Moskalev, V.N. Gladyshev, and A. Zhavoronkov. 2020b. Human gut microbiome aging clock based on taxonomic profiling and deep learning. *iScience*. 23:101199. doi:10.1016/j.isci.2020.101199.

Galkin, F., P. Mamoshina, K. Kochetov, D. Sidorenko, and A. Zhavoronkov. 2021. DeepMAge: a methylation aging clock developed with deep learning. *Aging Dis.* 12:1252–1262. doi:10.14336/ad.2020.1202.

Gan, W., X.-L. Liu, T. Yu, Y.-G. Zou, T.-T. Li, S. Wang, J. Deng, L.-L. Wang, and J.-P. Cai. 2018. Urinary 8-oxo-7,8-dihydroguanosine as a potential biomarker of aging. *Front. Aging Neurosci.* 10:34. doi:10.3389/fnagi.2018.00034.

Gan, W., B. Nie, F. Shi, X.-M. Xu, J.-C. Qian, Y. Takagi, H. Hayakawa, M. Sekiguchi, and J.-P. Cai.

2012. Age-dependent increases in the oxidative damage of DNA, RNA, and their metabolites in normal and senescence-accelerated mice analyzed by LC-MS/MS: urinary 8-oxoguanosine as a novel biomarker of aging. *Free Radic. Biol. Med.* 52:1700–1707. doi:10.1016/j.freeradbiomed.2012.02.016.

Garagnani, P., M.G. Bacalini, C. Pirazzini, D. Gori, C. Giuliani, D. Mari, A.M. Di Blasio, D. Gentilini, G. Vitale, S. Collino, S. Rezzi, G. Castellani, M. Capri, S. Salvioli, and C. Franceschi. 2012. Methylation of ELOVL2 gene as a new epigenetic marker of age. *Aging Cell*. 11:1132–1134. doi:10.1111/acel.12005.

GBD 2019 Diseases and Injuries Collaborators. 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Lond. Engl.* 396:1204–1222. doi:10.1016/S0140-6736(20)30925-9.

GBD 2019 Risk Factors Collaborators. 2020. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Lond. Engl.* 396:1223–1249. doi:10.1016/S0140-6736(20)30752-2.

Gellhaus, B., K.O. Böker, A.F. Schilling, and D. Saul. 2023. Therapeutic consequences of targeting the IGF-1/PI3K/AKT/FOXO3 axis in sarcopenia: a narrative review. *Cells*. 12:2787. doi:10.3390/cells12242787.

Gerstein, H.C., J.F.E. Mann, Q.L. Yi, B. Zinman, S.F. Dinneen, B. Hoogwerf, J.P. Halle, J. Young, A. Rashkow, C. Joyce, S. Nawaz, and S. Yusuf. 2001. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA-J. Am. Med. Assoc.* 286:421–426. doi:10.1001/jama.286.4.421.

Glasser, S.P., D.K. Arnett, G.E. McVeigh, S.M. Finkelstein, A.J. Bank, D.J. Morgan, and J.N. Cohn. 1997. Vascular compliance and cardiovascular disease: a risk factor or a marker? *Am. J. Hypertens.* 10:1175–1189. doi:10.1016/s0895-7061(97)00311-7.

Go, A.S., G.M. Chertow, D.J. Fan, C.E. McCulloch, and C.Y. Hsu. 2004. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med.* 351:1296–1305. doi:10.1056/nejmoa041031.

Goodpaster, B.H., S. Krishnaswami, T.B. Harris, A. Katsiaras, S.B. Kritchevsky, E.M. Simonsick, M. Nevitt, P. Holvoet, and A.B. Newman. 2005. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. *Arch. Intern. Med.* 165:777–783. doi:10.1001/archinte.165.7.777.

Goodpaster, B.H., S. Krishnaswami, H. Resnick, D.E. Kelley, C. Haggerty, T.B. Harris, A.V. Schwartz, S. Kritchevsky, and A.B. Newman. 2003. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care*. 26:372–379. doi:10.2337/diacare.26.2.372.

Goodpaster, B.H., S.W. Park, T.B. Harris, S.B. Kritchevsky, M. Nevitt, A.V. Schwartz, E.M. Simonsick, F.A. Tylavsky, M. Visser, and A.B. Newman. 2006. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J. Gerontol. A. Biol. Sci. Med. Sci.* 61:1059–1064. doi:10.1093/gerona/61.10.1059.

Greenland, P., M.J. Blaha, M.J. Budoff, R. Erbel, and K.E. Watson. 2018. Coronary calcium score and cardiovascular risk. *J. Am. Coll. Cardiol.* 72:434–447. doi:10.1016/j.jacc.2018.05.027.

Guo, S.-M., Y.-T. Liu, S.-R. He, M.-S. Wu, W.-T. Tseng, R.-C. Wu, and I.-C. Wu. 2021. Differential relationship of uric acid to mortality and clinical biomarkers of aging according to grip strength in older adults: a cohort study. *Aging*. 13:10555–10583. doi:10.18632/aging.202820.

Guralnik, J.M., L. Ferrucci, C.F. Pieper, S.G. Leveille, K.S. Markides, G.V. Ostir, S. Studenski, L.F. Berkman, and R.B. Wallace. 2000. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *J. Gerontol. A. Biol. Sci. Med. Sci.* 55:M221-231. doi:10.1093/gerona/55.4.m221.

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

Harber, M.P., L.A. Kaminsky, R. Arena, S.N. Blair, B.A. Franklin, J. Myers, and R. Ross. 2017. Impact of cardiorespiratory ritness on all-cause and disease-specific mortality: advances since 2009. *Prog. Cardiovasc. Dis.* 60:11–20. doi:10.1016/j.pcad.2017.03.001.

Harris, S.E., S.R. Cox, S. Bell, R.E. Marioni, B.P. Prins, A. Pattie, J. Corley, S. Muñoz Maniega, M. Valdés Hernández, Z. Morris, S. John, P.G. Bronson, E.M. Tucker-Drob, J.M. Starr, M.E. Bastin, J.M. Wardlaw, A.S. Butterworth, and I.J. Deary. 2020. Neurology-related protein biomarkers are associated with cognitive ability and brain volume in older age. *Nat. Commun.* 11:800. doi:10.1038/s41467-019-14161-7.

Hawkins, M.N., P.B. Raven, P.G. Snell, J. Stray-Gundersen, and B.D. Levine. 2007. Maximal oxygen uptake as a parametric measure of cardiorespiratory capacity. *Med. Sci. Sports Exerc.* 39:103–107. doi:10.1249/01.mss.0000241641.75101.64.

Heijmans, B.T., M. Beekman, J.J. Houwing-Duistermaat, M.R. Cobain, J. Powell, G.J. Blauw, F. van der Ouderaa, R.G.J. Westendorp, and P.E. Slagboom. 2006. Lipoprotein particle profiles mark familial and sporadic human longevity. *PLoS Med.* 3:e495. doi:10.1371/journal.pmed.0030495.

Hillege, H.L., V. Fidler, G.F.H. Diercks, W.H. van Gilst, D. de Zeeuw, D.J. van Veldhuisen, R.O.B. Gans, W.M.T. Janssen, D.E. Grobbee, and P.E. de Jong. 2002. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation*. 106:1777–1782. doi:10.1161/01.cir.0000031732.78052.81.

Hippisley-Cox, J., C. Coupland, and P. Brindle. 2017. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *BMJ*. 357:j2099. doi:10.1136/bmj.j2099.

vB Hjelmborg, J., I. Iachine, A. Skytthe, J.W. Vaupel, M. McGue, M. Koskenvuo, J. Kaprio, N.L. Pedersen, and K. Christensen. 2006. Genetic influence on human lifespan and longevity. *Hum. Genet.* 119:312–321. doi:10.1007/s00439-006-0144-y.

Holland, J., S. Bandelow, and E. Hogervorst. 2011. Testosterone levels and cognition in elderly men: a review. *Maturitas*. 69:322–337. doi:10.1016/j.maturitas.2011.05.012.

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

Horvath, S., and K. Raj. 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* 19:371–384. doi:10.1038/s41576-018-0004-3.

Hubert, H.B. 1986. The importance of obesity in the development of coronary risk factors and disease: the epidemiologic evidence. *Annu. Rev. Public Health*. 7:493–502. doi:10.1146/annurev.pu.07.050186.002425.

Hubert, H.B., M. Feinleib, P.M. McNamara, and W.P. Castelli. 1983. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham

Heart Study. *Circulation*. 67:968–977. doi:10.1161/01.cir.67.5.968.

Inker, L.A., C.H. Schmid, H. Tighiouart, J.H. Eckfeldt, H.I. Feldman, T. Greene, J.W. Kusek, J. Manzi, F. Van Lente, Y.L. Zhang, J. Coresh, and A.S. Levey. 2012. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N. Engl. J. Med.* 367:20–29. doi:10.1056/nejmoa1114248.

Isiozor, N.M., S.K. Kunutsor, A. Voutilainen, and J.A. Laukkanen. 2023. Life's Essential 8 and the risk of cardiovascular disease death and all-cause mortality in Finnish men. *Eur. J. Prev. Cardiol.* 30:658–667. doi:10.1093/eurjpc/zwad040.

Jansen, R., L.K. Han, J.E. Verhoeven, K.A. Aberg, E.C. van den Oord, Y. Milaneschi, and B.W. Penninx. 2021. An integrative study of five biological clocks in somatic and mental health. *eLife*. 10:e59479. doi:10.7554/eLife.59479.

Janssen, I., S.B. Heymsfield, D.B. Allison, D.P. Kotler, and R. Ross. 2002. Body mass index and waist circumference independently contribute to the prediction of nonabdominal, abdominal subcutaneous, and visceral fat. *Am. J. Clin. Nutr.* 75:683–688. doi:10.1093/ajcn/75.4.683.

Jetté, M., K. Sidney, and G. Blümchen. 1990. Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin. Cardiol.* 13:555–565. doi:10.1002/clc.4960130809.

Jiang, S., J. Cui, L.-Q. Zhang, Z. Liu, Y. Zhang, Y. Shi, and J.-P. Cai. 2022. Role of a urinary biomarker in the common mechanism of physical performance and cognitive function. *Front. Med.* 9:816822. doi:10.3389/fmed.2022.816822.

Jin, Y., W. Wang, Q. Wang, Y. Zhang, K.R. Zahid, U. Raza, and Y. Gong. 2022. Alpha-1-antichymotrypsin as a novel biomarker for diagnosis, prognosis, and therapy prediction in human diseases. *Cancer Cell Int.* 22:156. doi:10.1186/s12935-022-02572-4.

Johnson, A.A., M.N. Shokhirev, T. Wyss-Coray, and B. Lehallier. 2020. Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. *Ageing Res. Rev.* 60:101070. doi:10.1016/j.arr.2020.101070.

Jones, C.J., R.E. Rikli, and W.C. Beam. 1999. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res. Q. Exerc. Sport*. 70:113–119. doi:10.1080/02701367.1999.10608028.

Jouven, X., J.-P. Empana, P.J. Schwartz, M. Desnos, D. Courbon, and P. Ducimetière. 2005. Heart-rate profile during exercise as a predictor of sudden death. *N. Engl. J. Med.* 352:1951–1958. doi:10.1056/nejmoa043012.

Justice, J.N., L. Ferrucci, A.B. Newman, V.R. Aroda, J.L. Bahnson, J. Divers, M.A. Espeland, S. Marcovina, M.N. Pollak, S.B. Kritchevsky, N. Barzilai, and G.A. Kuchel. 2018. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. *GeroScience*. 40:419–436. doi:10.1007/s11357-018-0042-y.

Jylhävä, J., N.L. Pedersen, and S. Hägg. 2017. Biological age predictors. *EBioMedicine*. 21:29–36. doi:10.1016/j.ebiom.2017.03.046.

Kim, K., and H.K. Choe. 2019. Role of hypothalamus in aging and its underlying cellular mechanisms. *Mech. Ageing Dev.* 177:74–79. doi:10.1016/j.mad.2018.04.008.

Kim, W.Y., and N.E. Sharpless. 2006. The regulation of INK4/ARF in cancer and aging. *Cell*. 127:265–275. doi:10.1016/j.cell.2006.10.003.

Klausen, K., K. Borch-Johnsen, B. Feldt-Rasmussen, G. Jensen, P. Clausen, H. Scharling, M. Appleyard, and J.S. Jensen. 2004. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation*. 110:32–35. doi:10.1161/01.cir.0000133312.96477.48.

Knight, E.L., J.C. Verhave, D. Spiegelman, H.L. Hillege, D. De Zeeuw, G.C. Curhan, and P.E. De Jong. 2004. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int.* 65:1416–1421. doi:10.1111/j.1523-1755.2004.00517.x.

Kopelman, P.G. 2000. Obesity as a medical problem. *Nature*. 404:635–643. doi:10.1038/35007508.

Kuk, J.L., P.T. Katzmarzyk, M.Z. Nichaman, T.S. Church, S.N. Blair, and R. Ross. 2006. Visceral fat is an independent predictor of all-cause mortality in men. *Obes. Silver Spring Md*. 14:336–341. doi:10.1038/oby.2006.43.

Kuk, J.L., T.J. Saunders, L.E. Davidson, and R. Ross. 2009. Age-related changes in total and regional fat distribution. *Ageing Res. Rev.* 8:339–348. doi:10.1016/j.arr.2009.06.001.

Kurdyukov, S., and M. Bullock. 2016. DNA methylation analysis: choosing the right method. *Biology*. 5:3. doi:10.3390/biology5010003.

Kuzuya, M., F. Ando, A. Iguchi, and H. Shimokata. 2002. Effect of aging on serum uric acid levels: longitudinal changes in a large Japanese population group. *J. Gerontol. A. Biol. Sci. Med. Sci.* 57:M660–M664. doi:10.1093/gerona/57.10.m660.

Kyle, U.G., I. Bosaeus, A.D. De Lorenzo, P. Deurenberg, M. Elia, J.M. Gómez, B.L. Heitmann, L. Kent-Smith, J.-C. Melchior, M. Pirlich, H. Scharfetter, A.M.W.J. Schols, C. Pichard, and Composition of the ESPEN Working Group. 2004. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin. Nutr. Edinb. Scotl.* 23:1226–1243. doi:10.1016/j.clnu.2004.06.004.

Lakka, H.M., T.A. Lakka, J. Tuomilehto, and J.T. Salonen. 2002. Abdominal obesity is associated with increased risk of acute coronary events in men. *Eur. Heart J.* 23:706–713. doi:10.1053/euhj.2001.2889.

Laurent, S., P. Boutouyrie, R. Asmar, I. Gautier, B. Laloux, L. Guize, P. Ducimetiere, and A. Benetos. 2001. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertens. Dallas Tex 1979*. 37:1236–1241. doi:10.1161/01.hyp.37.5.1236.

Lehallier, B., D. Gate, N. Schaum, T. Nanasi, S.E. Lee, H. Yousef, P. Moran Losada, D. Berdnik, A. Keller, J. Verghese, S. Sathyan, C. Franceschi, S. Milman, N. Barzilai, and T. Wyss-Coray. 2019. Undulating changes in human plasma proteome profiles across the lifespan. *Nat. Med.* 25:1843–1850. doi:10.1038/s41591-019-0673-2.

Lehallier, B., M.N. Shokhirev, T. Wyss-Coray, and A.A. Johnson. 2020. Data mining of human plasma proteins generates a multitude of highly predictive aging clocks that reflect different aspects of aging. *Aging Cell*. 19:e13256. doi:10.1111/acel.13256.

Lemaitre, H., A.L. Goldman, F. Sambataro, B.A. Verchinski, A. Meyer-Lindenberg, D.R. Weinberger, and V.S. Mattay. 2012. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol. Aging*. 33:617.e1–9. doi:10.1016/j.neurobiolaging.2010.07.013.

Leong, D.P., K.K. Teo, S. Rangarajan, P. Lopez-Jaramillo, A. Avezum, A. Orlandini, P. Seron, S.H.

Ahmed, A. Rosengren, R. Kelishadi, O. Rahman, S. Swaminathan, R. Iqbal, R. Gupta, S.A. Lear, A. Oguz, K. Yusoff, K. Zatonska, J. Chifamba, E. Igumbor, V. Mohan, R.M. Anjana, H. Gu, W. Li, S. Yusuf, and Prospective Urban Rural Epidemiology (PURE) Study investigators. 2015. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *Lancet Lond. Engl.* 386:266–273. doi:10.1016/s0140-6736(14)62000-6.

Levine, M.E. 2013. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? *J. Gerontol. A. Biol. Sci. Med. Sci.* 68:667–674. doi:10.1093/gerona/gls233.

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

Lewington, S., R. Clarke, N. Qizilbash, R. Peto, R. Collins, and Prospective Studies Collaboration. 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet Lond. Engl.* 360:1903–1913. doi:10.1016/s0140-6736(02)11911-8.

Li, H., S. Wu, J. Li, Z. Xiong, K. Yang, W. Ye, J. Ren, Q. Wang, M. Xiong, Z. Zheng, S. Zhang, Z. Han, P. Yang, B. Jiang, J. Ping, Y. Zuo, X. Lu, Q. Zhai, H. Yan, S. Wang, S. Ma, B. Zhang, J. Ye, J. Qu, Y.-G. Yang, F. Zhang, G.-H. Liu, Y. Bao, and W. Zhang. 2024. HALL: a comprehensive database for human aging and longevity studies. *Nucleic Acids Res.* 52:D909–D918. doi:10.1093/nar/gkad880.

Li, X., A. Ploner, Y. Wang, P.K.E. Magnusson, C.A. Reynolds, D. Finkel, N.L. Pedersen, J. Jylhävä, and S. Hägg. 2020. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. *eLife*. doi:10.7554/elife.51507.

Liang, Y.-D., Q. Liu, M.-H. Du, Z. Liu, S.-M. Yao, P.-P. Zheng, Y.-H. Wan, N. Sun, Y.-Y. Li, J.-P. Liu, Y. Luo, J.-P. Cai, J.-F. Yang, and H. Wang. 2020. Urinary 8-oxo-7,8-dihydroguanosine as a potential biomarker of frailty for elderly patients with cardiovascular disease. *Free Radic. Biol. Med.* 152:248–254. doi:10.1016/j.freeradbiomed.2020.03.011.

Liang, Z., T. Zhang, H. Liu, Z. Li, L. Peng, C. Wang, and T. Wang. 2022. Inflammaging: the ground for sarcopenia? *Exp. Gerontol.* 168:111931. doi:10.1016/j.exger.2022.111931.

Libby, P., J.E. Buring, L. Badimon, G.K. Hansson, J. Deanfield, M.S. Bittencourt, L. Tokgözoğlu, and E.F. Lewis. 2019. Atherosclerosis. *Nat. Rev. Dis. Primer*. 5:56. doi:10.1038/s41572-019-0106-z.

Liem, F., G. Varoquaux, J. Kynast, F. Beyer, S.K. Masouleh, J.M. Huntenburg, L. Lampe, M. Rahim, A. Abraham, R.C. Craddock, S.G. Riedel-Heller, S.G. Riedel-Heller, T. Luck, T. Luck, M. Loeffler, M. Loeffler, M. Loeffler, M.L. Schroeter, M.L. Schroeter, A.V. Witte, A.V. Witte, A. Villringer, A. Villringer, A. Villringer, D.S. Margulies, and D.S. Margulies. 2017. Predicting brain-age from multimodal imaging data captures cognitive impairment. *NeuroImage*. doi:10.1016/j.neuroimage.2016.11.005.

de Lima Camillo, L.P. 2024. pyaging: a Python-based compendium of GPU-optimized aging clocks. *Bioinforma. Oxf. Engl.* 40:btae200. doi:10.1093/bioinformatics/btae200.

Lin, F.R., L. Ferrucci, E.J. Metter, Y. An, A.B. Zonderman, and S.M. Resnick. 2011. Hearing loss and cognition in the Baltimore Longitudinal Study of Aging. *Neuropsychology*. 25:763–770. doi:10.1037/a0024238.

Lin, F.R., K. Yaffe, J. Xia, Q.L. Xue, T.B. Harris, E. Purchase-Helzner, S. Satterfield, H.N.

Ayonayon, L. Ferrucci, and E.M. Simonsick. 2013. Hearing loss and cognitive decline in older adults. *JAMA Intern. Med.* doi:10.1001/jamainternmed.2013.1868.

Lin, J.-R., P. Sin-Chan, V. Napolioni, G.G. Torres, J. Mitra, Q. Zhang, M.R. Jabalameli, Z. Wang, N. Nguyen, T. Gao, Regeneron Genetics Center, M. Laudes, S. Görg, A. Franke, A. Nebel, M.D. Greicius, G. Atzmon, K. Ye, V. Gorbunova, W.C. Ladiges, A.R. Shuldiner, L.J. Niedernhofer, P.D. Robbins, S. Milman, Y. Suh, J. Vijg, N. Barzilai, and Z.D. Zhang. 2021. Rare genetic coding variants associated with human longevity and protection against age-related diseases. *Nat. Aging*. 1:783–794. doi:10.1038/s43587-021-00108-5.

Lindenberger, U., and P.B. Baltes. 1994. Sensory functioning and intelligence in old age: a strong connection. *Psychol. Aging*. doi:10.1037/0882-7974.9.3.339.

Lippi, G., G.L. Salvagno, and G.C. Guidi. 2014. Red blood cell distribution width is significantly associated with aging and gender. *Clin. Chem. Lab. Med.* 52:e197-199. doi:10.1515/cclm-2014-0353.

Liu, C.-C., C.-C. Liu, T. Kanekiyo, H. Xu, and G. Bu. 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat. Rev. Neurol.* 9:106–118. doi:10.1038/nrneurol.2012.263.

Liu, R.S.N., L. Lemieux, G.S. Bell, S.M. Sisodiya, S.D. Shorvon, J.W. a. S. Sander, and J.S. Duncan. 2003. A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging and difference image analysis. *NeuroImage*. 20:22–33. doi:10.1016/s1053-8119(03)00219-2.

Liu, X., X. Chen, F. Hu, X. Xia, L. Hou, G. Zhang, X. Peng, X. Sun, S. Luo, J. Yue, and B. Dong. 2022. Higher uric acid serum levels are associated with sarcopenia in west China: a cross-sectional study. *BMC Geriatr.* 22:121. doi:10.1186/s12877-022-02817-x.

Liu, Z., P.-L. Kuo, S. Horvath, E. Crimmins, L. Ferrucci, and M. Levine. 2018. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: A cohort study. *PLoS Med.* 15:e1002718. doi:10.1371/journal.pmed.1002718.

Lloyd-Jones, D.M., N.B. Allen, C.A.M. Anderson, T. Black, L.C. Brewer, R.E. Foraker, M.A. Grandner, H. Lavretsky, A.M. Perak, G. Sharma, W. Rosamond, and American Heart Association. 2022. Life's Essential 8: updating and enhancing the American Heart Association's construct of cardiovascular health: a presidential advisory from the American Heart Association. *Circulation*. 146:e18–e43. doi:10.1161/CIR.0000000000001078.

Lloyd-Jones, D.M., Y. Hong, D. Labarthe, D. Mozaffarian, L.J. Appel, L. Van Horn, K. Greenlund, S. Daniels, G. Nichol, G.F. Tomaselli, D.K. Arnett, G.C. Fonarow, P.M. Ho, M.S. Lauer, F.A. Masoudi, R.M. Robertson, V. Roger, L.H. Schwamm, P. Sorlie, C.W. Yancy, W.D. Rosamond, and American Heart Association Strategic Planning Task Force and Statistics Committee. 2010. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*. 121:586–613. doi:10.1161/CIRCULATIONAHA.109.192703.

López-Otín, C., L. Galluzzi, J.M.P. Freije, F. Madeo, and G. Kroemer. 2016. Metabolic control of longevity. *Cell*. 166:802–821. doi:10.1016/j.cell.2016.07.031.

Lowe, R., N. Shirley, M. Bleackley, S. Dolan, and T. Shafee. 2017. Transcriptomics technologies. *PLoS Comput. Biol.* 13:e1005457. doi:10.1371/journal.pcbi.1005457.

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

Mamoshina, P., K. Kochetov, F. Cortese, A. Kovalchuk, A. Aliper, E. Putin, M. Scheibye-Knudsen, C.R. Cantor, N.M. Skjodt, O. Kovalchuk, and A. Zhavoronkov. 2019. Blood biochemistry analysis to detect smoking status and quantify accelerated aging in smokers. *Sci. Rep.* 9:142. doi:10.1038/s41598-018-35704-w.

Mandsager, K., S. Harb, P. Cremer, D. Phelan, S.E. Nissen, and W. Jaber. 2018. Association of cardiorespiratory fitness with long-term mortality among adults undergoing exercise treadmill testing. *JAMA Netw. Open*. 1:e183605. doi:10.1001/jamanetworkopen.2018.3605.

Marioni, R.E., S. Shah, A.F. McRae, B.H. Chen, E. Colicino, S.E. Harris, J. Gibson, A.K. Henders, P. Redmond, S.R. Cox, A. Pattie, J. Corley, L. Murphy, N.G. Martin, G.W. Montgomery, A.P. Feinberg, M.D. Fallin, M.L. Multhaup, A.E. Jaffe, R. Joehanes, J. Schwartz, A.C. Just, K.L. Lunetta, J.M. Murabito, J.M. Starr, S. Horvath, A.A. Baccarelli, D. Levy, P.M. Visscher, N.R. Wray, and I.J. Deary. 2015. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.* 16:25. doi:10.1186/s13059-015-0584-6.

Martinon, F., V. Petrilli, A. Mayor, A. Tardivel, and J. Tschopp. 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 440:237–241. doi:10.1038/nature04516.

Mavaddat, N., S. Peock, D. Frost, S. Ellis, R. Platte, E. Fineberg, D.G. Evans, L. Izatt, R.A. Eeles, J. Adlard, R. Davidson, D. Eccles, T. Cole, J. Cook, C. Brewer, M. Tischkowitz, F. Douglas, S. Hodgson, L. Walker, M.E. Porteous, P.J. Morrison, L.E. Side, M.J. Kennedy, C. Houghton, A. Donaldson, M.T. Rogers, H. Dorkins, Z. Miedzybrodzka, H. Gregory, J. Eason, J. Barwell, E. McCann, A. Murray, A.C. Antoniou, D.F. Easton, and EMBRACE. 2013. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *J. Natl. Cancer Inst.* 105:812–822. doi:10.1093/jnci/djt095.

Mavromatis, L.A., D.B. Rosoff, A.S. Bell, J. Jung, J. Wagner, and F.W. Lohoff. 2023. Multi-omic underpinnings of epigenetic aging and human longevity. *Nat. Commun.* 14:2236. doi:10.1038/s41467-023-37729-w.

McGreevy, K.M., Z. Radak, F. Torma, M. Jokai, A.T. Lu, D.W. Belsky, A. Binder, R.E. Marioni, L. Ferrucci, E. Pośpiech, W. Branicki, A. Ossowski, A. Sitek, M. Spólnicka, L.M. Raffield, A.P. Reiner, S. Cox, M. Kobor, D.L. Corcoran, and S. Horvath. 2023. DNAmFitAge: biological age indicator incorporating physical fitness. *Aging*. 15:3904–3938. doi:10.18632/aging.204538.

Metter, E.J., L.A. Talbot, M. Schrager, and R. Conwit. 2002. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J. Gerontol. A. Biol. Sci. Med. Sci.* 57:B359-365. doi:10.1093/gerona/57.10.b359.

Mihaylova, B., J. Emberson, L. Blackwell, A. Keech, J. Simes, E.H. Barnes, M. Voysey, A. Gray, R. Collins, C. Baigent, J. de Lemos, E. Braunwald, M. Blazing, S. Murphy, J.R. Downs, A. Gotto, M. Clearfield, H. Holdaas, D. Gordon, B. Davis, M. Koren, B. Dahlof, N. Poulter, P. Sever, R.H. Knopp, B. Fellstrom, H. Holdaas, A. Jardine, R. Schmieder, F. Zannad, U. Goldbourt, E. Kaplinsky, H.M. Colhoun, D.J. Betteridge, P.N. Durrington, G.A. Hitman, J. Fuller, A. Neil, C. Wanner, V. Krane, F. Sacks, L. Moye, M. Pfeffer, C.M. Hawkins, E. Braunwald, J. Kjekshus, H. Wedel, J. Wikstrand, P. Barter, A. Keech, L. Tavazzi, A. Maggioni, R. Marchioli, G. Tognoni, M.G. Franzosi, A. Maggioni, H. Bloomfield, S. Robins, R. Collins, J. Armitage, A. Keech, S. Parish, R. Peto, P. Sleight, T.R. Pedersen, P.M. Ridker, R. Holman, T. Meade, J. Simes, A. Keech, S. MacMahon, I. Marschner, A. Tonkin, J. Shaw, P.W. Serruys, H. Nakamura, G. Knatterud, C. Furberg, R. Byington, P. Macfarlane, S. Cobbe, I. Ford, M. Murphy, G.J. Blauw, C. Packard, J. Shepherd, J. Kjekshus, T. Pedersen, L. Wilhelmsen, E. Braunwald, C. Cannon, S. Murphy, R. Collins, J. Armitage, L. Bowman, S. Parish, R. Peto, P. Sleight, et al. 2012. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet*. 380:581–590. doi:10.1016/s0140-6736(12)60367-5.

Mitchell, G.F., C.-Y. Guo, E.J. Benjamin, M.G. Larson, M.J. Keyes, J.A. Vita, R.S. Vasan, and D. Levy. 2007. Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. *Circulation*. 115:2628–2636. doi:10.1161/CIRCULATIONAHA.106.667733.

Mitchell, G.F., S.-J. Hwang, R.S. Vasan, M.G. Larson, M.J. Pencina, N.M. Hamburg, J.A. Vita, D. Levy, and E.J. Benjamin. 2010. Arterial stiffness and cardiovascular events - The Framingham Heart Study. *Circulation*. 121:505–511. doi:10.1161/circulationaha.109.886655.

Mitchell, G.F., H. Parise, E.J. Benjamin, M.G. Larson, M.J. Keyes, J.A. Vita, R.S. Vasan, and D. Levy. 2004. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertens. Dallas Tex 1979*. 43:1239–1245. doi:10.1161/01.HYP.0000128420.01881.aa.

Monnier, L., E. Mas, C. Ginet, F. Michel, L. Villon, J.P. Cristol, and C. Colette. 2006. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA-J. Am. Med. Assoc.* 295:1681–1687. doi:10.1001/jama.295.14.1681.

Moore, L.D., T. Le, and G. Fan. 2013. DNA methylation and its basic function. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 38:23–38. doi:10.1038/npp.2012.112.

Moqri, M., C. Herzog, J.R. Poganik, Biomarkers of Aging Consortium, J. Justice, D.W. Belsky, A. Higgins-Chen, A. Moskalev, G. Fuellen, A.A. Cohen, I. Bautmans, M. Widschwendter, J. Ding, A. Fleming, J. Mannick, J.-D.J. Han, A. Zhavoronkov, N. Barzilai, M. Kaeberlein, S. Cummings, B.K. Kennedy, L. Ferrucci, S. Horvath, E. Verdin, A.B. Maier, M.P. Snyder, V. Sebastiano, and V.N. Gladyshev. 2023. Biomarkers of aging for the identification and evaluation of longevity interventions. *Cell*. 186:3758–3775. doi:10.1016/j.cell.2023.08.003.

Moqri, M., C. Herzog, J.R. Poganik, K. Ying, J.N. Justice, D.W. Belsky, A.T. Higgins-Chen, B.H. Chen, A.A. Cohen, G. Fuellen, S. Hägg, R.E. Marioni, M. Widschwendter, K. Fortney, P.O. Fedichev, A. Zhavoronkov, N. Barzilai, J. Lasky-Su, D.P. Kiel, B.K. Kennedy, S. Cummings, P.E. Slagboom, E. Verdin, A.B. Maier, V. Sebastiano, M.P. Snyder, V.N. Gladyshev, S. Horvath, and L. Ferrucci. 2024. Validation of biomarkers of aging. *Nat. Med.* 30:360–372. doi:10.1038/s41591-023-02784-9.

More, S., G. Antonopoulos, F. Hoffstaedter, J. Caspers, S.B. Eickhoff, K.R. Patil, and Alzheimer's Disease Neuroimaging Initiative. 2023. Brain-age prediction: a systematic comparison of machine learning workflows. *NeuroImage*. 270:119947. doi:10.1016/j.neuroimage.2023.119947.

Muñoz-Bermejo, L., J.C. Adsuar, M. Mendoza-Muñoz, S. Barrios-Fernández, M.A. Garcia-Gordillo, J. Pérez-Gómez, and J. Carlos-Vivas. 2021. Test-retest reliability of five times sit to stand test (FTSST) in adults: a systematic review and meta-analysis. *Biology*. 10:510. doi:10.3390/biology10060510.

Murata, S., M. Ebeling, A.C. Meyer, K. Schmidt-Mende, N. Hammar, and K. Modig. 2024. Blood biomarker profiles and exceptional longevity: comparison of centenarians and non-centenarians in a 35-year follow-up of the Swedish AMORIS cohort. *GeroScience*. 46:1693–1702. doi:10.1007/s11357-023-00936-w.

Myers, J., M. Prakash, V. Froelicher, D. Do, S. Partington, and J.E. Atwood. 2002. Exercise capacity and mortality among men referred for exercise testing. *N. Engl. J. Med.* 346:793–801. doi:10.1056/nejmoa011858.

Nasreddine, Z.S., N.A. Phillips, V. Bédirian, S. Charbonneau, V. Whitehead, I. Collin, J.L.

Cummings, and H. Chertkow. 2005. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53:695–699. doi:10.1111/j.1532-5415.2005.53221.x.

NCD Risk Factor Collaboration (NCD-RisC). 2021. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet Lond. Engl.* 398:957–980. doi:10.1016/S0140-6736(21)01330-1.

Newman, A.B., V. Kupelian, M. Visser, E.M. Simonsick, B.H. Goodpaster, S.B. Kritchevsky, F.A. Tylavsky, S.M. Rubin, and T.B. Harris. 2006. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J. Gerontol. A. Biol. Sci. Med. Sci.* 61:72–77. doi:10.1093/gerona/61.1.72.

Nordestgaard, B.G., M. Benn, P. Schnohr, and A. Tybjaerg-Hansen. 2007. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA-J. Am. Med. Assoc.* 298:299–308. doi:10.1001/jama.298.3.299.

Odamaki, T., K. Kato, H. Sugahara, N. Hashikura, S. Takahashi, J.-Z. Xiao, F. Abe, and R. Osawa. 2016. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol.* 16:90. doi:10.1186/s12866-016-0708-5.

Oschwald, J., S. Guye, F. Liem, P. Rast, S. Willis, C. Röcke, L. Jäncke, M. Martin, and S. Mérillat. 2019. Brain structure and cognitive ability in healthy aging: a review on longitudinal correlated change. *Rev. Neurosci.* 31:1–57. doi:10.1515/revneuro-2018-0096.

Pai, J.K., T. Pischon, J. Ma, J.E. Manson, S.E. Hankinson, K. Joshipura, G.C. Curhan, N. Rifai, C.C. Cannuscio, M.J. Stampfer, and E.B. Rimm. 2004. Inflammatory markers and the risk of coronary heart disease in men and women. *N. Engl. J. Med.* 351:2599–2610. doi:10.1056/nejmoa040967.

Palliyaguru, D.L., E.J. Shiroma, J.K. Nam, E. Duregon, C. Vieira Ligo Teixeira, N.L. Price, M. Bernier, S. Camandola, K.L. Vaughan, R.J. Colman, A. Deighan, R. Korstanje, L.L. Peters, S.L. Dickinson, K. Ejima, E.M. Simonsick, L.J. Launer, C.W. Chia, J. Egan, D.B. Allison, G.A. Churchill, R.M. Anderson, L. Ferrucci, J.A. Mattison, and R. de Cabo. 2021. Fasting blood glucose as a predictor of mortality: lost in translation. *Cell Metab.* 33:2189-2200.e3. doi:10.1016/j.cmet.2021.08.013.

Panyard, D.J., B. Yu, and M.P. Snyder. 2022. The metabolomics of human aging: advances, challenges, and opportunities. *Sci. Adv.* 8:eadd6155. doi:10.1126/sciadv.add6155.

Patel, K.V., R.D. Semba, L. Ferrucci, A.B. Newman, L.P. Fried, R.B. Wallace, S. Bandinelli, C.S. Phillips, B. Yu, S. Connelly, M.G. Shlipak, P.H.M. Chaves, L.J. Launer, W.B. Ershler, T.B. Harris, D.L. Longo, and J.M. Guralnik. 2010. Red cell distribution width and mortality in older adults: a meta-analysis. *J. Gerontol. A. Biol. Sci. Med. Sci.* 65:258–265. doi:10.1093/gerona/glp163.

Peters, M.J., R. Joehanes, L.C. Pilling, C. Schurmann, K.N. Conneely, J. Powell, E. Reinmaa, G.L. Sutphin, A. Zhernakova, K. Schramm, Y.A. Wilson, S. Kobes, T. Tukiainen, NABEC/UKBEC Consortium, Y.F. Ramos, H.H.H. Göring, M. Fornage, Y. Liu, S.A. Gharib, B.E. Stranger, P.L. De Jager, A. Aviv, D. Levy, J.M. Murabito, P.J. Munson, T. Huan, A. Hofman, A.G. Uitterlinden, F. Rivadeneira, J. van Rooij, L. Stolk, L. Broer, M.M.P.J. Verbiest, M. Jhamai, P. Arp, A. Metspalu, L. Tserel, L. Milani, N.J. Samani, P. Peterson, S. Kasela, V. Codd, A. Peters, C.K. Ward-Caviness, C. Herder, M. Waldenberger, M. Roden, P. Singmann, S. Zeilinger, T. Illig, G. Homuth, H.-J. Grabe, H. Völzke, L. Steil, T. Kocher, A. Murray, D. Melzer, H. Yaghootkar, S. Bandinelli, E.K. Moses, J.W. Kent, J.E. Curran, M.P. Johnson, S. Williams-Blangero, H.-J. Westra, A.F. McRae, J.A. Smith, S.L.R. Kardia, I. Hovatta, M. Perola, S. Ripatti, V. Salomaa, A.K. Henders, N.G. Martin, A.K. Smith, D. Mehta, E.B.

Binder, K.M. Nylocks, E.M. Kennedy, T. Klengel, J. Ding, A.M. Suchy-Dicey, D.A. Enquobahrie, J. Brody, J.I. Rotter, Y.-D.I. Chen, J. Houwing-Duistermaat, M. Kloppenburg, P.E. Slagboom, Q. Helmer, W. den Hollander, S. Bean, T. Raj, N. Bakhshi, Q.P. Wang, L.J. Oyston, B.M. Psaty, R.P. Tracy, et al. 2015. The transcriptional landscape of age in human peripheral blood. *Nat. Commun.* 6:8570. doi:10.1038/ncomms9570.

Peters, R. 2006. Ageing and the brain. *Postgrad. Med. J.* 82:84–88. doi:10.1136/pgmj.2005.036665.

Porter, H.L., C.A. Brown, X. Roopnarinesingh, C.B. Giles, C. Georgescu, W.M. Freeman, and J.D. Wren. 2021. Many chronological aging clocks can be found throughout the epigenome: implications for quantifying biological aging. *Aging Cell*. 20:e13492. doi:10.1111/acel.13492.

Potvin, A.R., A.R. Potvin, K. Syndulko, K. Syndulko, W.W. Tourtellotte, W.W. Tourtellotte, W.W. Tourtellotte, J.A. Lemmon, J.H. Potvin, and J.H. Potvin. 1980. Human neurologic function and the aging process. *J. Am. Geriatr. Soc.* doi:10.1111/j.1532-5415.1980.tb00115.x.

Pou, K.M., J.M. Massaro, U. Hoffmann, R.S. Vasan, P. Maurovich-Horvat, M.G. Larson, J.F. Keaney, J.B. Meigs, I. Lipinska, S. Kathiresan, J.M. Murabito, C.J. O'Donnell, E.J. Benjamin, and C.S. Fox. 2007. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. *Circulation*. 116:1234–1241. doi:10.1161/circulationaha.107.710509.

Prospective Studies Collaboration, S. Lewington, G. Whitlock, R. Clarke, P. Sherliker, J. Emberson, J. Halsey, N. Qizilbash, R. Peto, and R. Collins. 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet Lond. Engl.* 370:1829–1839. doi:10.1016/s0140-6736(07)61778-4.

Prospective Studies Collaboration, G. Whitlock, S. Lewington, P. Sherliker, R. Clarke, J. Emberson, J. Halsey, N. Qizilbash, R. Collins, and R. Peto. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet Lond. Engl.* 373:1083–1096. doi:10.1016/s0140-6736(09)60318-4.

Putin, E., P. Mamoshina, A. Aliper, M. Korzinkin, A. Moskalev, A. Kolosov, A. Ostrovskiy, C. Cantor, J. Vijg, and A. Zhavoronkov. 2016. Deep biomarkers of human aging: application of deep neural networks to biomarker development. *Aging*. 8:1021–1033. doi:10.18632/aging.100968.

Pyrkov, T.V., and P.O. Fedichev. 2019. Biological age is a universal marker of aging, stress, and frailty. *In* Biomarkers of Human Aging. A. Moskalev, editor. Springer International Publishing Ag, Cham. 23–36.

Pyrkov, T.V., E. Getmantsev, B. Zhurov, K. Avchaciov, M. Pyatnitskiy, L. Menshikov, K. Khodova, A.V. Gudkov, and P.O. Fedichev. 2018a. Quantitative characterization of biological age and frailty based on locomotor activity records. *Aging*. 10:2973–2990. doi:10.18632/aging.101603.

Pyrkov, T.V., K. Slipensky, M. Barg, A. Kondrashin, B. Zhurov, A. Zenin, M. Pyatnitskiy, L. Menshikov, S. Markov, and P.O. Fedichev. 2018b. Extracting biological age from biomedical data via deep learning: too much of a good thing? *Sci. Rep.* 8:5210. doi:10.1038/s41598-018-23534-9.

Pyrkov, T.V., I.S. Sokolov, and P.O. Fedichev. 2021. Deep longitudinal phenotyping of wearable sensor data reveals independent markers of longevity, stress, and resilience. *Aging*. 13:7900–7913. doi:10.18632/aging.202816.

Quach, A., M.E. Levine, T. Tanaka, A.T. Lu, B.H. Chen, L. Ferrucci, B. Ritz, S. Bandinelli, M.L.

Neuhouser, J.M. Beasley, L. Snetselaar, R.B. Wallace, P.S. Tsao, D. Absher, T.L. Assimes, J.D. Stewart, Y. Li, L. Hou, A.A. Baccarelli, E.A. Whitsel, and S. Horvath. 2017. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging*. 9:419–446. doi:10.18632/aging.101168.

Raghavan, S., R.I. Reid, S.A. Przybelski, T.G. Lesnick, J. Graff-Radford, C.G. Schwarz, D.S. Knopman, M.M. Mielke, M.M. Machulda, R.C. Petersen, C.R. Jack, and P. Vemuri. 2021. Diffusion models reveal white matter microstructural changes with ageing, pathology and cognition. *Brain Commun.* 3:fcab106. doi:10.1093/braincomms/fcab106.

Rajpathak, S.N., Y. Liu, O. Ben-David, S. Reddy, G. Atzmon, J. Crandall, and N. Barzilai. 2011. Lifestyle factors of people with exceptional longevity. *J. Am. Geriatr. Soc.* 59:1509–1512. doi:10.1111/j.1532-5415.2011.03498.x.

Rantanen, T., J.M. Guralnik, D. Foley, K. Masaki, S. Leveille, J.D. Curb, and L. White. 1999. Midlife hand grip strength as a predictor of old age disability. *JAMA*. 281:558–560. doi:10.1001/jama.281.6.558.

Rantanen, T., T. Harris, S.G. Leveille, M. Visser, D. Foley, K. Masaki, and J.M. Guralnik. 2000. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. *J. Gerontol. A. Biol. Sci. Med. Sci.* 55:M168-173. doi:10.1093/gerona/55.3.m168.

Raz, N., F.M. Gunning, D. Head, J.H. Dupuis, J. McQuain, S.D. Briggs, W.J. Loken, A.E. Thornton, and J.D. Acker. 1997. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. *Cereb. Cortex N. Y. N 1991*. 7:268–282. doi:10.1093/cercor/7.3.268.

Reeve, E.H., J.N. Barnes, M.E. Moir, and A.E. Walker. 2024. Impact of arterial stiffness on cerebrovascular function: a review of evidence from humans and preclincal models. *Am. J. Physiol. Heart Circ. Physiol.* doi:10.1152/ajpheart.00592.2023.

Ren, J., M. Song, W. Zhang, J.-P. Cai, F. Cao, Z. Cao, P. Chan, C. Chen, G. Chen, H.-Z. Chen, J. Chen, X.-C. Chen, W. Ci, B.-S. Ding, Q. Ding, F. Gao, S. Gao, J.-D.J. Han, Q.-Y. He, K. Huang, Z. Ju, Q.-P. Kong, J. Li, J. Li, J. Li, X. Li, B. Liu, F. Liu, J.-P. Liu, L. Liu, Q. Liu, Q. Liu, X. Liu, Y. Liu, X. Luo, S. Ma, X. Ma, Z. Mao, J. Nie, Y. Peng, J. Qu, R. Ren, W. Song, Z. Songyang, L. Sun, Y.E. Sun, Y. Sun, M. Tian, X.-L. Tian, Y. Tian, J. Wang, S. Wang, S. Wang, W. Wang, X. Wang, X. Wang, Y.-J. Wang, Y. Wang, C.C.L. Wong, A.P. Xiang, Y. Xiao, Z.-X. Xiao, Z. Xie, W. Xiong, D. Xu, Z. Yang, J. Ye, W. Yu, R. Yue, C. Zhang, H. Zhang, L. Zhang, X. Zhang, Y. Zhang, Y.-W. Zhang, Z. Zhang, T. Zhao, Y. Zhao, Z. Zhou, D. Zhu, W. Zou, G. Pei, and G.-H. Liu. 2023. The Aging Biomarker Consortium represents a new era for aging research in China. *Nat. Med.* 29:2162–2165. doi:10.1038/s41591-023-02444-y.

Ridker, P.M., J.E. Buring, N.R. Cook, and N. Rifai. 2003. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events. *Circulation*. 107:391–397. doi:10.1161/01.cir.0000055014.62083.05.

Ridker, P.M., N. Rifai, L. Rose, J.E. Buring, and N.R. Cook. 2002. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.* 347:1557–1565. doi:10.1056/nejmoa021993.

Roberts, H.C., H.J. Denison, H.J. Martin, H.P. Patel, H. Syddall, C. Cooper, and A.A. Sayer. 2011. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. *Age Ageing*. 40:423–429. doi:10.1093/ageing/afr051.

Robinson, O., and C.E. Lau. 2023. How do metabolic processes age: evidence from human metabolomic studies. *Curr. Opin. Chem. Biol.* 76:102360. doi:10.1016/j.cbpa.2023.102360.

Ross, R., S.N. Blair, R. Arena, T.S. Church, J.-P. Després, B.A. Franklin, W.L. Haskell, L.A. Kaminsky, B.D. Levine, C.J. Lavie, J. Myers, J. Niebauer, R. Sallis, S.S. Sawada, X. Sui, U. Wisløff, American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health, Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular and Stroke Nursing, Council on Functional Genomics and Translational Biology, and Stroke Council. 2016. Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign. A scientific statement from the American Heart Association. *Circulation*. 134:e653–e699. doi:10.1161/CIR.0000000000000461.

Roy, B., E. Lee, T. Li, and M. Rampersaud. 2022. Role of miRNAs in neurodegeneration: from disease cause to tools of biomarker discovery and therapeutics. *Genes*. 13:425. doi:10.3390/genes13030425.

Rozing, M.P., R.G.J. Westendorp, A.J.M. de Craen, M. Frölich, M.C.M. de Goeij, B.T. Heijmans, M. Beekman, C.A. Wijsman, S.P. Mooijaart, G.-J. Blauw, P.E. Slagboom, D. van Heemst, and Leiden Longevity Study Group. 2010a. Favorable glucose tolerance and lower prevalence of metabolic syndrome in offspring without diabetes mellitus of nonagenarian siblings: the Leiden longevity study. *J. Am. Geriatr. Soc.* 58:564–569. doi:10.1111/j.1532-5415.2010.02725.x.

Vasudevan, S., A. Saha, M.E. Tarver, and B. Patel. 2022. Digital biomarkers: convergence of digital health technologies and biomarkers. *Npj Digit. Med.* 5:36. doi:10.1038/s41746-022-00583-z.

Wanigatunga, A.A., J. Di, V. Zipunnikov, J.K. Urbanek, P.-L. Kuo, E.M. Simonsick, L. Ferrucci, and J.A. Schrack. 2019. Association of total daily physical activity and fragmented physical activity with mortality in older adults. *JAMA Netw. Open*. 2:e1912352. doi:10.1001/jamanetworkopen.2019.12352.

Warburton, D.E.R., C.W. Nicol, and S.S.D. Bredin. 2006. Health benefits of physical activity: the evidence. *CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can.* 174:801–809. doi:10.1503/cmaj.051351.

Wassel, C.L., E. Barrett-Connor, and G.A. Laughlin. 2010. Association of circulating C-reactive protein and interleukin-6 with longevity into the 80s and 90s: The Rancho Bernardo Study. *J. Clin. Endocrinol. Metab.* 95:4748–4755. doi:10.1210/jc.2010-0473.

Weaver, J.D., M.H. Huang, M. Albert, T. Harris, J.W. Rowe, and T.E. Seeman. 2002. Interleukin-6 and risk of cognitive decline - MacArthur studies of successful aging. *Neurology*. 59:371–378. doi:10.1212/wnl.59.3.371.

Willcox, B.J., T.A. Donlon, Q. He, R. Chen, J.S. Grove, K. Yano, K.H. Masaki, D.C. Willcox, B. Rodriguez, and J.D. Curb. 2008. FOXO3A genotype is strongly associated with human longevity. *Proc. Natl. Acad. Sci. U. S. A.* 105:13987–13992. doi:10.1073/pnas.0801030105.

Williams, S.R., E. Jones, W. Bell, B. Davies, and M.W. Bourne. 1997. Body habitus and coronary heart disease in men. A review with reference to methods of body habitus assessment. *Eur. Heart J.* 18:376–393. doi:10.1093/oxfordjournals.eurheartj.a015258.

Wilson, P.W., R.B. D'Agostino, D. Levy, A.M. Belanger, H. Silbershatz, and W.B. Kannel. 1998. Prediction of coronary heart disease using risk factor categories. *Circulation*. 97:1837–1847. doi:10.1161/01.cir.97.18.1837.

Woerle, H.J., C. Neumann, S. Zschau, S. Tenner, A. Irsigler, J. Schirra, J.E. Gerich, and B. Göke. 2007. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes Importance of postprandial glycemia to achieve target HbA1c levels. *Diabetes Res. Clin. Pract.* 77:280–285. doi:10.1016/j.diabres.2006.11.011.

Wolska, A., and A.T. Remaley. 2022. CRP and high-sensitivity CRP: “What’s in a name?” *J. Appl. Lab. Med.* 7:1255–1258. doi:10.1093/jalm/jfac076.

Xia, S., X. Zhang, S. Zheng, R. Khanabdali, B. Kalionis, J. Wu, W. Wan, and X. Tai. 2016. An update on inflamm-aging: mechanisms, prevention, and treatment. *J. Immunol. Res.* 2016:8426874. doi:10.1155/2016/8426874.

Xia, X., W. Chen, J. McDermott, and J.-D.J. Han. 2017. Molecular and phenotypic biomarkers of aging. *F1000Research*. 6:860. doi:10.12688/f1000research.10692.1.

Yadav, S., null Deepika, and P.K. Maurya. 2024. A systematic review of red blood cells biomarkers in human aging. *J. Gerontol. A. Biol. Sci. Med. Sci.* glae004. doi:10.1093/gerona/glae004.

Yatsunenko, T., F.E. Rey, M.J. Manary, I. Trehan, M.G. Dominguez-Bello, M. Contreras, M. Magris, G. Hidalgo, R.N. Baldassano, A.P. Anokhin, A.C. Heath, B. Warner, J. Reeder, J. Kuczynski, J.G. Caporaso, C.A. Lozupone, C. Lauber, J.C. Clemente, D. Knights, R. Knight, and J.I. Gordon. 2012. Human gut microbiome viewed across age and geography. *Nature*. 486:222–227. doi:10.1038/nature11053.

Ying, K., J.P. Castro, A.V. Shindyapina, A. Tyshkovskiy, M. Moqri, L.J.E. Goeminne, S. Milman, Z.D. Zhang, N. Barzilai, and V.N. Gladyshev. 2024a. Depletion of loss-of-function germline mutations in centenarians reveals longevity genes. *Nat. Commun.* 15:9030. doi:10.1038/s41467-024-52967-2.

Ying, K., S. Paulson, A. Eames, A. Tyshkovskiy, S. Li, M. Perez-Guevara, M. Emamifar, M.C. Martínez, D. Kwon, A. Kosheleva, M.P. Snyder, D. Gobel, C. Herzog, J.R. Poganik, B. of A. Consortium, M. Moqri, and V.N. Gladyshev. 2024b. A unified framework for systematic curation and evaluation of aging biomarkers. doi:10.1101/2023.12.02.569722.

Ying, K., A. Tyshkovskiy, A. Trapp, H. Liu, M. Moqri, C. Kerepesi, and V.N. Gladyshev. 2023. ClockBase: a comprehensive platform for biological age profiling in human and mouse. doi:10.1101/2023.02.28.530532.

Yoshikawa, T.T. 2000. Epidemiology and unique aspects of aging and infectious diseases. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 30:931–933. doi:10.1086/313792.

Yusuf, S., S. Hawken, S. Ounpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen, A. Budaj, P. Pais, J. Varigos, L. Lisheng, and INTERHEART Study Investigators. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet Lond. Engl.* 364:937–952. doi:10.1016/S0140-6736(04)17018-9.

Zenin, A., Y. Tsepilov, S. Sharapov, E. Getmantsev, L. Menshikov, P.O. Fedichev, and Y. Aulchenko. 2019. Identification of 12 genetic loci associated with human healthspan. *Commun. Biol.* 2:41. doi:10.1038/s42003-019-0290-0.

Zhang, Q., C.L. Vallerga, R.M. Walker, T. Lin, A.K. Henders, G.W. Montgomery, J. He, D. Fan, J. Fowdar, M. Kennedy, T. Pitcher, J. Pearson, G. Halliday, J.B. Kwok, I. Hickie, S. Lewis, T. Anderson, P.A. Silburn, G.D. Mellick, S.E. Harris, P. Redmond, A.D. Murray, D.J. Porteous, C.S. Haley, K.L. Evans, A.M. McIntosh, J. Yang, J. Gratten, R.E. Marioni, N.R. Wray, I.J. Deary, A.F. McRae, and P.M. Visscher. 2019. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. *Genome Med.* 11:54. doi:10.1186/s13073-019-0667-1.

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

Zhavoronkov, A., R. Li, C. Ma, and P. Mamoshina. 2019. Deep biomarkers of aging and longevity: from research to applications. *Aging*. 11:10771–10780. doi:10.18632/aging.102475.

Zhavoronkov, A., and P. Mamoshina. 2019. Deep aging clocks: the emergence of AI-based biomarkers of aging and longevity. *Trends Pharmacol. Sci.* 40:546–549. doi:10.1016/j.tips.2019.05.004.

Zhou, Y., G. Hu, and M.C. Wang. 2021. Host and microbiota metabolic signals in aging and longevity. *Nat. Chem. Biol.* 17:1027–1036. doi:10.1038/s41589-021-00837-z.

Zieman, S.J., V. Melenovsky, and D.A. Kass. 2005. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler. Thromb. Vasc. Biol.* 25:932–943. doi:10.1161/01.ATV.0000160548.78317.29.

Zitt, E., A. Fischer, K. Lhotta, H. Concin, and G. Nagel. 2020. Sex- and age-specific variations, temporal trends and metabolic determinants of serum uric acid concentrations in a large population-based Austrian cohort. *Sci. Rep.* 10:7578. doi:10.1038/s41598-020-64587-z.

Rozing, M.P., R.G.J. Westendorp, A.J.M. de Craen, M. Frölich, B.T. Heijmans, M. Beekman, C. Wijsman, S.P. Mooijaart, G.-J. Blauw, P.E. Slagboom, D. van Heemst, and Leiden Longevity Study (LLS) Group. 2010b. Low serum free triiodothyronine levels mark familial longevity: the Leiden Longevity Study. *J. Gerontol. A. Biol. Sci. Med. Sci.* 65:365–368. doi:10.1093/gerona/glp200.

Ruby, J.G., K.M. Wright, K.A. Rand, A. Kermany, K. Noto, D. Curtis, N. Varner, D. Garrigan, D. Slinkov, I. Dorfman, J.M. Granka, J. Byrnes, N. Myres, and C. Ball. 2018. Estimates of the heritability of human longevity are substantially inflated due to assortative mating. *Genetics*. 210:1109–1124. doi:10.1534/genetics.118.301613.

Rutledge, J., H. Oh, and T. Wyss-Coray. 2022. Measuring biological age using omics data. *Nat. Rev. Genet.* 23:715–727. doi:10.1038/s41576-022-00511-7.

Sarwar, N., P. Gao, S.R.K. Seshasai, R. Gobin, S. Kaptoge, E. Di Angelantonio, E. Ingelsson, D.A. Lawlor, E. Selvin, M. Stampfer, C.D.A. Stehouwer, S. Lewington, L. Pennells, A. Thompson, N. Sattar, I.R. White, K.K. Ray, J. Danesh, R.W. Tipping, C.E. Ford, S.L. Pressel, A.R. Folsom, L.E. Chambless, L.E. Wagenknecht, D.B. Panagiotakos, C. Pitsavos, C. Chrysohoou, C. Stefanadis, M. Knuiman, P.H. Whincup, S.G. Wannamethee, R.W. Morris, S. Kiechl, J. Willeit, F. Oberhollenzer, A. Mayr, N. Wald, S. Ebrahim, J.W. Yarnell, J. Gallacher, E. Casiglia, V. Tikhonoff, P.J. Nietert, S.E. Sutherland, D.L. Bachman, J.E. Keil, I.H. de Boer, J.R. Kizer, K.J. Mukamal, A. Tybjaerg-Hansen, B.G. Nordestgaard, M. Benn, R. Frikke-Schmidt, S. Giampaoli, L. Palmieri, S. Panico, D. Vanuzzo, L. Pilotto, A.G. de la Camara, M.A. Rubio, L. Simons, J. McCallum, Y. Friedlander, F.G.R. Fowkes, A.J. Lee, J. Taylor, J.M. Guralnik, C.L. Phillips, R. Wallace, J.M. Guralnik, C.L. Phillips, D.G. Blazer, J.M. Guralnik, C.L. Phillips, C.L. Phillips, J.M. Guralnik, K.T. Khaw, H. Brenner, E. Raum, H. Mueller, D. Rothenbacher, J.H. Jansson, P. Wennberg, A. Nissinen, C. Donfrancesco, S. Giampaoli, V. Salomaa, K. Harald, P. Jousilahti, E. Vartiainen, M. Woodward, R.B. D'Agostino, R.S. Vasan, C.S. Fox, M.J. Pencina, E. Bladbjerg, T. Jorgensen, L. Moller, et al. 2010. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet*. 375:2215–2222. doi:10.1016/s0140-6736(10)60484-9.

Scahill, R.I., C. Frost, R. Jenkins, J.L. Whitwell, M.N. Rossor, and N.C. Fox. 2003. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch. Neurol.* 60:989–994. doi:10.1001/archneur.60.7.989.

Schiffrin, E.L., M.L. Lipman, and J.F.E. Mann. 2007. Chronic kidney disease - Effects on the cardiovascular system. *Circulation*. 116:85–97. doi:10.1161/circulationaha.106.678342.

Schmidt, R., H. Schmidt, J.D. Curb, K. Masaki, L.R. White, and L.J. Launer. 2002. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia aging study. *Ann. Neurol.* 52:168–174. doi:10.1002/ana.10265.

Schnell, A., D.R. Littman, and V.K. Kuchroo. 2023. TH17 cell heterogeneity and its role in tissue inflammation. *Nat. Immunol.* 24:19–29. doi:10.1038/s41590-022-01387-9.

Schrack, J.A., P.-L. Kuo, A.A. Wanigatunga, J. Di, E.M. Simonsick, A.P. Spira, L. Ferrucci, and V. Zipunnikov. 2019. Active-to-sedentary behavior transitions, fatigability, and physical functioning in older adults. *J. Gerontol. A. Biol. Sci. Med. Sci.* 74:560–567. doi:10.1093/gerona/gly243.

Schram, M.T., S.M. Euser, A.J.M. de Craen, J.C. Witteman, M. Frölich, A. Hofman, J. Jolles, M.M.B. Breteler, and R.G.J. Westendorp. 2007. Systemic markers of inflammation and cognitive decline in old age. *J. Am. Geriatr. Soc.* 55:708–716. doi:10.1111/j.1532-5415.2007.01159.x.

Sebastiani, P., N. Solovieff, A.T. Dewan, K.M. Walsh, A. Puca, S.W. Hartley, E. Melista, S. Andersen, D.A. Dworkis, J.B. Wilk, R.H. Myers, M.H. Steinberg, M. Montano, C.T. Baldwin, J. Hoh, and T.T. Perls. 2012. Genetic signatures of exceptional longevity in humans. *PLoS One*. 7:e29848. doi:10.1371/journal.pone.0029848.

Sergi, G., M. De Rui, B. Stubbs, N. Veronese, and E. Manzato. 2017. Measurement of lean body mass using bioelectrical impedance analysis: a consideration of the pros and cons. *Aging Clin. Exp. Res.* 29:591–597. doi:10.1007/s40520-016-0622-6.

Serhan, C.N., and B.D. Levy. 2018. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J. Clin. Invest.* 128:2657–2669. doi:10.1172/JCI97943.

Shaffer, F., and J.P. Ginsberg. 2017. An overview of heart rate variability metrics and norms. *Front. Public Health*. 5:258. doi:10.3389/fpubh.2017.00258.

Shamir, L. 2015. Composite aging markers can be used for quantitative profiling of aging. *Gerontology*. 62:66–68. doi:10.1159/000433466.

Sharma, N., R. Schwendimann, O. Endrich, D. Ausserhofer, and M. Simon. 2021. Comparing Charlson and Elixhauser comorbidity indices with different weightings to predict in-hospital mortality: an analysis of national inpatient data. *BMC Health Serv. Res.* 21:13. doi:10.1186/s12913-020-05999-5.

Sheinerman, K.S., and S.R. Umansky. 2013. Circulating cell-free microRNA as biomarkers for screening, diagnosis and monitoring of neurodegenerative diseases and other neurologic pathologies. *Front. Cell. Neurosci.* doi:10.3389/fncel.2013.00150.

Shlipak, M.G., M.J. Sarnak, R. Katz, L.F. Fried, S.L. Seliger, A.B. Newman, D.S. Siscovick, and C. Stehman-Breen. 2005. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N. Engl. J. Med.* 352:2049–2060. doi:10.1056/nejmoa043161.

Solovev, I., M. Shaposhnikov, and A. Moskalev. 2020. Multi-omics approaches to human biological age estimation. *Mech. Ageing Dev.* 185:111192. doi:10.1016/j.mad.2019.111192.

Sprott, R.L. 2010. Biomarkers of aging and disease: introduction and definitions. *Exp. Gerontol.* 45:2–4. doi:10.1016/j.exger.2009.07.008.

Stevens, L.A., J. Coresh, T. Greene, and A.S. Levey. 2006. Assessing kidney function--measured and estimated glomerular filtration rate. *N. Engl. J. Med.* 354:2473–2483. doi:10.1056/nejmra054415.

Stevens, L.A., C.H. Schmid, T. Greene, L. Li, G.J. Beck, M.M. Joffe, M. Froissart, J.W. Kusek, Y.L. Zhang, J. Coresh, and A.S. Levey. 2009. Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int.* 75:652–660. doi:10.1038/ki.2008.638.

Steves, C.J., T.D. Spector, and S.H.D. Jackson. 2012. Ageing, genes, environment and epigenetics: what twin studies tell us now, and in the future. *Age Ageing*. 41:581–586. doi:10.1093/ageing/afs097.

Studenski, S., S. Perera, K. Patel, C. Rosano, K. Faulkner, M. Inzitari, J. Brach, J. Chandler, P. Cawthon, E.B. Connor, M. Nevitt, M. Visser, S. Kritchevsky, S. Badinelli, T. Harris, A.B. Newman, J. Cauley, L. Ferrucci, and J. Guralnik. 2011. Gait speed and survival in older adults. *JAMA*. 305:50–58. doi:10.1001/jama.2010.1923.

Studenski, S., S. Perera, D. Wallace, J.M. Chandler, P.W. Duncan, E. Rooney, M. Fox, and J.M. Guralnik. 2003. Physical performance measures in the clinical setting. *J. Am. Geriatr. Soc.* 51:314–322. doi:10.1046/j.1532-5415.2003.51104.x.

Suh, Y., G. Atzmon, M.-O. Cho, D. Hwang, B. Liu, D.J. Leahy, N. Barzilai, and P. Cohen. 2008. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl. Acad. Sci. U. S. A.* 105:3438–3442. doi:10.1073/pnas.0705467105.

Sullivan, E.V., and A. Pfefferbaum. 2006. Diffusion tensor imaging and aging. *Neurosci. Biobehav. Rev.* 30:749–761. doi:10.1016/j.neubiorev.2006.06.002.

Sun, E.D., Y. Qian, R. Oppong, T.J. Butler, J. Zhao, B.H. Chen, T. Tanaka, J. Kang, C. Sidore, F. Cucca, S. Bandinelli, G.R. Abecasis, M. Gorospe, L. Ferrucci, D. Schlessinger, I. Goldberg, and J. Ding. 2021. Predicting physiological aging rates from a range of quantitative traits using machine learning. *Aging*. 13:23471–23516. doi:10.18632/aging.203660.

Sun, J., Y. Li, M. Zhao, X. Yu, C. Zhang, C.G. Magnussen, and B. Xi. 2023. Association of the American Heart Association's new "Life's Essential 8" with all-cause and cardiovascular disease-specific mortality: prospective cohort study. *BMC Med.* 21:116. doi:10.1186/s12916-023-02824-8.

Sundstrom, J., L. Sullivan, R.B. D'Agostino, D. Levy, W.B. Kannel, and R.S. Vasan. 2005. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. *Hypertension*. 45:28–33. doi:10.1161/01.hyp.0000150784.92944.9a.

Tanaka, T., A. Biancotto, R. Moaddel, A.Z. Moore, M. Gonzalez-Freire, M.A. Aon, J. Candia, P. Zhang, F. Cheung, G. Fantoni, CHI consortium, R.D. Semba, and L. Ferrucci. 2018. Plasma proteomic signature of age in healthy humans. *Aging Cell*. 17:e12799. doi:10.1111/acel.12799.

Temelkova-Kurktschiev, T.S., C. Koehler, E. Henkel, W. Leonhardt, K. Fuecker, and M. Hanefeld. 2000. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA(1c) level. *Diabetes Care*. 23:1830–1834. doi:10.2337/diacare.23.12.1830.

Terry, D.F., J.C. Evans, M.J. Pencina, J.M. Murabito, R.S. Vasan, P.A. Wolf, M. Kelly-Hayes, D. Levy, R.B. D'Agostino, and E.J. Benjamin. 2007. Characteristics of Framingham offspring participants with long-lived parents. *Arch. Intern. Med.* 167:438–444. doi:10.1001/archinte.167.5.438.

Tsao, C.W., A.W. Aday, Z.I. Almarzooq, C.A.M. Anderson, P. Arora, C.L. Avery, C.M. Baker-Smith, A.Z. Beaton, A.K. Boehme, A.E. Buxton, Y. Commodore-Mensah, M.S.V. Elkind, K.R. Evenson, C. Eze-Nliam, S. Fugar, G. Generoso, D.G. Heard, S. Hiremath, J.E. Ho, R. Kalani, D.S. Kazi, D. Ko, D.A. Levine, J. Liu, J. Ma, J.W. Magnani, E.D. Michos, M.E. Mussolino, S.D. Navaneethan, N.I. Parikh, R. Poudel, M. Rezk-Hanna, G.A. Roth, N.S.

Shah, M.-P. St-Onge, E.L. Thacker, S.S. Virani, J.H. Voeks, N.-Y. Wang, N.D. Wong, S.S. Wong, K. Yaffe, S.S. Martin, and American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. 2023. Heart disease and stroke statistics-2023 update: a report from the American Heart Association. *Circulation*. 147:e93–e621. doi:10.1161/CIR.0000000000001123.

Uchai, S., L.F. Andersen, L.A. Hopstock, and A. Hjartåker. 2023. Body mass index, waist circumference and pre-frailty/frailty: the Tromsø study 1994-2016. *BMJ Open*. 13:e065707. doi:10.1136/bmjopen-2022-065707.

Umetani, K., D.H. Singer, R. McCraty, and M. Atkinson. 1998. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31:593–601. doi:10.1016/s0735-1097(97)00554-8.

Van Nguyen, A., and J.W. Pollard. 2002. Colony stimulating factor-1 is required to recruit macrophages into the mammary gland to facilitate mammary ductal outgrowth. *Dev. Biol.* 247:11–25. doi:10.1006/dbio.2002.0669.

Varol, C., A. Mildner, and S. Jung. 2015. Macrophages: development and tissue specialization. *Annu. Rev. Immunol.* 33:643–675. doi:10.1146/annurev-immunol-032414-112220.

Table 1. Longevity biomarker summary table.

Category	Biomarker	Physiological association	Measurement method	Application	Limitation	References
Visual	Overall appearance	Adiposity; Sarcopenia; Posture; Skin changes	Visual inspection: photography, video, mirror, etc.	- Easy at-home self-assessment of general fitness, body composition, and visible aging signs. - Provides a quick overview of overall physical appearance and musculoskeletal changes.	- Does not account for visceral or intramuscular fat, functional capacity, nor existing pathology. - Assessment is subjective and can miss subtle changes.	
	Eye corners	General aging; Skin aging; Tissue elasticity	ML-aided image analysis	- Non-invasive visual biomarker of aging: automated assessment of wrinkles and tissue properties around the eye can provide an objective measure of biological age.	- Requires specialized software. - May be affected by temporary factors, e.g. hydration, sleep, and environment.	(Bobrov et al., 2018)
Physiological	Grip strength	Sarcopenia; Upper extremity muscle function; Frailty; Overall strength	Handgrip dynamometry	- Direct quantifiable measure of muscle function and strength. - Strong predictor of mortality, morbidity, and functional decline.	- Can be influenced by acute conditions, e.g. inflammation, joint/muscle injuries. - Limited by pre-existing joint/neuromuscular problems. - Variations in measurement make standardizing difficult. - Optimization for grip strength reduces utility.	(Rantanen et al., 1999; Metter et al., 2002; Newman et al., 2006; Rantanen et al., 2000; Roberts et al., 2011; Leong et al., 2015; Bohannon, 2019)
	Muscle mass	Sarcopenia; Metabolic function; Global physical function	Bioelectric impedance analysis (BIA)	- Accurate and objective measure of body composition, i.e. the muscle/fat mass ratio, total body water content.	- Function and performance matter more than muscle mass alone; not as informative as direct measures of muscle strength and quality. - Can be affected by hydration status. - Requires specialized equipment.	(Kyle et al., 2004; Sergi et al., 2017)
	5x sit-to-stand	Sarcopenia; Lower extremity musculoskeletal function; Frailty;	Stopwatch measurement: time (s)	- Standardized assessment of functional lower body strength,	- Limited utility in highly functional younger or middle-aged adults.	(Muñoz-Bermejo et al., 2021)

		Balance		power, and dynamic balance. - Predictive of fall risk and functional decline, especially in older adults.	- Requires careful standardization testing protocol, e.g. technique and chair height.	
	30-second chair stand	Sarcopenia; Lower extremity musculoskeletal function; Frailty; Balance	Stopwatch measurement: number of repetitions in 30 s (times)	- Evaluation of functional lower body strength and endurance. - Suitable for assessing a wide range of fitness levels from frail to highly active. - Provides insights into mobility and fall risk.	- May not be sensitive enough to detect subtle changes in very fit individuals. - Requires careful standardization testing protocol, e.g. technique and chair height.	(Jones et al., 1999)
	6-min walk test	Sarcopenia; Lower extremity musculoskeletal function; Frailty	Stopwatch measurement: distance in 6 min (m)	- Distance walked within predefined time as a proxy for overall physical performance. - Submaximal exercise test that measures functional exercise capacity and correlates with activities of daily living. - Provides insights into overall physical performance and cardiovascular fitness.	- Requires adequate space. - May be limited by joint pain, balance issues, or other physical impairments.	(Enright et al., 2003; Enright, 2003)
	Gait speed	Sarcopenia; Global physical function; Frailty	Stopwatch measurement: speed (m/s)	- Time to walk a predefined distance as a proxy for overall physical performance. - Quick assessment of functional mobility and fall risk. - Strong predictor of adverse outcomes like disability, hospitalization and mortality. - Provides insights into neuromuscular control and cognitive function.	- Can be affected by acute medical conditions, neurological issues, or pain. - Variations in testing protocols make standardization challenging. - Limited utility in highly functional adults.	(Guralnik et al., 2000; Studenski et al., 2011)
	VO_2max	Cardiovascular function; Pulmonary function; Musculoskeletal function; Metabolic function	Open-circuit spirometry	- Gold standard measurement of cardiorespiratory fitness and aerobic capacity. - Provides precise quantification of an individual's maximal	- Requires maximal physical exertion, specialized equipment, as well as skilled personnel to administer the test.	(Hawkins et al., 2007; Franklin et al., 2018)

				oxygen uptake under peak exercise load. - Reflects the integrated function of the cardiovascular, pulmonary, and musculoskeletal systems.	- Not feasible for routine clinical use in all populations.	
	Body weight	Overall health status; Metabolic function; Nutrition status	Weight measurement (kg)	- Simple, accessible measure of overall body mass. - Can track changes over time as an indicator of general health status, nutritional status, and metabolic function.	- Does not differentiate between muscle and fat mass. - Can be affected by hydration status and time of day.	(Hubert et al., 1983; Hubert, 1986; Kopelman, 2000)
	Waist circumference (WC)	Central adiposity; Metabolic function; Cardiovascular risk	Girth measurement (cm)	- Easy, low-cost assessment of abdominal adiposity. - Strong predictor of metabolic disease risk and cardiovascular health.	- Measurement technique can vary, leading to inconsistencies. - May miss metabolically unhealthy individuals with normal weight ("skinny fat").	(Williams et al., 1997; Lakka et al., 2002; Janssen et al., 2002; Kuk et al., 2006)
	Waist-to-hip ratio (WHR)	Fat distribution; Metabolic function; Cardiovascular risk	Waist/hip circumference measurement ratio	- Better predictor of health risks than waist circumference alone, as it accounts for body shape and fat distribution. - Provides insights into metabolic and cardiovascular health.	- Measurement standardization can be challenging. - May not fully reflect changes in overall adiposity over time, e.g. subcutaneous vs. visceral fat.	(Williams et al., 1997; Lakka et al., 2002)
	Body mass index (BMI)	Overall adiposity	Weight (kg)/height $(m)^2$	- Calculation based on height and weight according to a standard formula. - Simple, widely-used screening tool for assessing overall body adiposity at the population level. - Provides a general indication of health risks associated with being underweight, overweight, or obese.	- Poor at differentiating muscle from fat mass, limiting its utility for athletic or muscular individuals. - Not an accurate measure of body composition for all ethnic groups.	(Kopelman, 2000; Lakka et al., 2002; Prospective Studies Collaboration et al., 2009; Flegal et al., 2013)

	Coronary artery calcium (CAC)	Vascular health; Atherosclerosis Cardiovascular risk	Computer tomography (CT)	- Multislice CT scan without contrast to measure coronary artery calcium deposits - Direct, quantitative measure of coronary artery calcification, which is a marker of atherosclerosis. - Strong predictor of future cardiovascular events. - Provides insights into subclinical vascular aging.	- Requires exposure to ionizing radiation from CT imaging. - Expensive procedure that is not suitable for frequent monitoring; limited availability and access.	(Greenland et al., 2018)
	Pulse wave velocity (PWV)	Vascular health; Arterial stiffness; Cardiovascular risk	Specialized pulse wave analysis	- Non-invasive measure of arterial stiffness, which is an early marker of cardiovascular disease and vascular aging, based on pulse wave propagation speed. - Provides insights into overall arterial health.	- Requires specialized equipment and technical expertise to perform and interpret the measurements. - Multiple testing protocols exist, making standardization challenging.	(Laurent et al., 2001; Mitchell et al., 2004, 2007, 2010)
	Heart rate variability (HRV)	Autonomic nervous system function; Recovery capacity	Continuous ECG monitoring	- Continuous measurement of physical activity in 5 min to 24 h timeframes (with wearables). - Non-invasive assessment of autonomic nervous system function. - Provides insights into an individual's physiological stress response, recovery capacity, and overall cardiovascular health.	- Utility declines with advanced age; less useful at ages >65 years. - Can be affected by medications or physical conditions. - Lack of standardized testing conditions.	(Umetani et al., 1998; Cole et al., 1999; Jouven et al., 2005; Shaffer and Ginsberg, 2017)
	Blood pressure	Cardiovascular function; Arterial health; Autonomic nervous system function	Sphygmomanometry	- Simple, widely available screening tool for assessing cardiovascular function, arterial health, and autonomic regulation, using a manual or automatic sphygmomanometer. - Provides a basic indication of	- Measures comorbidity rather than longevity potential. - Highly variable measurements due to factors like "white coat effect", improper technique, and diurnal fluctuations.	(Lewington et al., 2002; Yusuf et al., 2004; Buford, 2016; Fuchs and Whelton, 2020)

				hypertension risk and overall cardiovascular health.	- Requires careful standardization of measurement protocol.	
Neurological	Sensory acuity	Neural function; Neural aging; Cognitive processing	Various sensory tests	- Assessment of the integrity and function of the sensory systems, including vision, hearing, touch, and smell. - Can provide early detection of neural decline associated with aging or neurodegeneration.	- Multiple testing methods are required to comprehensively assess different sensory modalities. - Subjective components make standardization challenging. - Hard to differentiate between general neural aging and the deterioration of associated sensory organs.	(Lindenberger and Baltes, 1994; Baltes and Lindenberger, 1997; Brenowitz et al., 2020; Aging Biomarker Consortium et al., 2023b)
	Total brain volume (TBV)	Brain aging; Neurodegeneration	MRI volumetric analysis	- Quantitative measure of overall brain structure that can be used to track age-related changes and assess cognitive reserve. - Provides insights into global brain aging and risk of neurodegeneration.	- Expensive and requires specialized neuroimaging equipment and expertise. - Does not account for differential regional volume changes during aging.	(Liu et al., 2003; Oschwald et al., 2019; Blinkouskaya et al., 2021; Aging Biomarker Consortium et al., 2023b)
	Grey matter volume (GMV)	Cognitive function; Neural plasticity; Brain aging	MRI volumetric analysis	- Specific measure of neural tissue volume that is associated with cognitive performance and brain plasticity. - Can provide insights into regional brain aging and neurodegeneration.	- Technically complex analysis, requiring high-resolution imaging and specialized expertise. - Costs associated with advanced neuroimaging techniques.	(Raz et al., 1997; Lemaitre et al., 2012; Farokhian et al., 2017)
	White matter volume (WMV)	Neural connectivity; Brain aging	MRI volumetric analysis	- Assessment of white matter volume, which reflects the integrity of neural networks and information processing capabilities. - Can indicate changes in brain connectivity associated with aging.	- Technically complex analysis, requiring high-resolution imaging and specialized expertise. - Costs associated with advanced neuroimaging techniques. - Age-related changes in white matter volume may be modest or even negligible.	(Liu et al., 2003; Oschwald et al., 2019; Blinkouskaya et al., 2021)
	Hippocampal volume (HV)	Memory function; Brain aging; Neurodegeneration	MRI volumetric analysis	- Targeted assessment of the hippocampus, a	- Technically complex analysis, requiring high-resolution imaging	(Scahill et al., 2003; Liu et al., 2003; Oschwald et al., 2019)

				critical brain structure for memory function. - Can serve as an early marker of cognitive decline and neurodegenerative processes.	and specialized expertise to accurately measure hippocampal volume. - Costs associated with advanced neuroimaging techniques.	
	Fractional anisotropy (FA); Mean diffusivity (MD)	White matter integrity; Neural connectivity; Brain aging	Diffusion tensor MRI	- Detailed assessment of white matter microstructure and integrity, providing insights into neural connectivity and brain network function. - Can indicate changes in white matter associated with aging and neurodegeneration.	- Complex analysis techniques, expensive imaging, and specialized expertise are required to obtain and interpret diffusion-based metrics.	(Sullivan and Pfefferbaum, 2006; Raghavan et al., 2021)
	Brain age	Overall brain health; Brain aging	ML-aided analysis of brain images	- Composite measure that combines multiple neuroimaging biomarkers to provide an estimate of an individual's biological brain age, which can be compared to their chronological age. - Offers a holistic assessment of brain health and aging.	- Dependent on the specific machine learning model used, the quality of input neuroimaging data, and the need for standardization across different imaging protocols and populations.	(More et al., 2023; Liem et al., 2017; Cole et al., 2017, 2018; Franke and Gaser, 2019)
Laboratory	Fasting blood glucose (FBG)	Glucose metabolism; Insulin sensitivity; Metabolic function	Blood test	- Basic screening test for assessing glucose metabolism and insulin sensitivity, providing insights into overall metabolic health and diabetes risk.	- Can be affected by diurnal variation, physical activity, stress, and recent dietary intake. - Limited in its ability to capture the dynamic aspects of glucose regulation.	(Sarwar et al., 2010; Palliyaguru et al., 2021)
	Postprandial glucose	Glucose metabolism; Insulin sensitivity; Metabolic function	Blood test after meal	- Dynamic assessment of glucose handling and metabolic flexibility in response to a standardized meal challenge. - Provides more comprehensive evaluation of glucose metabolism and insulin function.	- Largely depends on the meal composition taken before the test. - Requires timed blood sampling, making it more time-consuming and resource-intensive than fasting glucose. - Variations in testing protocols can affect results.	(Woerle et al., 2007)

	2-hour post-challenge blood glucose (2h-BG)	Glucose metabolism; Insulin sensitivity; Metabolic function	Blood test after glucose challenge	- Gold standard for diagnosing diabetes and assessing overall glucose metabolism and insulin sensitivity. - Offers a dynamic evaluation of an individual's ability to handle a glucose load.	- Time-consuming procedure that requires standardized glucose challenge, patient preparation, and timed blood sampling. - Not feasible for frequent monitoring.	(Borch-Johnsen et al., 2001)
	Glycated hemoglobin (HbA1c)	Glucose metabolism; Long-term glucose control; Metabolic function	Blood test	- Measures average blood glucose levels over the past 2-3 months, providing a stable marker of long-term glucose control and metabolic health. - Can indicate risk of diabetes and associated complications.	- Can be affected by factors like red blood cell lifespan, limiting its utility in certain medical conditions.	(Bennett et al., 2007; Currie et al., 2010)
	Total triglycerides	Lipid metabolism; Cardiovascular risk; Metabolic function	Blood test	- Assessment of triglyceride levels, which reflect lipid metabolism and can provide insights into cardiovascular disease risk and overall metabolic health.	- Can be affected by diurnal variation and recent dietary intake. - Can be affected by individual and population-level confounders, e.g. medication or ethnic background	(Prospective Studies Collaboration et al., 2007; Nordestgaard et al., 2007)
	Total cholesterol (TC)	Lipid metabolism; Cardiovascular risk; Metabolic function	Blood test	- Basic lipid screening test that provides a measure of total cholesterol levels. - Can be used for initial cardiovascular risk assessment; requires context of other lipid markers.	- Limited utility as a standalone marker, as it does not differentiate between "good" and "bad" cholesterol fractions: HDL-C and LDL-C, respectively.	(Prospective Studies Collaboration et al., 2007)
	HDL-cholesterol (HDL-C)	Lipid metabolism; Cardiovascular risk; Metabolic function	Blood test	- Provides insights into cardiovascular protective mechanisms and overall metabolic health.	- Genetic and physiological factors can influence HDL levels, making its interpretation and utility complex.	(Prospective Studies Collaboration et al., 2007)
	LDL-cholesterol (LDL-C)	Lipid metabolism; Cardiovascular risk; Metabolic function	Blood test	- Primary target for cardiovascular risk reduction and prevention. - Measurement of the "bad" cholesterol fraction is essential for assessing	- LDL is composed of multiple subtypes with differing atherogenic potential.	(Prospective Studies Collaboration et al., 2007)

				and managing cardiovascular disease risk.		
	C-reactive protein (CRP)	Systemic inflammation; Inflammatory signaling; Immune function	Blood test	- Sensitive marker of systemic inflammation, with associations with aging processes and various disease states. - Can provide insights into overall health and disease risk.	- A non-specific marker, as CRP can be elevated in response to acute conditions, infections, or other inflammatory processes.	(Frohlich et al., 2000; Ridker et al., 2002, 2003; Pai et al., 2004; Wolska and Remaley, 2022)
	Interleukin-6 (IL-6)	Systemic inflammation Inflammatory signaling; Immune function	Blood test	- Specific inflammatory cytokine that plays a role in aging, immune function, and disease processes. - Can offer more targeted insights into inflammatory mechanisms underlying health and longevity.	- Non-specific marker, as IL-6 is universally elevated in response to a wide range of inflammatory conditions. - Mainly suited for the diagnosis of acute conditions, e.g. sepsis, rather than routine monitoring. - More technical than routine blood biochemistry, as it relies on the ELISA assay.	(Pai et al., 2004; Wassel et al., 2010; Fabbri et al., 2015)
	α_1-antichymotrypsin (ACT)	Acute phase response; Inflammation; Protein homeostasis	Blood test	- Specific inflammatory marker that is part of the acute phase response, providing insights into protein homeostasis and systemic inflammation. - Can complement other inflammatory biomarkers.	- Limited availability and technical complexity of the assay (ELISA), as well as higher costs (antibodies required), restrict the routine use of ACT testing. - Post-translational modifications of ACT, such as glycosylation, complicate unambiguous interpretation	(Engelhart et al., 2004; Dik et al., 2005; Schram et al., 2007; Jin et al., 2022)
	Serum uric acid (SUA)	Purine metabolism; Oxidative stress; Metabolic function	Blood test	- Marker of metabolic health, with associations to purine metabolism, oxidative stress, and cardiovascular risk. - Can provide insights into an individual's overall metabolic status and estimate risk for comorbidities, such as hypertension and sarcopenia.	- Subject to dietary factors, diurnal variation, and gender differences, complicating the interpretation.	(Kuzuya et al., 2002; Sundstrom et al., 2005; Bos et al., 2006; Chen et al., 2009; Zitt et al., 2020; Guo et al., 2021; Liu et al., 2022)

	8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxo-7,8-dihydroguanosine	DNA/RNA oxidative damage; Cellular stress	Mass spectrometry of urine samples	- Direct measure of oxidative damage to nucleic acids, offering insights into cellular stress and aging processes at the molecular level.	- Complex sample handling and analysis techniques, requiring specialized expertise and equipment. - Higher costs limit the widespread use of this biomarker.	(Gan et al., 2012, 2018; Liang et al., 2020; Jiang et al., 2022)
	Glomerular filtration rate (GFR)	Kidney function; Vascular health	Blood test/calculation	- Assessment of kidney function, based on serum creatinine or cystatin C levels, which is also an indicator of vascular health and can change with aging.	- Estimation formulas used to calculate GFR can vary, and age-related decline in GFR may be within the normal range.	(Fastbom et al., 1996; Dharnidharka et al., 2002; Go et al., 2004; Knight et al., 2004; Stevens et al., 2006, 2009; Inker et al., 2012)
	Urinary albumin	Kidney function; Vascular health	Urine test	- Early marker of kidney damage and subclinical vascular dysfunction. - Provides insights into overall health and disease risk.	- Requires standardization of urine collection and testing procedures. - Normal variation in urinary albumin levels can complicate interpretation. - Albumin levels well below clinical thresholds for kidney damage can also have diagnostic value, complicating differentiation between norm and pathology.	(Gerstein et al., 2001; Hillege et al., 2002; Klausen et al., 2004; Arnlov et al., 2005)
	Red cell distribution width (RCDW)	Hematopoiesis; Overall health	Complete blood count	- General health marker that provides insights into hematopoietic function, aging processes, and overall health status. - Has been associated with mortality risk.	- A non-specific marker that can be influenced by multiple factors, limiting its utility as a standalone biomarker.	(Patel et al., 2010; Lippi et al., 2014)
	Neutrophil-to-lymphocyte ratio (NLR)	Immune function; Inflammation	Complete blood count	- Simple marker of systemic inflammation and immune status. - Reflects balance between innate and adaptive immunity.	- A non-specific marker that can be affected by acute conditions, such as infections. - Limited value as a standalone measure.	(Cataudella et al., 2017)
Genetic	*BRCA1*; *BRCA2*	DNA repair; Cellular maintenance; Cancer risk	DNA sequencing	- Some genetic variants, i.e. polymorphisms, are associated with reduced DNA repair capacity and	- Primarily relevant to prophylaxis of specific cancer types with limited impact on overall longevity.	(Antoniou et al., 2003; Mavaddat et al., 2013)

				elevated breast and ovarian cancer risk. - Important for personalized risk assessment.	- Complex interpretation with other genetic factors.	
	APOE	Cardiovascular risk; Cognitive function; Metabolic function	DNA sequencing	- Certain genetic variants have been shown to be associated with cardiovascular disease and risk for Alzheimer's disease. - Important for personalized health strategies.	- Environmental factors, such as lifestyle, strongly influence outcomes. - Single gene provides limited insight.	(Liu et al., 2013; Reeve et al., 2024)
	IGFRI	Growth signaling; Metabolic function	DNA sequencing	- A gene for the IGF-1 growth factor receptor, involved in cellular growth signaling, metabolism and aging. - Associated with longevity in multiple species.	- Complex interaction with other genes and environment. - Limited predictive value alone.	(Suh et al., 2008)
	FOXO3	Stress resistance; Cellular maintenance	DNA sequencing	- Longevity-associated gene involved in stress response and cellular maintenance. - Has been linked to healthy aging.	- Complex interaction with other genes and environment. - Limited predictive value alone.	(Willcox et al., 2008; Gellhaus et al., 2023)
	INK4A	Cell cycle regulation; Cellular senescence;	DNA sequencing	- Cell cycle regulator involved in cellular senescence and aging. - Marker of cellular senescence.	- Complex interaction with other genes and environment. - Limited predictive value alone.	(Kim and Sharpless, 2006)
Epigenetic	DNA methylation	Gene regulation; Environmental response; Cellular function	On-chip methylation profiling	- Measurement of the abundance of 5mC at select CpG sites across the genome to impute biological age	- Requires sophisticated analysis and equipment. - Temporal and tissue-specific variations complicate interpretation. - Earlier blood-based versions were affected by blood cell composition.	(Kurdyukov and Bullock, 2016)
	Histone modification	Gene regulation; Chromatin structure; Cellular function	ChIP-seq	- Mapping the histone modification landscape to gain insight into the “histone code” and associated gene expression patterns	- Requires sophisticated analysis techniques; time- and labor-intensive. - Tissue-specific variations and environmental effects	(Furey, 2012; Fadri et al., 2024)

					may complicate interpretation.	
	Telomere length	Cellular senescence; Stress exposure	TRF, qPCR, etc.	- Assessment of a cell's replicative potential by measuring non-gene chromosome ends and chromosomal integrity.	- Utility can be influenced by technical variability in measurement. - Complex relationship with health outcomes, as telomere length correlates with environmental confounders, such as psychological stress or infectious diseases.	(Coulter et al., 2024)
	Non-coding RNA profile	Gene regulation; Cellular function	Microarrays, RNA-seq	- Analysis of the non-coding regulatory RNA pool involved in the aging process. - Provides insights into gene regulation networks that may underlie and/or mediate aging.	- Technically complex and laborious analysis. - Complicated interpretation; although agnostic approaches are possible, disentanglement of regulatory networks may be "noise-prone", i.e. it is challenging to infer the target coding mRNA from a microRNA's sequence alone.	(Lowe et al., 2017)
Composite scores	Framingham Risk Score (FRS)	Cardiovascular risk; Overall health	Multiple parameter assessment	- Validated risk assessment tool for cardiovascular disease.	- Limited by included parameters. - May not be applicable to all populations.	(Wilson et al., 1998; Beekman et al., 2016)
	QRISK3	Cardiovascular risk; Overall health	Multiple parameter assessment	- Updated cardiovascular risk assessment based on an expanded panel of additional factors.	- Requires detailed medical history. - May not be globally applicable.	(Hippisley-Cox et al., 2017)
	Life's Essential 8 (LE8)	Cardiovascular health; Overall health; Healthcare outcomes	Multiple parameter assessment	- Modern measure of cardiovascular health incorporating lifestyle factors. - Comprehensive health assessment.	- Requires multiple measurements. - May not capture all relevant health domains.	(Lloyd-Jones et al., 2022)
	Fried's Frailty Index	Frailty; Functional decline	Multiple parameter assessment	- Standardized assessment of physical frailty. - Predicts adverse health outcomes.	- Requires multiple measurements. - May not capture early stages of frailty.	(Fried et al., 2001)
	Charlson Comorbidity Index	Disease burden; Healthcare outcomes	Clinical assessment	- Weighted index of comorbid conditions.	- May not capture severity of conditions. - Limited by included comorbidities.	(Charlson et al., 1987)

				- Predicts mortality risk and healthcare utilization.		
	Elixhauser Comorbidity Index	Disease burden; Healthcare outcomes	Clinical assessment	- Comprehensive assessment of comorbid conditions. - Better discrimination for some outcomes than Charlson.	- Complex scoring system. - Requires detailed medical history.	(Elixhauser et al., 1998)

Figure 1

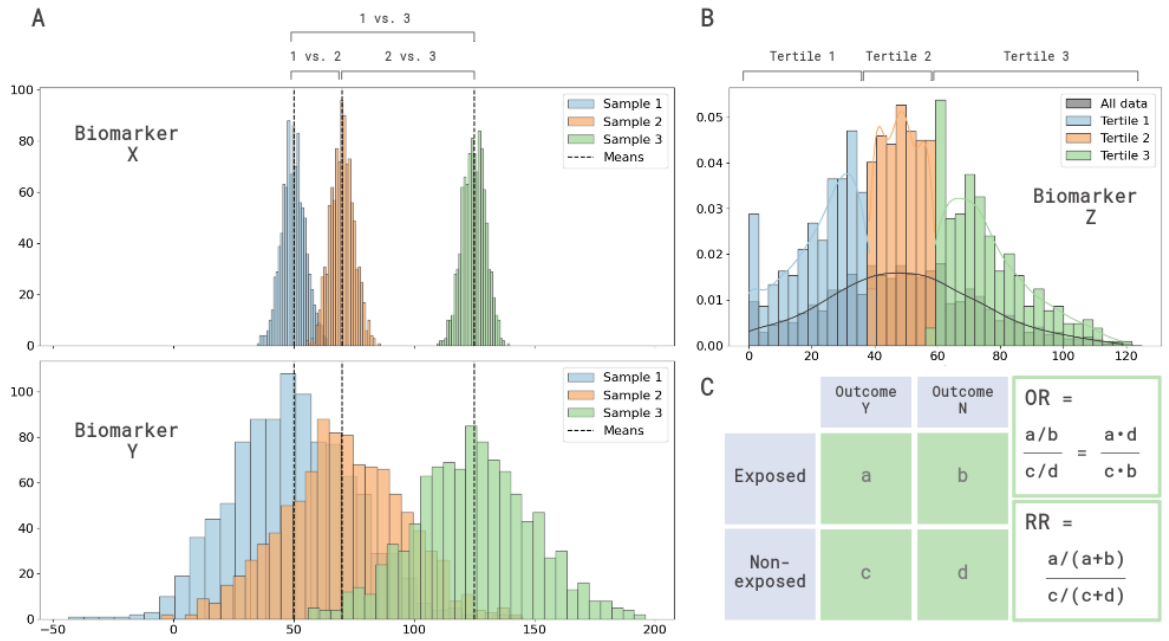

Figure 1. Good biomarkers have strong discriminative ability, correlate with biological function, and have strong predictive power. (A) In three different theoretical sampled populations (blue, orange, and green), with sample size n = 1000 each, the means of biomarker X (top) and Y (bottom) values are the same for each sample taken from the given population (50, 70, and 125, respectively); differences in the biomarker means between the samples are statistically significant for X and Y in each case (1 vs. 2, 2 vs. 3, and 1 vs. 3). The greater variation in Y (standard deviation (SD) = 25) as compared to X (SD = 5) makes the former less useful as a biomarker due to overlap between the sample values from different populations. **(B)** Biomarker Z values within the population follow a normal distribution and can be divided into tertiles according to the biomarker Z readout. **(C)** Calculation of the odds ratio (OR) and relative risk ratio (RR) for an outcome (Y = yes, N = no), e.g. for the highest vs. the lowest tertile from (B), using absolute numbers of persons either exposed or non-exposed to the given tertile biomarker value range. The data were simulated and visualized using Python in JupyterLab. Created with BioRender.com.

Figure 2

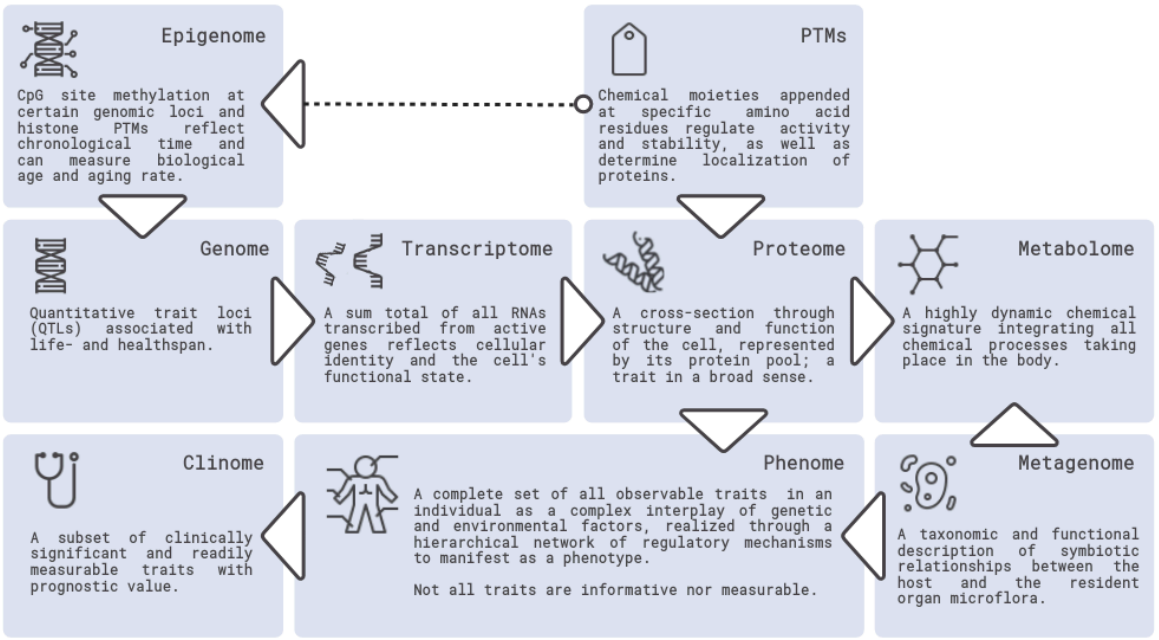

Figure 2. The hierarchical relationship connecting clinical phenotype to the expression and regulation of genetic information. The flow of information in a cell proceeds from DNA (genome) to RNA (transcriptome) to protein (proteome). The epigenome modifies the hosts' genome, regulating gene expression without altering the DNA nucleotide sequence. Genes, expressed as coding and non-coding RNA by the genome, form the transcriptome. Translation of protein-coding mRNAs creates the proteome, which is also influenced by post-translational modifications (PTMs) of proteins. PTMs also regulate the epigenome via histone modification. The proteome can directly impact the organism's overall phenotype (phenome), along with the metabolites produced in biochemical enzyme-catalyzed reactions. In addition, the metagenome, which includes the microbes living on and in an organism, feeds into the metabolome and phenome. Finally, the phenome underlies the clinome, which is the set of clinical traits used to predict and measure disease. Created with BioRender.com. The icons were downloaded from icons8.com.

Figure 3

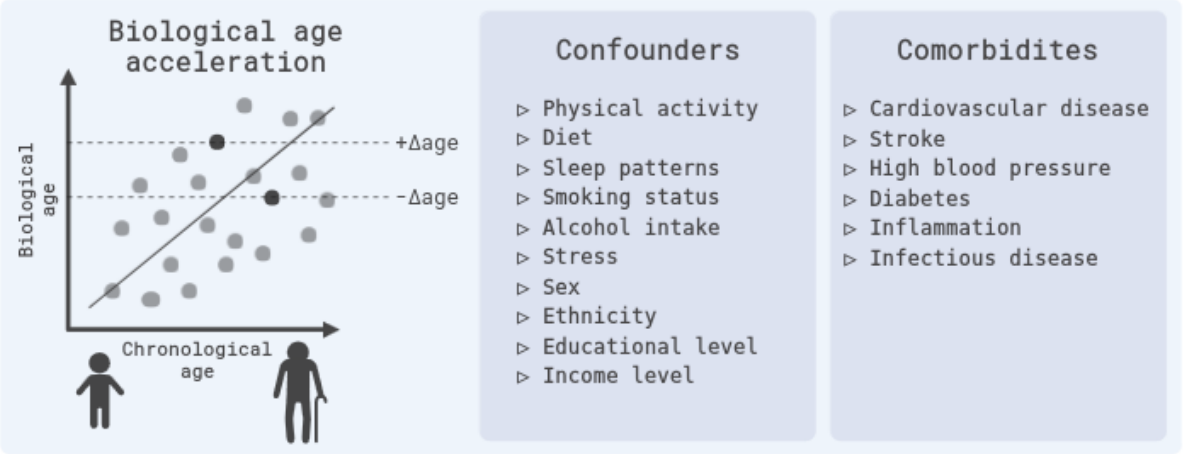

Figure 3. Biological age vs. chronological age. Biological age is an estimate of a person's position on a molecular-genetic, physiological, and/or functional scale in relation to analogous positions of persons of the same age. In case the estimated biological age does not match chronological age—as expressed by positive or negative age delta—biological age acceleration or deceleration has occurred. Estimation of biological status can be potentially confounded by lifestyle factors, socioeconomic background, as well as the presence, absence, or degree of comorbidities. Created with BioRender.com.

Figure 4

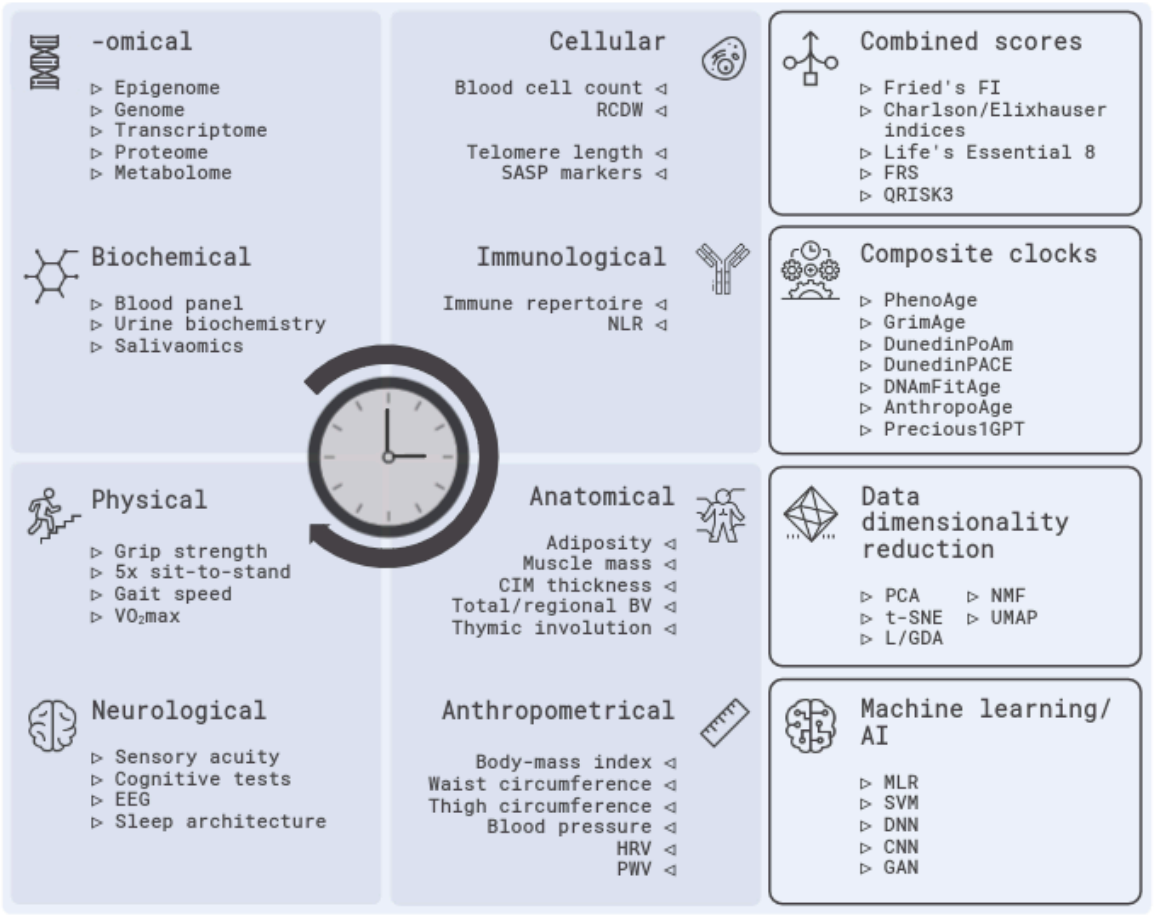

Figure 4. Biomarkers and concepts used to measure longevity. Aging unfolds from the molecular/genetic level to the cellular level to the organismal level of organization, encompassing hierarchically more complex and extensive physiological networks. Individual and composite biomarkers, along with statistical and machine learning/artificial intelligence (AI) methods, can be used to measure the degree and speed of aging. BV, brain volume; CIM, carotid intima-media thickness; CNN, convolutional neural network; DNN, deep neural network; EEG, electroencephalogram; FI, frailty index; FRS, Framingham risk score; GAN, generative adversarial network; HRV, heart rate variability; L/GDA, linear/Gaussian discriminant analysis; MLR, multiple linear regression; NLR, neutrophil-to-lymphocyte ratio; NMF, non-negative matrix factorization; PCA, principal component analysis; PWV, pulse wave velocity; RCDW, red cell distribution width; SASP, senescence-associated secretory phenotype; SVM, support vector machine; t-SNE, t-distributed stochastic neighbor embedding; UMAP, uniform manifold approximation and projection. Created with BioRender.com. The icons were downloaded from icons8.com.