WHITE PAPER

Biological Age, Healthspan, and Longevity

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Abstract

Aging starts at the molecular level, leading to visible changes like wrinkles, muscle loss, chronic diseases, and functional decline. However, lifestyle and environment can influence aging by affecting gene expression, causing "epigenetic" changes that influence health and aging. Epigenetic measures of aging help evaluate "biological age" and chronic disease risk.

Biological age indicates how well your body functions compared to your chronological age, reflecting "healthspan" rather than lifespan. Biological age can be assessed using blood biomarkers, DNA methylation, and telomere length, which reflect organ condition and function and, ultimately, chronic disease risk.

Researchers have identified nine blood biomarkers that reflect the physiological function of various systems, including cardiovascular, liver, kidney, immune, and metabolic systems. Changes in these systems contribute to aging and disease susceptibility. This set of biomarkers can help predict 10-year mortality and is more indicative of true health rather than chronological age.

Adopting healthy lifestyle habits, such as a balanced diet, regular exercise, good sleep, and stress management, can improve biological age and reduce the risk of chronic diseases, even with small changes.

Biological Age, A True Measure of Health

This white paper will review the difference between chronological age, which is predictable, and biological age, which varies depending on lifestyle and physiological function at a given point in time. Biological age decreases with less-than-optimal nutrition, physical activity, and lifestyle habits.

Fortunately, the biological age of our organs and systems can be evaluated and improved upon if needed, independent of one's chronological age.

The goal is to reduce the risk of chronic disease and dysfunction and optimize one's healthful years by adopting beneficial habits associated with wellness and longevity.

This ODX white paper will address

- ✓ What is Biological Age?
- ✓ How to Measure Biological Age: Overview
- ✓ How to Measure Biological Age: Key Biomarkers
- ✓ How to Measure Biological Age: DNA Methylation (DNAm)
- ✓ How to Measure Biological Age: Telomere Length
- ✓ How to Improve Biological Age
- ✓ Optimal Takeaways
- ✓ Biological Age Biomarkers In Depth

What is Biological Age?

Age is just a number, but it means so much to so many.

Chronological age is based on how many years someone has been alive. However, biological age reflects how many of those years were healthy and how many years may lie ahead. The National Institute on Aging states, "Biological age means the true age that our cells, tissues, and organ systems appear to be, based on biochemistry (NIH NIA 2021). Therefore, chronological and biological age can differ depending on an individual's health status. Chronological age reflects lifespan, while biological age reflects healthspan.

Biological age may also be called "Phenotypic Age" or PhenoAge.

While chronological age is measured in annual birthdays, biological age can be calculated using blood biomarkers and specific genetic markers, such as those associated with DNA methylation and telomere length.

A higher biological age is associated with inflammation, disrupted DNA repair, and impaired mitochondrial function, leading to altered energy metabolism and accelerated aging. Biological age reflects organ function and physical capacity. Biological age can help predict susceptibility to chronic disease and even all-cause mortality independent of chronological age (Levine 2018).

Biological age is considered a valuable marker for identifying high-risk populations who will be increasingly susceptible to chronic disease and mortality as they age chronologically. Research suggests that age and cardiovascular disease, a primary cause of death, are closely interconnected and may share mutual pathways. However, accelerated biological aging can be linked to cardiovascular dysfunction, disease susceptibility, and a more significant number of chronic disorders in subjects with coronary artery disease despite their chronological age. Biological age calculations may be beneficial in predicting cardiovascular risk in young subjects with a lower chronological age than traditional cardiovascular patients (Ma 2022).

The main factors that can be used to evaluate biological age include:

- Blood biomarkers
    - Nine primary biomarkers: Albumin, alkaline phosphatase, creatinine, C-reactive protein, fasting glucose, lymphocyte %, MCV, RDW, and white blood cell count
- DNA methylation
- Telomere length

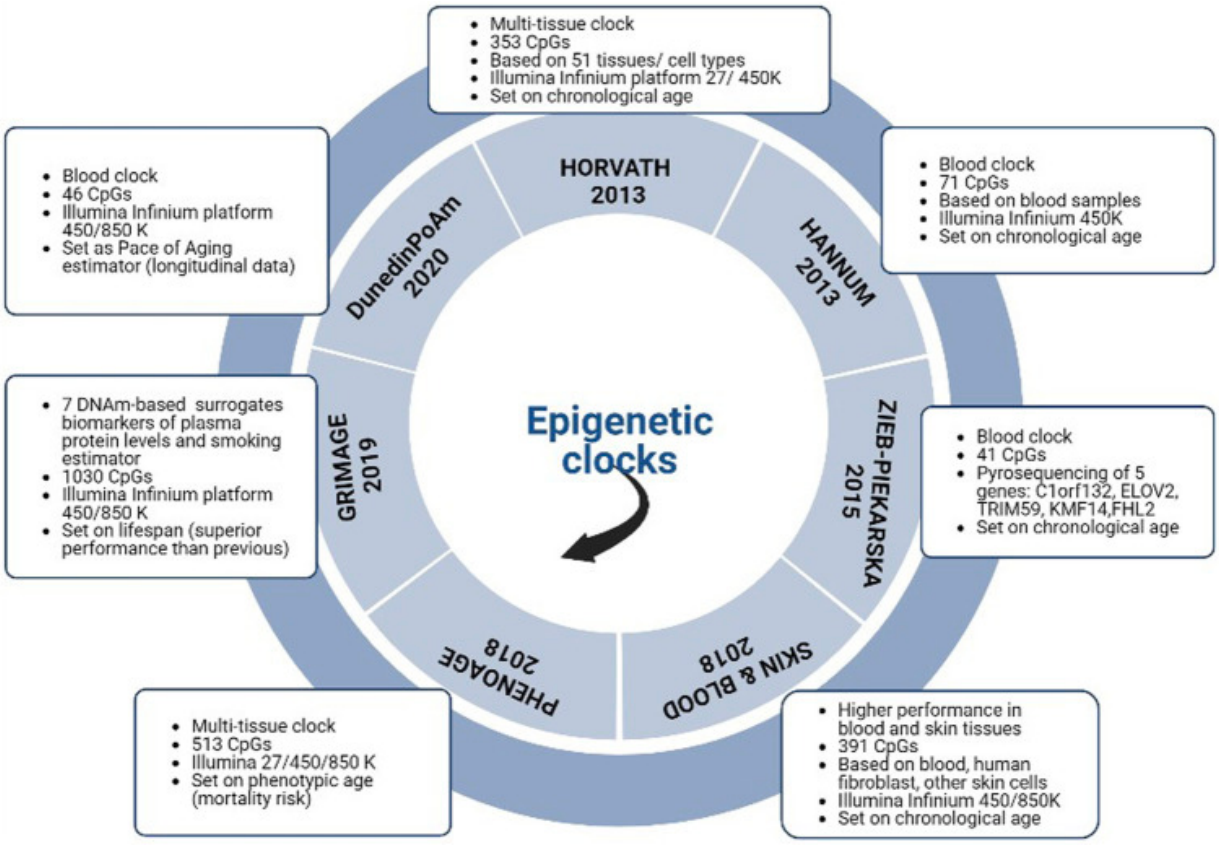
Higher biological age is associated with less-than-desirable lifestyle habits, including poor sleep hygiene, unhealthy eating habits, higher body mass index, nicotine exposure, limited physical activity, unhealthy blood glucose levels, blood lipids, and blood pressure. Fortunately, biological age can be reduced by optimizing these factors (Zhang 2023).

It is important to note that even small steps toward a healthy lifestyle can improve biological age and reduce the risk of chronic disease.

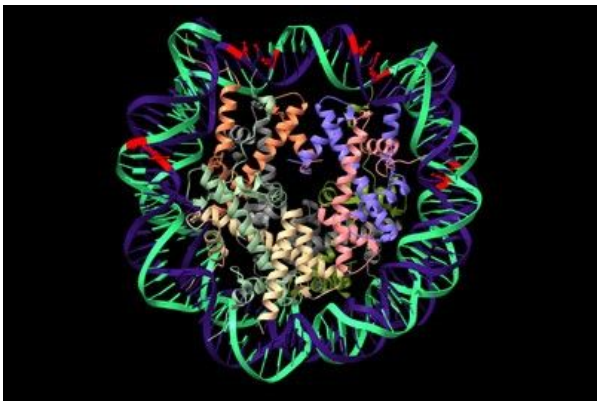
How to Measure Biological Age Overview

The changes associated with "aging" begin at the molecular level and eventually manifest as chronic disease, functional decline, wrinkled skin, muscle loss, compromised athletic ability, and biomarker changes reflected in blood work. Early approaches called "epigenetic clocks" were used to measure biological age based on DNA methylation patterns or "age predictors" and included the Hannum Clock and the Horvath Clock (Levine 2023).

Description of the characteristics of the most common epigenetic clocks.



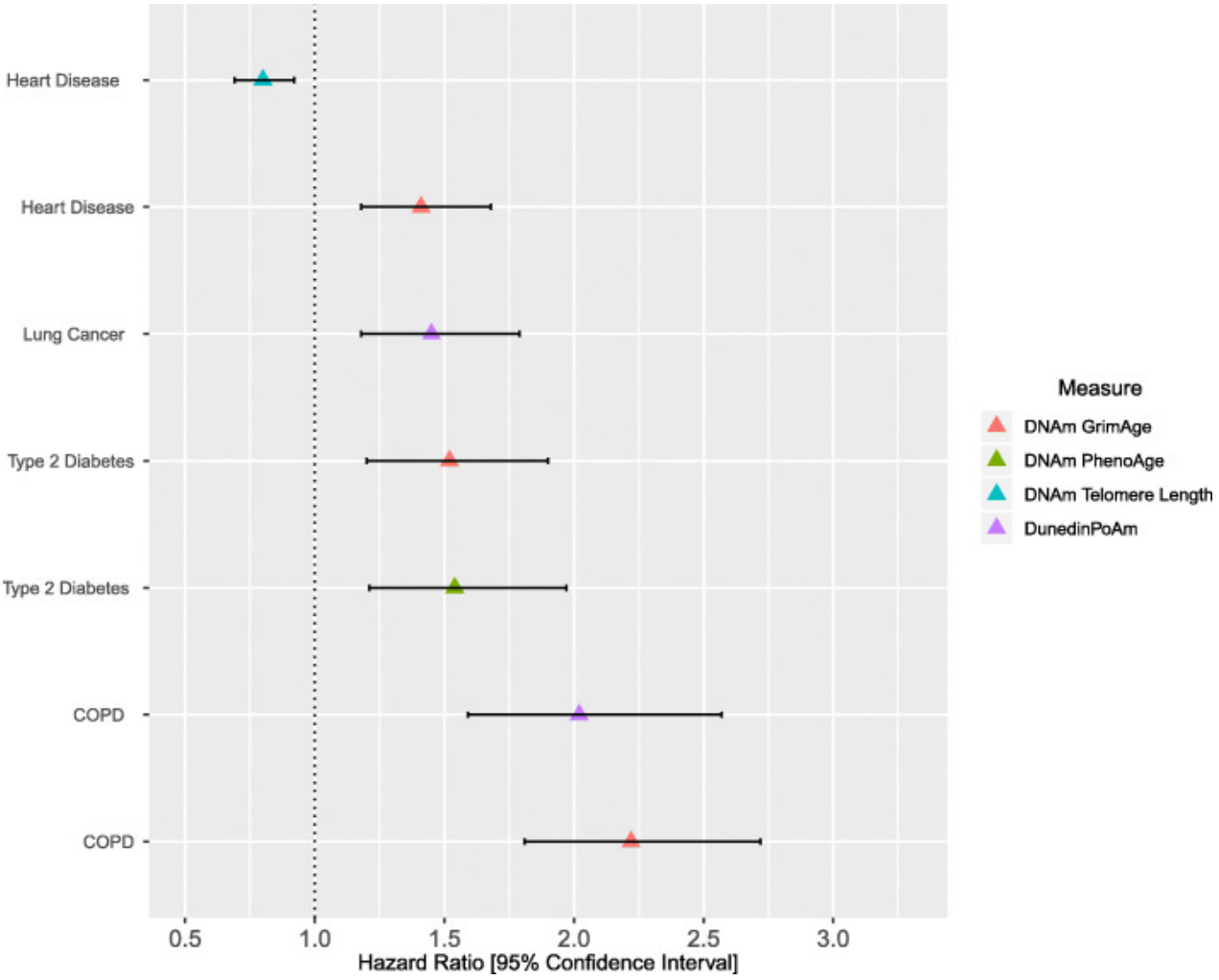
Source: Li Piani, Letizia et al. "Epigenetic clocks and female fertility timeline: A new approach to an old issue?." Frontiers in cell and developmental biology vol. 11 1121231. 21 Mar. 2023, doi:10.3389/fcell.2023.1121231 This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).



Epigenetics

Epigenetics refers to how lifestyle behaviors and environmental factors can influence how genes are expressed. Epigenetic changes occur on a regular basis and help regulate the lifecycle and renewal of cells. Diet, sleep, exercise, smoking, alcohol consumption, drugs, toxins, stress, and trauma promote epigenetic changes that can be beneficial or detrimental, depending on lifestyle choices, toxin exposure, and even social factors. Healthy choices and circumstances can promote beneficial epigenetic changes and likely expand one's healthspan, i.e., the amount of time associated with good health. DNA methylation (DNAm) and certain blood biomarkers can provide insight into health at the cellular level and help determine an individual's internal biological age (NIH NIA 2021).

The associations between epigenetic measures of aging and incidence of common disease states



Age-adjusted DNAm GrimAge was associated with the incidence of COPD, type 2 diabetes and ischemic heart disease after 13 years of follow-up. Age-adjusted DNAm PhenoAge associated with the incidence of type 2 diabetes. Age-adjusted measures of DNAm Telomere Length associated with the incidence of ischemic heart disease. Higher DunedinPoAm values, indicating a faster pace of ageing, were associated with the incidence of COPD and lung cancer. Associations represent a one standard deviation increase in the respective epigenetic measure of ageing. Models were adjusted for age, sex, alcohol consumption, body mass index, deprivation, education and smoking. COPD (chronic obstructive pulmonary disease)

Source: Hillary, Robert F et al. "Epigenetic measures of ageing predict the prevalence and incidence of leading causes of death and disease burden." Clinical epigenetics vol. 12,1 115. 31 Jul. 2020, doi:10.1186/s13148-020-00905-6 This article is licensed under a Creative Commons Attribution 4.0 International License

Epigenetic clock research revealed the following (Levine 2023):

- Early menopause and surgical menopause were associated with an epigenetic age (biological age) that was older than chronological age
- Signs of Alzheimer's disease were associated with older epigenetic age
- Insomnia was associated with accelerated epigenetic aging
- Remaining life expectancy was lower in those with a higher epigenetic age
- Eating leafy greens, exercising, and obtaining a higher education were associated with a lower epigenetic age
- A new epigenetic clock based solely on nine blood biomarkers was recognized as the best predictor of health outcomes, the number of years remaining, accumulation of chronic diseases, and inflammatory processes.
- A higher biological age is often associated with a higher Frailty Index, a measure that takes into account the presence of chronic conditions, functional status, activity level, lipid and glucose regulation, and blood pressure

The main approaches to calculating biological age that we will cover include

- Nine key biomarkers
- DNA methylation
- Telomere length measurement

Note: The most straightforward and practical approach to calculating biological age is the nine key biomarker pattern based on albumin, alkaline phosphatase, creatinine, C-reactive protein, fasting glucose, lymphocyte %, MCV, RDW, and white blood cell count. This is the pattern calculated in the ODX Functional Blood Chemistry Analysis software.

How to Measure Biological Age: Key Biomarkers

Researchers identified a pattern of blood biomarkers that considers physiological processes and changes contributing to aging and disease susceptibility. This set of biomarkers reflects physiological function and biological age and can help predict 10-year mortality. Biological age may also be known as "phenotypic age" or "PhenoAge."

The biomarkers measured assess the physiological state of various systems, including cardiovascular, liver, kidney, immune, and metabolic systems. Evaluating biomarkers associated with physiological aging and dysfunction can help predict individual differences in cause-specific mortality, all-cause mortality, physical function, cognitive performance, facial aging, and remaining life expectancy. This evaluation is more reflective of true health than chronological age. Researchers reveal that the nine-biomarker panel biological age evaluation was superior and most predictive of (Levine 2023, Levine 2018):

- Lifespan years remaining
- Accumulation of chronic diseases
- Changes in physical and cognitive function
- Pro-inflammatory processes
- Accelerated aging in those with obesity or metabolic syndrome
- Higher biological age in those with Parkinson's, Alzheimer's, breast cancer, and HIV

Biological age predicted 10-year survival with 90% accuracy using the epigenetic clock based on these nine biomarkers (Levine 2023, Levine 2018):

- Fasting glucose: Reflects metabolic health
- C-reactive protein (CRP): Reflects inflammation
- Albumin: Reflects liver function, inflammation, malnutrition
- Alkaline phosphatase: Reflects liver function
- Creatinine: Reflects kidney function
- Red cell distribution width (RDW): Reflects immune and hematological function
- Lymphocyte %: Reflects immune function
- White blood cell count (WBC): Reflects immune function
- Mean corpuscular volume (MCV): Reflects hematological function

The same nine-biomarker pattern was used to determine the biological age of 9,926 subjects from the third National Health and Nutrition Examination Survey (NHANES III). Measurement units for the nine biomarkers that reflect physiological health include (Levine 2018):

- Albumin g/L
- Alkaline phosphatase U/L

- Creatinine umol/L
- CRP mg/dL
- Glucose, serum, mmol/L
- Lymphocyte %
- MCV fL
- Red cell distribution width (RDW) %
- White blood cell count (WBC) 1000 cells/uL

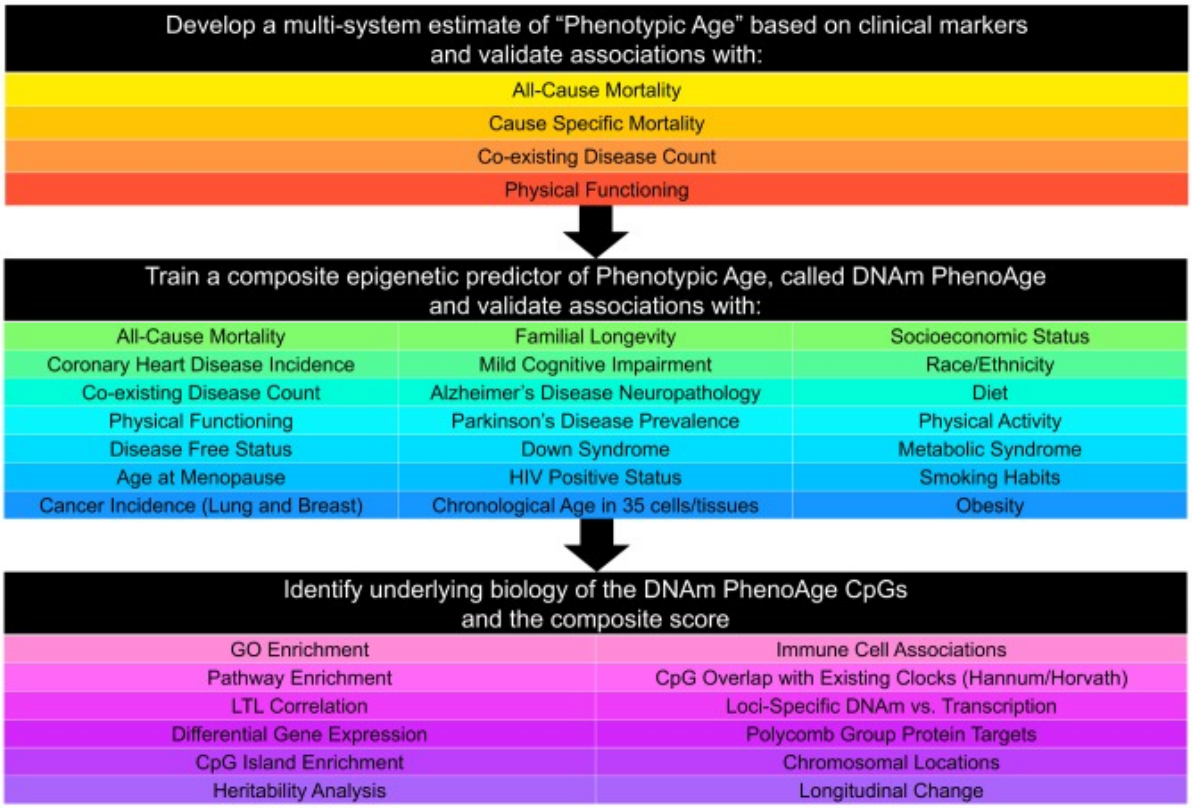
The validity of this calculation for estimating morbidity and mortality was confirmed in NHANES IV. Follow-up confirmed that for each one-year increase in biological age, the risk of:

- All-cause mortality increased by 9%
- Mortality from aging-related diseases increased by 9%
- CVD mortality increased by 10%
- Cancer mortality increased by 7%
- Diabetes mortality increased by 20%
- Mortality from chronic lower respiratory disease increased by 9%

Retrospective calculation of biological age using the nine established biomarkers revealed a strong association between biological age and all-cause mortality in 609 multivessel coronary artery disease PCI patients. Each 10-year increase in biological age correlated with a 51% increase in mortality risk. Researchers note those with a higher biological age had more disease comorbidities (Ma 2022).

A biological age higher than chronological age helped differentiate non-survivors from survivors in a retrospective study of 2,950 critically ill hospitalized patients. Being phenotypically older was associated with an increased risk of mortality. This effect was “accelerated” in those with pre-existing chronic conditions, including cardiovascular disease, end-stage renal failure, diabetes mellitus, cirrhosis, immune disease, or those undergoing immunosuppressive therapy (Ho 2023).

Factors affecting biological age



Source: Levine, Morgan E et al. "An epigenetic biomarker of aging for lifespan and healthspan." Aging vol. 10,4 (2018): 573-591. doi:10.18632/aging.101414 https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/29676998/ This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY) 3.0 License

Physiological function and deterioration

The nine biomarkers used to calculate biological age reflect crucial physiological changes associated with inflammation, impaired glucose regulation, compromised liver and kidney function, malnutrition, hematological alterations, and immune competence.

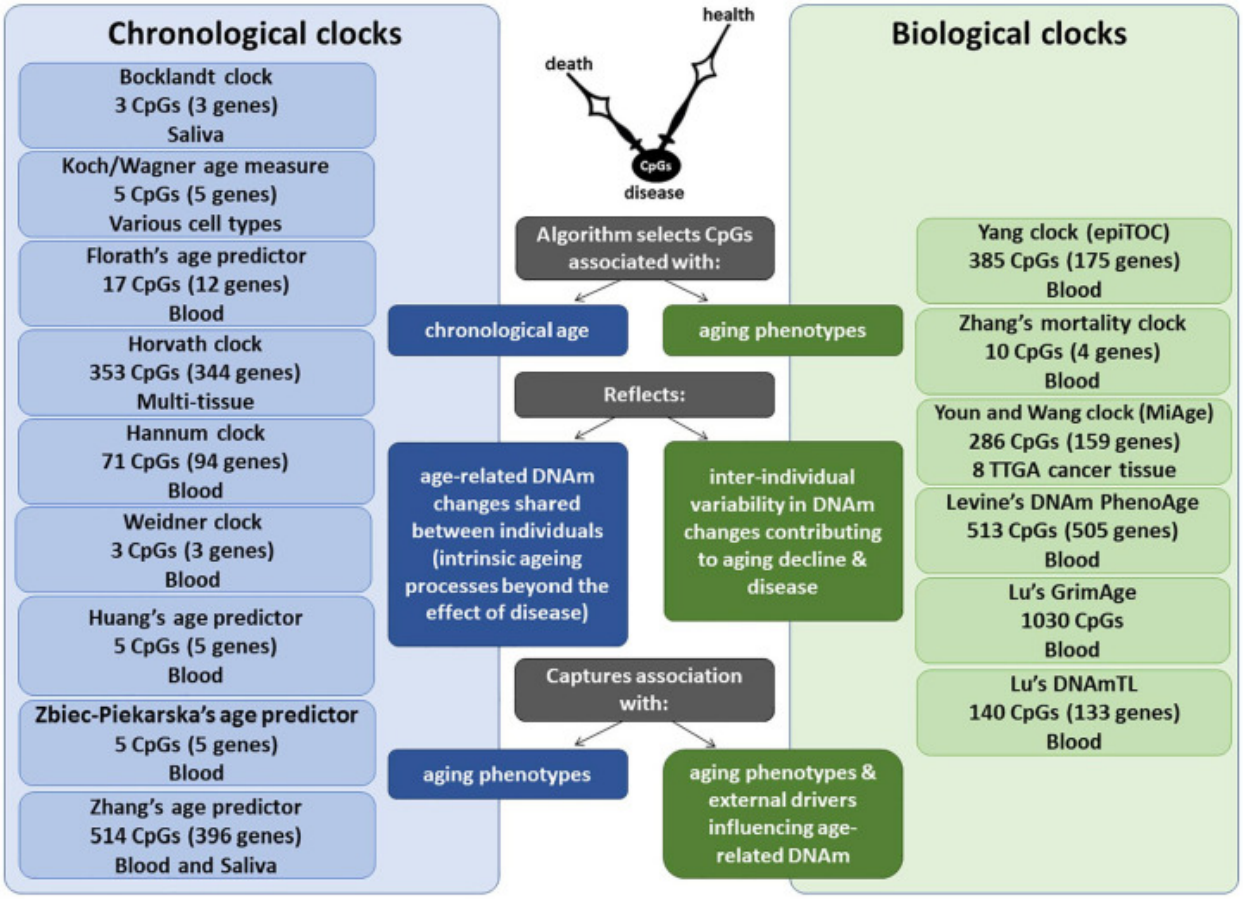
Calculating, addressing, and improving biological age can help reduce the risk of chronic disease and increase the likelihood of a longer healthspan.

How to Measure Biological Age: DNA Methylation (DNAm)

Biological age can be further evaluated using an epigenetic biomarker known as DNA methylation (DNAm). Epigenetics reflects how chemical changes alter how genes are expressed and whether they are turned off or on. DNA methylation refers to adding a methyl group to a specific portion of DNA, which usually turns a gene off or removing a methyl group, which turns a gene on. DNAm also reflects how cells are aging; DNAm patterns can be significantly altered with aging and appear to be associated with cancerous changes (Levine, 2023).

However, potential cofounders associated with DNA methylation evaluation and accuracy include genetic factors, environmental factors, and variations in specific DNAm arrays (Bergsma 2020).

Comparison of chronological vs biological DNAm clocks.



Each DNAm clock is developed using a unique training model, including a variable number of CpGs, tissue source of DNA and corresponding age-related measures. While chronological DNAm clocks reflect age-related DNAm changes that are shared between individuals and are expected to reflect the intrinsic aging process, biological DNAm clocks reflect age-related DNAm changes that vary between individuals and are expected to capture associations with specific age-related phenotypes and external drivers that may influence age-related DNAm.

Source: Bergsma, Tessa, and Ekaterina Rogaeva. "DNA Methylation Clocks and Their Predictive Capacity for Aging Phenotypes and Healthspan." Neuroscience insights vol. 15 2633105520942221. 21 Jul. 2020, doi:10.1177/2633105520942221 This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License

DNAm PhenoAge

DNAm combined with phenotypic (biological) age calculations yield a DNAm PhenoAge. Ultimately, changes in DNAm PhenoAge were highly correlated with changes in biological age, a more straightforward, more attainable equation. Research indicates that DNAm PhenoAge can help predict aging outcomes, including (Levine 2018):

- All-cause mortality
- Alzheimer's disease.
- Cancers
- Healthspan
- Physical functioning
- Aging at the cell and tissue level

The DNAm PhenoAge was significantly associated with subsequent mortality in 5 large-scale study samples independent of chronological age.

- A one-year increase in DNAm PhenoAge was associated with a 4.5% increase in all-cause mortality risk, increased coexisting morbidities, increased CHD risk, improved physical functioning issues, and decreased disease-free likelihood.
- The DNAm PhenoAge biomarker predicted the risk of lung cancer incidence and mortality in smokers. It was also positively associated with neuropathological hallmarks of Alzheimer's disease, including amyloid load, neuritic plaques, and neurofibrillary tangles.

- Lower DNAm PhenoAge is associated with more exercise; increased markers of increased fruit and vegetable intake; lower levels of C-reactive protein, insulin, glucose, triglycerides, and waist-to-hip ratio; higher HDL cholesterol; higher education; and higher income, demonstrating a negative association between DNAm PhenoAge and these factors.
- A positive association is observed with a higher DNAm PhenoAge and elevated C-reactive protein, insulin, glucose, triglycerides, waist-to-hip ratio, BMI, blood pressure, and smoking.
- A higher DNAm PhenoAge was also found to correlate weakly with shorter leukocyte telomere length, a marker of aging.

Researchers conclude that increased DNAm PhenoAge, relative to chronological age, is associated with;

- Activation of pro-inflammatory pathways, e.g., NF-kappaB
- Increased interferon signaling, a marker of DNA damage and cellular senescence
- Increased DNA damage and decreased DNA repair
- Transcriptional/translational signaling
- Various markers of immunosenescence
- Decline of naïve T cells
- Shortened leukocyte telomere length

How to Measure Biological Age: Telomere Length

Telomeres are the protective “caps” located at the tail end of a chromosome that promotes genomic stability and integrity. Research suggests that telomere length may reflect an individual’s risk for chronic disease, considering shorter telomeres are associated with atherosclerosis, impaired blood vessel repair, cardiovascular disease, Alzheimer’s, and mortality (Herrmann 2018). Extreme telomere shortening is associated with cellular senescence, a cellular stress response that disrupts normal cell replication (Ferrucci 2020).

Telomeres become shorter during cell division or exposure to damage or stress, and severe shortening contributes to the loss of cell and tissue integrity and cell death and may be a hallmark of aging. One study found that telomere shortening in leukocytes (WBCs) was associated with higher stress

levels, including perceived stress, and was associated with an age ten years older than chronological age. However, measuring leukocyte telomere length appears to be unable to differentiate between different types of white blood cells, which may limit its use as a general marker of aging (Levine 2023).

Several research studies have demonstrated the association between shorter telomeres and malignancy. Even when measured in the same individual, telomere length was shorter in cancer cells than in healthy cells from the same organ or elsewhere in the body. Decreased telomere length observed in breast, colon, and prostate tumor tissue was associated with more advanced disease, faster progression, and poor survival (Hermann 2018).

Research reveals that the protein telomerase can restore some of the lost genetic material at the end of chromosomes. However, enhanced telomerase activity is a hallmark of cancer, and it is possible that artificially lengthening telomeres may increase cancer risk (Levine 2023).

Lifestyle may also impact telomere length. In a review of NHANES data from 35,575 subjects participating from 1999 to 2018, shorter telomeres were associated with a higher score on the Dietary Inflammatory Index. This data may reflect a healthy versus an unhealthy diet (Xie 2023).

Longer telomere length was associated with the American Heart Association's 2010 Life's Simple 7 (LS7), which includes a balanced diet, smoking abstinence, healthy BMI, physical activity, desirable fasting glucose and total cholesterol, and healthy blood pressure. A higher LS7 score is associated with a more desirable biological age, as is a higher LS8 score, which incorporates restorative sleep into the score (Zhang 2023).

How to Improve Biological Age

Chronological age cannot be changed…. But biological age can.

The most influential factors contributing to biological age are individual health behaviors regarding nutrition, exercise, sleep, smoking, and alcohol. Recent adversities and stressors can also affect biological age, as can genetic factors, but lifestyle choices are the most prominent factors (Levine 2023).

If biological age is higher than chronological age, some simple tweaks and changes can improve it.

Life's Essential 8

The association between biological aging and lifestyle habits was investigated in a 20-year+ cross-sectional study of 11,729 subjects from the 2005-2010 NHANES study. Phenotypic aging was inversely correlated with Life's Essential 8 (LE8) factors, i.e., those with a higher LE8 score had a lower biological age. Life's Essential 8 scores are based on the following health behaviors and health factors (Zhang 2023):

Domain	CVH Metric	Measurement	Quantification and Scoring of CVH Metric
Health Behaviors	Diet	Healthy Eating Index-2015 diet score percentile	Quantiles of DASH-style diet adherence **Scoring (Population):** Points Quantile 100 ≥95th percentile (top/ideal diet) 80 75th – 94th percentile 50 50th – 74th percentile 25 25th – 49th percentile 0 1st – 24th percentile (bottom/least ideal quartile)
	Physical activity	Self-reported minutes of moderate or vigorous physical activity per week	**Metric:** Minutes of moderate (or greater) intensity activity per week **Scoring:** Points Minutes 100 ≥150 90 120 – 149 80 90 – 119 60 60 – 89 40 30 – 59 20 1 – 29 0 0
	Nicotine exposure	Self-reported use of cigarettes or inhaled nicotine-delivery system	**Metric:** Combustible tobacco use and/or inhaled NDS use; or secondhand smoke exposure **Scoring:** Points Status 100 Never smoker 75 Former smoker, quit ≥5 yrs 50 Former smoker, quit 1 - <5 yrs 25 Former smoker, quit <1 year, or currently using inhaled NDS 0 Current smoker Subtract 20 points (unless score is 0) for living with active indoor smoker in home
	Sleep health	Self-reported average hours of sleep per night	**Metric:** Average hours of sleep per night **Scoring:** Points Level 100 7 – <9 90 9 – <10 70 6 – <7 40 5 – <6 or ≥10 20 4 – <5 0 <4

Health Factors	Body mass index	Body weight (kg) divided by height squared (m^2)	**Metric:** Body mass index (kg/m^2) **Scoring:** Points Level 100 <25 70 25.0 – 29.9 30 30.0 – 34.9 15 35.0 – 39.9 0 ≥40.0
	Blood lipids	Plasma total and HDL-cholesterol with calculation of non-HDL-cholesterol	**Metric:** Non-HDL-cholesterol (mg/dL) **Scoring:** Points Level 100 <130 60 130 – 159 40 160 – 189 20 190 – 219 0 ≥220 If drug-treated level, subtract 20 points
	Blood glucose	Fasting blood glucose or casual hemoglobin A1c	**Metric:** Fasting blood glucose (mg/dL) or Hemoglobin A1c (%) **Scoring:** Points Level 100 No history of diabetes and FBG <100 (or HbA1c < 5.7) 60 No diabetes and FBG 100 – 125 (or HbA1c 5.7-6.4) (Pre-diabetes) 40 Diabetes with HbA1c <7.0 30 Diabetes with HbA1c 7.0 – 7.9 20 Diabetes with HbA1c 8.0 – 8.9 10 Diabetes with Hb A1c 9.0 – 9.9 0 Diabetes with HbA1c ≥10.0
	Blood pressure	Appropriately measured systolic and diastolic blood pressure	**Metric:** Systolic and diastolic blood pressure (mm Hg) **Scoring:** Points Level 100 <120/<80 (Optimal) 75 120-129/<80 (Elevated) 50 130-139 or 80-89 (Stage I HTN) 25 140-159 or 90-99 0 ≥160 or ≥100 Subtract 20 points if treated level

Source: Zhang, Ronghuai et al. "Association between life's essential 8 and biological ageing among US adults." Journal of translational medicine vol. 21,1 622. 14 Sep. 2023, doi:10.1186/s12967-023-04495-8 This article is licensed under a Creative Commons Attribution 4.0 International License

Diet and Nutrition

A highly inflammatory diet, measured by the Dietary Inflammatory Index (DII), is specifically associated with chronic disease and accelerated aging. This effect was demonstrated using NHANES data from 35,575 adult subjects, which found significant positive correlations between DII and biological age (Xie 2023).

The DII categorizes foods as pro-inflammatory or anti-inflammatory (Kanauchi 2019):

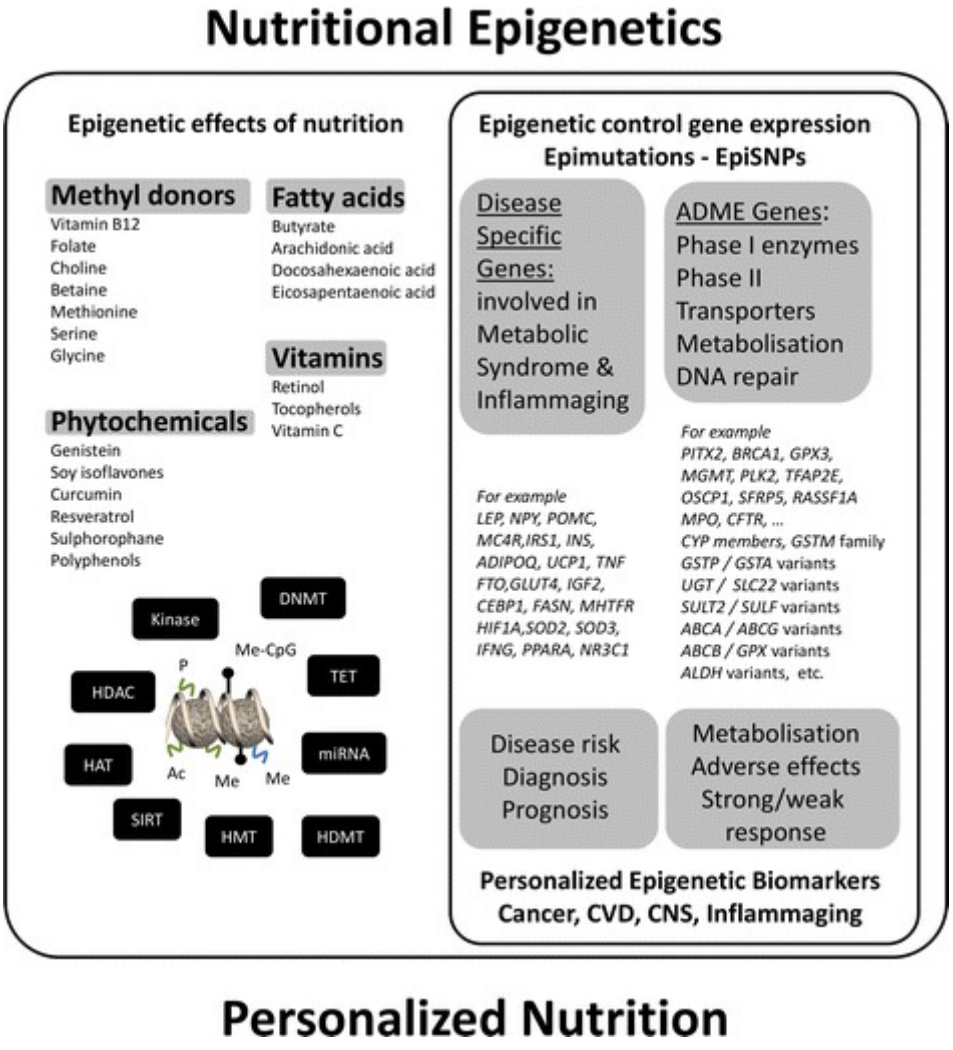
- ✓ Pro-inflammatory foods include red meats, processed meats, organ meats, non-oily fish, eggs, sugar-sweetened beverages, tomatoes, and refined grains (especially if these foods and beverages are consumed frequently).
- ✓ Anti-inflammatory foods include leafy green vegetables, dark yellow vegetables, fruit juice, oily fish, coffee, tea, wine, and beer or other alcoholic beverages.

A pro-inflammatory diet can be associated with persistent low-grade inflammation related to aging and chronic disease. In a cross-sectional study of 928 adults in Scotland, significantly higher blood levels of inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6) were associated with a higher DII (Corley 2019).

The premature aging that is associated with inflammation, known as inflammation, is associated with chronic conditions such as metabolic syndrome, diabetes, arthritis, dementia, cancer, osteoporosis, frailty, heart and lung diseases, and mortality. Several nutrients and plant-based compounds help attenuate the adverse effects of inflammation and oxidative stress, reduce inflammaging, and improve biological age. These constituents include (Szarc vel Szic 2015):

- Fatty acids
    - Butyrate, DHA, EPA, arachidonic acid [may also be pro-inflammatory]
- Methyl donors
    - Vitamin B12, folate, choline, betaine, methionine, serine, glycine
- Phytochemicals
    - Genistein, soy isoflavones, curcumin, resveratrol, sulforaphane, polyphenols
- Vitamins
    - B12, C, retinol, tocopherols

Overview of the mechanisms and consequences of epigenetic regulation by nutritional compounds.



Modulation of different classes of chromatin writers-erasers by phytochemicals (left panel). Genes encoding absorption, distribution, metabolism, and excretion (ADME) proteins can be epigenetically regulated and thereby determine individual nutritional responses. Epigenetic modification of disease-related genes can contribute to diagnosis (biomarker) as well as disease prevention or progression (right panel).

Source: Szarc vel Szic, Katarzyna et al. "From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition?." Clinical epigenetics vol. 7,1 33. 25 Mar. 2015, doi:10.1186/s13148-015-0068-2 This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)

Fasting, or abstaining from food for a set period of time, may help reduce biological age as well. Researchers observed a decrease in biological age of approximately 2.5 years when individuals participated in three short-term fasts over a few months, reaching an average of 15 fasting days per year. The reduction in biological would likely manifest as a reduced disease burden and longer healthspan and lifespan (Levine 2023).

The combination of diet and exercise improvements significantly decreased biological age and increased Healthy Aging Index scores in a randomized study of 93 adults with obesity, i.e., a BMI of 30 or higher. The positive effects of diet and exercise were observed by the sixth month of the 12-month study. The diet and exercise protocols were as follows (Ho 2022):

Diet protocol

- A diet approximately 500-750 kcals below daily energy requirements
- Weekly meetings with a dietitian to adjust and progress the diet

Exercise protocol

- Three exercise sessions per week for a total of 90 minutes, incorporating:
    - 15 minutes of flexibility
    - 15 minutes of balancing
    - 30 minutes of aerobics (treadmill, bike, or elliptical trainer)
    - 30 minutes of progressive resistance exercise (progressing to 2-3 sets at approximately 80% of the one-repetition maximum)
    - Supervision by facility exercise physiologists

Biological Age Biomarkers

How biomarker changes contribute to biological aging

Aging is a major contributor to chronic diseases and is a significant public health burden. However, individuals of the same chronological age can show considerable differences in age-related diseases and mortality risk, indicating variations in biological aging processes. Recent research suggests that biological age and aging acceleration predict morbidity and mortality risks better than chronological age (Tang 2024).

Aging is also associated with frailty characterized by weakness, reduced physical activity, muscle loss (sarcopenia), and decreased resistance and response to stress. In general, function deteriorates, and metabolic homeostasis is disrupted with aging. However, these changes may be better reflected by evaluating biological age, which considers physiological function and competence, versus chronological age, which only considers the passing of time. Genetic and environmental factors can influence pathological (premature) aging and biological age. Environmental and lifestyle factors are modifiable, including toxin exposure, smoking, excess alcohol, drug use, mental stress, education, sedentary lifestyle, and frailty (Figuer 2021).

How to Measure Biological Age: Key Biomarkers

Researchers have made a breakthrough in identifying a pattern of blood biomarkers that consider physiological processes and changes contributing to aging and disease susceptibility. This set of biomarkers, which reflects physiological function and biological age, cannot only help predict 10-year mortality but also empower individuals to take control of their health. Biological age, also known as “phenotypic age” or “PhenoAge,” is a powerful tool in this regard.

The biomarkers measured assess the physiological state of various systems, including cardiovascular, liver, kidney, immune, and metabolic systems. Evaluating biomarkers associated with physiological aging and dysfunction can help predict individual differences in cause-specific mortality, all-cause mortality, physical function, cognitive performance, facial aging, and remaining life expectancy. This evaluation is more reflective of true health

than chronological age. Researchers reveal that the nine-biomarker panel biological age evaluation was superior and most predictive of (Levine 2023, Levine 2018):

- Lifespan years remaining
- Accumulation of chronic diseases
- Changes in physical and cognitive function
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- Accelerated aging in those with obesity or metabolic syndrome
- Higher biological age in those with Parkinson's, Alzheimer's, breast cancer, and HIV

Biological age predicted 10-year survival with 90% accuracy using the epigenetic clock based on these nine biomarkers (Levine 2023, Levine 2018):

- Fasting glucose: Reflects metabolic health
- C-reactive protein (CRP): Reflects inflammation
- Albumin: Reflects liver function, inflammation, malnutrition
- Alkaline phosphatase: Reflects liver function
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- Lymphocyte %: Reflects immune function
- White blood cell count (WBC): Reflects immune function and inflammation
- Mean corpuscular volume (MCV): Reflects hematological function

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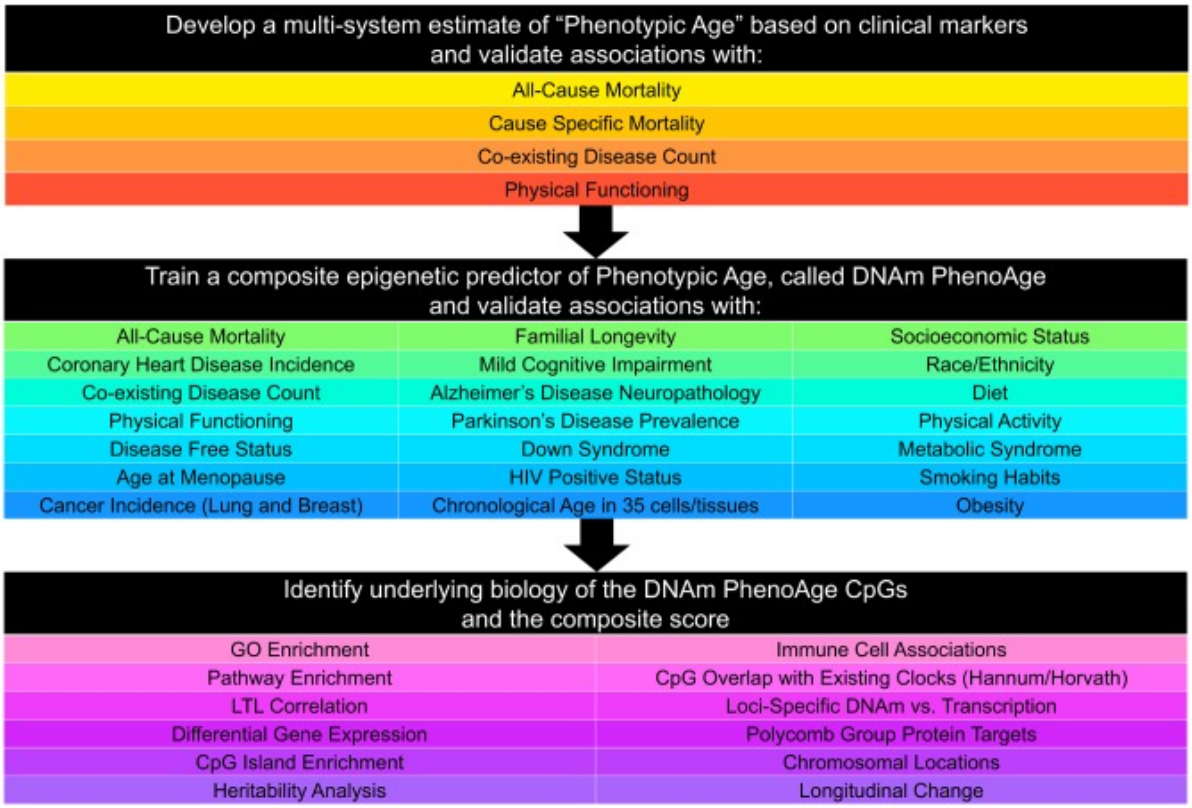
A biological age higher than chronological age helped differentiate non-survivors from survivors in a retrospective study of 2,950 critically ill hospitalized patients. Being phenotypically older was associated with an increased risk of mortality. This effect was “accelerated” in those with pre-existing chronic conditions, including cardiovascular disease, end-stage renal failure, diabetes mellitus, cirrhosis, immune disease, or those undergoing immunosuppressive therapy (Ho 2023).

Factors affecting biological age



Source: Levine, Morgan E et al. "An epigenetic biomarker of aging for lifespan and healthspan." Aging vol. 10,4 (2018): 573-591. doi:10.18632/aging.101414 https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/29676998/ This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY) 3.0 License

Physiological function and deterioration

Aging is associated with detrimental molecular and cellular changes often appearing after age 30. These changes are expressed as muscle, bone, and cartilage loss, adipose tissue increase, and hormonal changes (Dybiec 2022).

The nine biomarkers used to calculate biological age reflect crucial physiological changes associated with inflammation, impaired glucose regulation, compromised liver and kidney function, malnutrition, hematological alterations, and immune competence.

Calculating, addressing, and improving biological age can help reduce the risk of chronic disease and increase the likelihood of a longer healthspan.

Biological age predicted 10-year survival with 90% accuracy using the epigenetic clock based on these nine biomarkers (Levine 2023, Levine 2018):

- Fasting glucose: Reflects metabolic health
- C-reactive protein (CRP): Reflects inflammation
- Albumin: Reflects liver function, inflammation, malnutrition
- Alkaline phosphatase: Reflects liver function
- Creatinine: Reflects kidney function
- Red cell distribution width (RDW): Reflects inflammation and hematological function
- Lymphocyte %: Reflects immune function
- White blood cell count (WBC): Reflects immune function and inflammation
- Mean corpuscular volume (MCV): Reflects hematological function

Biological Age Biomarkers: Fasting Glucose Reflects Metabolic Health

Physiological changes associated with glucose exposure and hyperglycemia

Chronic exposure to high glucose levels results in numerous physiological and pathophysiological changes, affecting cells, tissues, and organ systems. Hyperglycemia exacerbates its toxic effects through several pathways, including inducing oxidative stress, upregulating the polyol pathway, activating protein kinase C (PKC), enhancing the hexosamine biosynthetic pathway (HBP), and promoting the formation of advanced glycation end-products (AGEs), which ultimately alter gene expression.

Prolonged hyperglycemia damages pancreatic β-cells and induces insulin resistance, leading to severe diabetic conditions. Diabetes is associated with various complications, such as cardiovascular and reproductive system dysfunction, nephropathy, retinopathy, neuropathy, and diabetic foot ulcers. Elevated glucose levels also encourage the proliferation of cancer cells and the development of osteoarthritis and create a favorable environment for infections. Hyperglycemia-induced reactive oxygen species (ROS) production leads to DNA damage and an inflammatory response, further contributing to its toxicity.

Chronic hyperglycemia is linked to both microvascular and macrovascular complications, including nephropathy, retinopathy, neuropathy, atherosclerosis, and an increased susceptibility to infections. Additionally, other complications such as hypothyroidism, hyperthyroidism, non-alcoholic fatty liver disease, limited joint mobility, and edema may arise.

Overall, the detrimental effects of hyperglycemia on various biomolecules, organelles, and cells highlight the importance of managing glucose levels to prevent the extensive damage caused by prolonged exposure to high glucose concentrations. The progression of glucose-induced physiological changes includes (Giri 2018):

- Glucose toxicity
- Oxidative stress
- Modification of biomolecules
- Beta cell damage and insulin resistance
- Organ dysfunction
- Chronic disorders, including cardiovascular disease, retinopathy, cataracts, nephropathy, chronic kidney disease, neuropathy, osteoarthritis, infertility, infection, inflammation, liver cirrhosis, and diabetic foot ulcers.

Glucose regulation and cognitive decline

Diabetes is a known risk factor for cognitive impairment and dementia. In the Atherosclerosis Risk in Communities (ARIC) study, nearly 13,000 participants were assessed over 20 years using neuropsychological tests.

The study found that low levels of 1,5-anhydroglucitol (1,5-AG), indicating glycemic peaks, were associated with an increased risk of dementia and more significant cognitive decline in persons with diabetes. Each 5 µg/mL increase in 1,5-AG was linked to a 16% higher risk of dementia. Among those with diabetes and HbA1c levels below 7%, more glucose peaks correlated with greater cognitive decline, although this finding was not statistically significant (Rawlings 2017).

The study suggests that glycemic variability, characterized by glucose peaks, may contribute to cognitive decline more than sustained hyperglycemia. This highlights the importance of targeting glucose peaks and average glycemia to

prevent cognitive decline and dementia in diabetes patients. Further research is needed to confirm these findings and explore the mechanisms involved

Glucose regulation and biological age

Chronological age (CA) is determined by the time of birth, while biological age (BA) is based on cellular changes and strongly correlates with morbidity, mortality, and longevity. In type 2 diabetes (T2D), increased morbidity and mortality are associated with a higher BA, calculated from routine clinical biomarkers. Studies found that the BA of individuals with T2D was, on average, 12.02 years higher than that of non-diabetics and 16.32 years higher in type 1 diabetes (T1D).

The biomarkers A1c and systolic blood pressure showed the strongest correlations with increased BA in T2D. The study validated these findings with mortality data, showing a significant correlation between higher BA and decreased survival.

Elevated BA in T2D and T1D indicates that glucose metabolism dysregulation accelerates aging rather than peripheral insulin resistance alone. Hyperglycemia contributes to oxidative stress, cellular senescence, and vascular aging, thereby promoting accelerated aging and increased risks for complications like cardiomyopathy, as reflected by elevated systolic blood pressure.

Even when A1c was excluded from analysis, the increase in BA persisted, suggesting that other diabetes-related cellular mechanisms contribute to accelerated aging. Understanding the relationship between glucose levels and biological age is crucial for predicting and managing age-related diseases in diabetic patients (Bahour 2022).

Approximately 25% of individuals with prediabetes will develop diabetes within 3 to 5 years, and as many as 70% will progress to diabetes in their lifetime. Progression from prediabetes to diabetes is associated with a higher risk of death. In a large cohort study of 45,782 individuals with prediabetes, it was found that reversion from prediabetes to normoglycemia within three years did not lower the risk of death compared to those with persistent prediabetes.

However, reversion to normoglycemia combined with healthy behaviors, such as higher physical activity levels and no smoking, was linked to a substantially lower risk of death and increased life expectancy. Evidence supports that lifestyle modifications like regular physical activity, a healthy diet, and maintaining a desirable weight are beneficial for stabilizing prediabetes and achieving normoglycemia. The primary reason for the reduced risk of death in those who reverted to normoglycemia is attributed to lifestyle changes (Cao 2023).

Biological Age Biomarkers: C-Reactive Protein (CRP) Reflects Inflammation

Physiological changes associated with elevated CRP

C-reactive protein is a key inflammation biomarker and an independent risk factor for ischemic cardiovascular diseases. It contributes to atherosclerosis through various mechanisms, including activation of vascular cells, accumulation of monocytes and lipids, apoptosis, and promotion of coronary artery thrombosis. Elevated CRP levels exacerbate ischemic damage by activating the complement system, leading to increased necrosis. CRP promotes monocyte mobilization into atheromatous plaques, suppressing nitric oxide release and causing endothelial dysfunction. CRP increases the expression of plasminogen activator inhibitor-1 and adhesion molecules and enhances cholesterol uptake by macrophages, directly contributing to atherosclerosis (Banai 2022). In peripheral tissues, CRP can dissociate from its native pentameric (pCRP) form to a monomeric form (mCRP) that can be synthesized *de novo* outside the liver. Both forms promote atherosclerosis: pCRP binds to oxidized LDL and apoptotic cells, and mCRP induces platelet aggregation and thrombosis (Salazar 2014).

Chronic inflammation can impair immune function and lead to significant alterations in cell and organ physiology, ultimately contributing to hyperglycemia, hypertension, depression, sarcopenia, osteoporosis, and cancer. Inflammation can cause a diversion of energy and nutrients away from maintenance metabolic processes and toward an activated immune system. This shift can lead to “sickness behaviors,” including fatigue, sadness, anhedonia, decreased libido, reduced food intake, altered sleep patterns,

social withdrawal, insulin resistance, increased blood pressure, and dyslipidemia (Furman 2019).

Increasing evidence shows that CRP is not only an inflammatory biomarker but also a significant risk factor associated with aging-related diseases, including cardiovascular disease, hypertension, diabetes mellitus, and kidney disease. Recent studies have demonstrated that CRP is pathogenic in numerous diseases, such as hypertensive cardiovascular and kidney complications, diabetic nephropathy, and acute and chronic kidney diseases. CRP is known to activate the NF-κB signaling pathway, mediate tissue fibrosis, impair cell regeneration, and promote aging via a Smad3-dependent p21/p27 mechanism (Tang 2017).

CRP and cognitive decline

Inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP), are closely associated with cognitive impairment (CI) and mortality. CRP, an acute-phase reactant, is a strong indicator of systemic inflammation and has been linked to both the initiation and progression of atherosclerosis. Elevated CRP levels, particularly those above 3.0 mg/L, are associated with an increased risk of cardiovascular events and mortality. Cognitive decline, commonly associated with aging, is also linked to increased mortality in the elderly. In patients with dementia or CI, CRP has been found in β-amyloid plaques and neurofibrillary tangles, suggesting that inflammation contributes to cognitive decline (Chen 2019)

Data from 1447 elderly adults in the 2012 Chinese Longitudinal Healthy Longevity Survey (CLHLS) demonstrated a graded association between higher hs-CRP levels and increased mortality, even after considering cognitive function. Hs-CRP and cognitive function were found to be independent predictors of all-cause mortality, with significant interaction effects observed, highlighting the critical role of inflammation in cognitive decline and mortality risk. Participants in the highest hs-CRP quartile (3.06 mg/L or above) had nearly double the risk of death compared to those in the lowest quartile (< 0.41 mg/L) (Chen 2019).

CRP and biological age

Chronic systemic inflammation underlies many chronic diseases, including CVD, cancer, diabetes, chronic kidney disease, non-alcoholic liver disease, and autoimmune and neurodegenerative disease. These disabling disorders are often associated with "aging" but can primarily be related to unhealthy lifestyle choices (Furman 2019).

Elevated CRP is a sign of systemic inflammation that can accelerate biological aging, especially when combined with metabolic dysfunction. A study of 41,634 adults examined the impact of C-reactive protein (CRP) levels and diabetes mellitus (DM) on biological aging and mortality. Key points include (Tang 2024):

- High CRP levels and diabetes significantly increase biological aging.
- Adults with high CRP and prediabetes or DM showed greater biological age acceleration.
    - In adults with CRP above 3 mg/L, biological age increased by 8.74 years in those with prediabetes and diabetes, compared to an acceleration of 1.66 in those without diabetes.
- High CRP and DM together raised the risk of all-cause and cardiovascular mortality by over three times compared to those without these conditions.
- Managing both inflammation and blood glucose in diabetics may improve healthy aging.

Elevated hs-CRP levels are linked to obesity and smoking, as expanded adipose tissue secretes proinflammatory cytokines that boost CRP synthesis. Moderate alcohol consumption and high physical activity are associated with lower hs-CRP levels, though evidence is not conclusive. While CRP is suggested as an independent marker of cardiovascular disease risk, its predictive capacity is debated. However, many studies show a higher risk of type 2 diabetes with elevated CRP, independent of obesity and other risk factors. Diet also influences CRP levels; high intakes of carotenoids and vitamin C, and high consumption of vegetables and fruits are linked to lower CRP levels, potentially due to their anti-inflammatory effects (Nanri 2007)

Lifestyle factors associated with the relative absence of chronic systemic inflammation include increased physical activity, fresh or minimally processed foods, limited exposure to toxins and pollution, circadian rhythms in sync with sunlight exposure, and limited social stressors (Furman 2019).

Biological Age Biomarkers: Albumin Reflects Liver Function, Inflammation, Malnutrition

Physiological changes associated with low albumin

Albumin, a vital protein produced in the liver, accounts for approximately 75% of serum antioxidant capacity and free radical scavenging. Albumin synthesis may be reduced, or its degradation may increase during illness, trauma, or inflammation. Although albumin has anti-inflammatory functions, persistent inflammation can cause albumin degradation and leakage into surrounding tissues, leading to hypoalbuminemia even with increased hepatic synthesis. Low serum albumin reflects physiological stress and predicts mortality, especially when a sign of "inflammaging," the subclinical process usually associated with advancing age. Low albumin is also associated with muscle mass loss, malnutrition, cirrhosis, kidney disease, and increased morbidity and mortality. Innate and adaptive immune responses depend on albumin and become impaired with an albumin deficit, increasing the risk of acute viral, bacterial, and fungal infection and infectious disease in general. Hypoalbuminemia is an independent risk factor for C. difficile-related mortality (Wiedermann 2021).

Damaged or oxidized albumin can be recycled in the liver, which is upregulated during inflammation. Albumin also serves as a reservoir of amino acids, a role that is escalated during stress or illness, contributing to a net deficit of functional albumin (Soeters 2019).

Albumin plays an essential role as a component of the endothelial glycocalyx, a protective layer within the blood vessel lining. Albumin reduces hydraulic conductivity across the vascular barrier and protects against glycocalyx degradation, preserving vascular integrity and normal capillary permeability. Disruption of this function and endothelial dysfunction are associated with oxidative stress, inflammation, aging, and chronic disease (Aldecoa 2020).

Albumin and cognitive decline

Cognitive decline and mild cognitive impairment (MCI) can be precursors to dementia, a potentially debilitating disorder. A decrease in albumin can contribute to cognitive impairment by interfering with the central nervous system's blood supply and decreasing the total antioxidant capacity of the blood, thereby increasing oxidative stress. Relatively low serum albumin below 4.05 g/dL was found to be an independent risk factor for MCI in adults over 60 years of age in a retrospective review of 1,800 subjects. Risk was further enhanced in those with two or more comorbidities, including hypertension, dyslipidemia, diabetes, CVD, or cerebrovascular disease. Elevated CRP, low total bilirubin, or low uric acid also enhanced the association between below-optimal albumin and MCI. The use of calcium blockers, ARBs, ACEIs, oral antidiabetic drugs, statins, non-steroidal anti-inflammatory drugs (NSAIDs), β-blockers, and nitrates was more common in those with MCI (Wang 2018).

A data review of 1,752 participants at least 65 years of age from a nationally representative population-based study found that a decreasing serum albumin level was significantly associated with cognitive impairment on a dose-response basis. Those with the lowest albumin (2.2-3.8 g/dL) were up to six times more likely to be cognitively impaired than those with an albumin of 4.4-5.3 g/dL (Llewellyn 2010). The association between albumin and impaired cognitive function may be enhanced in Apolipoprotein E (APOE) gene carriers. APOE is a significant genetic risk factor for Alzheimer's (Min 2022).

Alzheimer's disease is characterized by cerebral intraneuronal neurofibrillary tangles and extracellular deposits of beta-amyloid protein, a process that can begin 10-15 years before the onset of related cognitive symptomatology. Albumin sequesters beta-amyloid plaque and binds 90-95% of beta-amyloid in the blood. A reduction in albumin can impair the brain's ability to excrete beta-amyloid into circulation. Serum albumin was inversely associated with cerebral beta-amyloid deposition and beta-amyloid positivity in a study of 396 adults without dementia. An albumin below 4.4 g/dL was significantly associated with beta-amyloid positivity and retention in the brain compared to albumin above 4.5 g/dL (Kim 2020).

Lower albumin was observed in subjects with mild cognitive and Alzheimer's in the Oxford OPTIMA study. Median albumin was 4.3, 4.4, and 4.5 g/dL in Alzheimer's, MCI, and controls, respectively (Kellett 2011).

Albumin and biological age

Hypoalbuminemia is associated with poor quality of life and reduced longevity, especially in the stressed state, where increased degradation can reduce albumin half-life. Low albumin can also be associated with smoking, alcoholism, obesity, and muscle mass loss, which are also associated with accelerated aging (Soeters 2019).

Hypoalbuminemia is independently associated with persistent organ failure and mortality in cases of acute pancreatitis. It is also a predictor of mortality in community-acquired pneumonia, coronary heart disease, sepsis, and end-stage renal failure with hemodialysis (Hong 2017).

Albumin below 3.6 g/dL is associated with higher mortality rates among elderly subjects. When combined with low BMI, hypoalbuminemia can predict poor prognosis, with the highest mortality observed in those with an albumin below 2.8

Biological Age Biomarkers: Alkaline Phosphatase Reflects Liver Function

Physiological changes associated with elevated alkaline phosphatase

Alkaline phosphatase (ALP) represents a group of enzymes that function at an alkaline pH, primarily in the bone, liver, and kidneys (Yan 2023). These enzymes participate in protein phosphorylation, bone calcification and mineralization, cell growth, and cell apoptosis (Sharma 2014).

However, elevations in ALP are associated with all-cause mortality (Jia 2024) and pathological conditions that can reduce longevity, including coagulation (Yan 2023), inflammation, vascular calcification, endothelial dysfunction, cardiovascular disease, tissue fibrosis, and metabolic syndrome. Improved survival is associated with an ALP below 120 U/L (Haarhaus 2022).

A review of data from 34,147 adults participating in NHANES evaluations found that elevated ALP was significantly associated with all-cause and cardiovascular mortality. Subjects with an ALP above 82 U/L had more than twice the mortality rate of those an ALP of 55 U/L or below (Yan 2023).

Alkaline phosphatase and cognitive decline

ALP is critical in GABA metabolism, influences neuroplasticity and activity cortical functions, and is associated with neurodegenerative disease. In a cross-sectional study of 209 older adults, significantly higher ALP was also associated with subjective cognitive decline (SCD). Those with SCD had a mean ALP of 189.81 U/L, versus 164.52 in MCI and 139.91 in healthy controls (Boccardi 2021).

The increased alkaline phosphatase seen with brain injury and cerebrovascular dysfunction may be associated with neuronal loss and cognitive impairment. In the Oxford OPTIMA study, median serum ALP levels were higher in subjects with mild cognitive impairment and significantly higher in Alzheimer's patients versus controls. Alkaline phosphatase inversely correlated with cognitive function even in control subjects. Alkaline phosphatase dephosphorylation of tau proteins can create a byproduct that promotes neuronal death, possibly linking elevated serum ALP with cognitive impairment (Kellett 2011).

In a retrospective study of 1,019 acute ischemic stroke patients, significantly higher ALP was associated with post-stroke cognitive impairment (PSCI). Mean ALP was 86.5 in those with PSCI vs. 68.6 U/L in those without PSCI. The risk of cognitive impairment increased by 42% for each 1 U/L increase in ALP (Jia 2020).

Postoperative cognitive dysfunction (POCD) following general anesthesia was also associated with significantly higher serum ALP 3 months post-op in a retrospective study of 1,593 subjects. Those with POCD had a mean ALP of 87.3 U/L versus 60.3 in those without POCD. Higher ALP correlated with higher hs-CRP as well (Li 2022).

Alkaline phosphatase and biological age

Alkaline phosphatase (ALP) is incorporated into biomarker patterns used to evaluate biological age (Erema 2021, Levine 2013). As a biomarker of

biological aging, ALP reflects an upstream process associated with age-related disease. ALP is also associated with multiorgan failure and is used in the clinical setting to predict mortality (Wu 2021).

In a large NHANES study, higher alkaline phosphatase was associated with increased mortality per 1000 person-years, especially for all-cause, cardiovascular, and cerebrovascular mortality (Yan 2023). Circulatory diseases, chronic disorders, and mortality risk are, in turn, associated with a higher biological age (Liu 2023).

Alkaline phosphate promotes bone mineralization but may have similar pathological effects in other tissues, including the brain. High levels of ALP have been observed in the arteriole vascular endothelium in the brain and heart, which may contribute to vascular aging and disease and increase cardio- and cerebrovascular risk and mortality (Hui 1998).

Biological Age Biomarkers: Creatinine reflects kidney function, muscle metabolism

Physiological changes associated with elevated creatinine

The kidneys are responsible for removing metabolic waste products, drug metabolites, xenobiotics, and other toxins; regulating blood pressure; regulating blood levels of vital compounds, including glucose, sodium, potassium, and phosphate; and secreting hormones, including renin. erythropoietin, and calcitriol (active vitamin D). Risk factors that can contribute to kidney damage include oxidative stress, CVD, diabetes, hypertension, smoking, and excess red meat intake. Notable changes in the physiology and structure of the kidney are observed with the aging process. Renal parenchyma volume decreases, adipose tissue accumulates in the renal sinuses, glomerular basement membranes thicken, nephrosclerosis progresses, and mesangial widening and accumulation of extracellular matrix increase. Creatinine increases as the kidney's ability to filter decreases. This filtering ability is reflected in the estimated glomerular filtration rate (eGFR). The eGFR is expected to decline by 0.4-2.6 mL/min/year. An eGFR below 60 mL/min/1.73 m2 is associated with chronic kidney disease. A decline in glomerular filtration rate is also associated with obesity, doubling CKD risk

(Dybiec 2022). A doubling of serum creatinine likely represents a 50% reduction in glomerular filtration rate (Pagana 2022).

Chronic kidney disease promotes vascular damage, oxidative stress, systemic inflammation, and uremic toxicity, factors that may be directly involved in brain lesion development and cognitive decline (Xie 2022). Declining kidney function is also associated with declining visual memory, verbal memory, and learning (Seliger 2015). Creatinine is highly correlated with homocysteine, a metabolic waste product that may reflect compromised kidney function (Elias 2009).

Creatinine and cognitive decline

Declining kidney function is associated with declining cognitive function, and a decreased eGFR may be an independent indicator of cognitive dysfunction. Kidney and cognitive dysfunction were associated in TILDA, a large cohort study of 8,175 healthy individuals over 50. Researchers note that kidney dysfunction and cerebrovascular disease share common pathological pathways involving NT-proBNP and GDF15 (Nowak 2023).

Chronic kidney disease reflects accelerated vascular brain aging, and a lower eGFR is associated with brain atrophy. A strong relationship is seen between declining kidney function and declining cognitive function once eGFR drops below 45 mL/min/1.73 m2, with more moderate-to-severe cognitive impairment occurring when eGFR falls below 30. Cognitive impairment is independently associated with a urinary albumin to creatinine ratio above 30 mg/g. The UACR reflects kidney function and is also a measure of vascular endothelial inflammation and brain atrophy. Research also finds that cortical thinning, a measure of brain atrophy, was associated with an eGFR of 31-60 min/mL/1.73m2 versus an eGFR above 83 (Murray 2023).

Creatinine and biological age

Increasing creatinine is associated with increasing biological age which, in turn, is associated with cognitive decline, a potential indicator of early mortality (Erema 2022). Renal function is considered an essential predictor of longevity and early identification of renal dysfunction is critical. The kidney is

one of the organs most susceptible to aging and a linear decline in function can be seen in healthy adults after age 30 (Dybiec 2022).

Creatinine is significantly associated with mortality and chronological age and can be used to assess biological age (Erema 2022).. An eGFR of 90 mL/min/1.73 m2 or above is normal and considered "stage 1," 60-89 stage 2, 30-59 stage 3, 15-29 stage 2, and below 15 mL/min/1.73 m2 is stage 5, representing a very high risk of CKD. For stages 2-5, biological age is increased by 3-9 years, respectively (Abu 2022).

Chronic kidney disease is associated with increased morbidity and mortality and is considered the main warning sign of premature aging. It is closely associated with cardiovascular disease and GFR is inversely proportional to cardiovascular mortality worldwide (Figuer

2021). Exercise and a healthy diet, including sources of resveratrol, can help support renal function and healthy aging (Dybiec 2022).

Biological Age Biomarkers: Red Cell Distribution Width (RDW) reflects inflammation and hematological function

Physiological changes associated with elevated RDW

Red cell distribution width (RDW) reflects the degree of variation in size between red blood cells, i.e., anisocytosis. RDW has been used traditionally to differentiate various types of anemias. However, it is also strongly correlated with cardiovascular and all-cause morbidity and mortality as well as specific pathological conditions, including hypertension, atherosclerosis, heart failure, atrial fibrillation, ischemic stroke, pulmonary embolism, diabetes, acute pancreatitis, cancer, liver and kidney failure, Parkinsonism, sepsis, and COVID-19. An elevated RDW is considered a marker of inflammation and elevations are associated with atherosclerotic plaque, platelet activation, atherothrombotic incidents (Ananthaseshan 2022), progression to end-stage renal disease in diabetic subjects (Roumeliotis 2020). An RDW of 14.5% and above was a predictor of ischemic stroke mortality and poorer stroke rehabilitation outcomes in general (Zalyesov 2020).

An elevation in RDW may indicate anemia, abnormal RBC production, or congenital RBC pathology. Increased RDW interferes with RBC functions, namely the transport of oxygen, glucose, carbon dioxide, glucose, and amino acids (Yang 2024). Anemia associated with an elevated RDW is often due to nutrient deficiency (e.g., iron, vitamin B12, folate) or sideroblastic anemia. Anemia of chronic disease, aplastic anemia, and thalassemia heterozygosity are associated with a normal RDW (Jiang 2021).

The RDW is considered a useful biomarker for predicting chronic disease morbidity and mortality in both anemia patients and those without anemia (Beydoun 2021).

RDW and cognitive decline

RDW is closely associated with anemia, which is linked to cognitive decline, impaired reaction time, and impaired reasoning. However, elevated RDW is associated with cognitive impairment, including verbal memory deficits and dementia prevalence, in those without anemia. Elevated RDW also correlates independently with elevated homocysteine, a risk factor for Alzheimer's disease (Beydoun 2021).

In a study of 550 subjects 65 or older, RDW was negatively correlated with cognitive assessment scores. The most significant correlation observed was between elevated RDW and attention impairment, and researchers consider increased RDW to be a significant and sensitive biomarker of mild cognitive impairment. Elevated RDW has a strong association with Alzheimer's disease and the inflammatory pathogenesis of Alzheimer's dementia (Yang 2024).

A J-shaped curve was observed between RDW and dementia, including Alzheimer's and vascular dementia, despite the absence of anemia in a cross-sectional study of 5,115 subjects aged 65 and older. The risk of dementia was highest in those with an RDW above 14% and lowest with an RDW of 13.1-13.5% (Jiang 2021).

RDW and biological age

Red blood cell biomarkers, including RDW, hemoglobin, and MCHC, are associated with multimorbidity, disability, cognitive impairment, and mortality.

RDW is one of the most significant biomarkers of aging and mortality (Jia 2024).

Elevations in RDW may indicate oxidative stress, increased inflammation, reduced RBC lifespan, and anemia, all factors significantly associated with an increased mortality risk. Higher RDW was significantly associated with all-cause, cancer, CVD, and respiratory mortality based on a study of 27,063 participants from the Malmo Diet and Cancer cohort study. Those with the highest RDW were 27% and 39% more likely to die from cancer and cardiovascular disease, respectively (Pan 2019).

A comprehensive review of the literature found that elevated RDW was associated with many biomarkers and conditions related to aging and mortality, including inflammation (CRP, fibrinogen, WBCs), metabolic syndrome, dyslipidemia, ischemic heart disease, hypertension, peripheral artery disease, diabetes (Fava 2019), stroke, (Jiang 2021), impaired kidney function, and nutrient insufficiency. It is considered a significant and independent risk factor for mortality among the general population and a potential indicator of chronic disease (Yousefi 2020)

Biological Age Biomarkers: Mean corpuscular volume (MCV) reflects hematological function

Physiological changes associated with elevated MCV

The mean corpuscular volume reflects the average size or volume of a red blood cell and is a part of the RDW calculation. A decreased MCV indicates small “microcytic” RBCs which occur with iron-deficiency anemia, sideroblastic anemia, or thalassemia. A decreased MCV may occur in association with blood loss, lead poisoning, malabsorption, malignancy, and iron, copper, or vitamin B6 deficiency (Cappellini 2015, Maner 2021).

An elevated MCV indicates the RBC is larger than normal and is considered macrocytic, a condition most often associated with folate or B12 deficiency (Maner 2021), which impairs DNA synthesis, causing megaloblastic anemia. Non-megaloblastic anemia may be due to genetic factors, hepatic insufficiency, or alcohol abuse (Lee 2022).

Measurement of MCV is useful in determining the underlying cause of anemia, defined as a hemoglobin below 13 g/dL in males and below 12 g/dL in females. However, MCV may be elevated irrespective of hemoglobin in cases of alcoholism, liver disease, malignancy, chemotherapy, chronic kidney disease, ischemic stroke, and cardiac interventions (Lee 2022)

The increase in MCV is observed with aging may be partly due to a shorter RBC lifespan leading to a compensatory increase in RBCs and increased circulation of younger RBCs that tend to have a higher MCV (Gamaldo 2011). Larger RBCs may have difficulty passing through capillaries, impairing the delivery of oxygen and nutrients. Additional age-related factors that may contribute to a higher MCV include oxidative stress, inflammation, and folate or vitamin B12 insufficiency (Gamaldo 2013).

MCV and cognitive decline

Changes in MCV and in cognitive performance are observed with aging. Analysis of Baltimore Longitudinal Study of Aging data found that a higher MCV significantly correlated with worsening performance on standardized cognitive testing, including attention, global mental status, and verbal long delay memory performance. Circulatory impairment can magnify these deficit. The rate of cognitive decline was accelerated with an MCV of 97 or above. The association of poor cognitive performance and higher MCV was independent of the presence of anemia or inflammation (Gamaldo 2013).

MCV and biological age

Mean corpuscular volume values outside of optimal are associated with various age-related disorders including Alzheimer's, Parkinson's, and macular degeneration, with an increase in MCV correlating with aging itself. In vivo hematopoietic stem cell research has indicated that cell enlargement is causally related to aging (Davies 2022).

Elevated MCV is considered a risk factor for morbidity and mortality in certain clinical presentations. An MCV above 95 fL was associated with arterial stiffening in young healthy subjects. A higher MCV was associated with endothelial dysfunction, insulin resistance, and all-cause, CVD, and infection-associated mortality in chronic kidney disease patients. Elevated MCV is also

associated with cognitive decline. The link between higher MCV and aging has not been clearly defined, though researchers note a definitive increase after age 25, possibly linked to accelerated aging following the reproductive years (Lee 2022).

A mean MCV of 91 fL was associated with mortality in critical care myocardial infarction patients, whereas a mean MCV of 89 fL was associated with survival in a review of ICU hospital records (Huang 2016).

Biological Age Biomarkers: Lymphocytes reflect immune function

Physiological changes associated with low lymphocytes

The primary job of lymphocytes, a type of white blood cell, is to fight chronic bacterial or acute viral infections. Lymphocytes facilitate adaptive immunity and include B cells and T cells. Antibody-producing B-cells participate in humoral immunity, while T-cells enable cellular-type immune reactions (Pagana 2022).

Aging is associated with an overall reduction in B and T lymphocytes (Cisneros 2022). This reduction impairs the immune system's ability to clear viral and bacterial infections (naive lymphocytes) and decreases antibody response to vaccination and recurrent infection (memory lymphocytes). Although aging is associated with an overall reduction in lymphocytes, increased activation of lymphocytes, including natural killer lymphocytes, can be observed during the aging process (Corona 2012, Weng 2006). An aging immune system is also associated with impaired wound healing, unopposed tissue inflammation, cancer susceptibility, and reactivation of viral infections, including shingles (Weyland 2016).

A decreased lymphocyte count is associated with immune compromise and severe infection (Pagana 2022). However, it is also a marker for malnutrition, a risk factor for increased morbidity and mortality (Leandro-Merhi 2019). A low lymphocyte count is also associated with COVID-19. The SARS-CoV-2 virus that causes COVID-19 directly attacks lymphocytes, complicating recovery from this pandemic. A prospective study of 101 COVID-19 patients found that a decreased lymphocyte level was associated with malnutrition, anorexia,

organ failure, diabetes, and cancer (Zhang 2022). Lower lymphocytes were associated with overall mortality in sepsis and all-cause mortality in COVID-19 (Seshadri 2023).

Lymphocytes and cognitive decline

Lymphocytes may help protect cognitive function and are higher in those with normal cognition. Decreased lymphocytes are associated with vascular cognitive impairment which correlates with inflammation and cerebral white matter injury in those with non-disabling cerebrovascular events (Li 2022).

A decrease in lymphocytes may be associated with the early cognitive impairment observed with Parkinson's disease. Lower baseline circulating lymphocytes were associated with accelerated cognitive decline in individuals with Parkinson's who carry the APOE4 gene. Although lower levels of lymphocytes in the blood were associated with decline, infiltration of T lymphocytes across the blood-brain barrier appears to be a part of the pathological mechanism (Tsukita 2021).

Lymphocytes and biological age

Aging is associated with diminishing immune system function which can increase vulnerability to infection and immune dysfunction. T lymphocytes are especially affected by physiological aging. T cell senescence occurs over time, while T cell exhaustion, characterized by dysfunctional T cells, can be accelerated by chronic disease or chronic antigen exposure (Jia 2023).

Aging, at any rate, is associated with an overall reduction in lymphocytes. Stress can hasten this decline and contribute to altered lymphocyte differentiation. Exposure to stress, especially chronic stress, can accelerate the aging process and contribute to immune impairment, organ dysfunction, and premature mortality. The adverse effects of stress on lymphocytes can impair the immune system's ability to fight off viruses such as cytomegalovirus (CMV) (Klopack 2022)

Lymphocyte reduction may be considered a biomarker of frailty, a hallmark of physiological aging. A lower lymphocyte count and lymphocyte percentage of white blood cells may be associated with an increased incidence and severity of frailty. Chronic stress and activation of the HPA access can magnify this

effect, tying in elevated stress, elevated cortisol, decreased lymphocytes, and frailty. Conversely, physical activity, which is impaired or limited by frailty, is associated with higher lymphocyte counts in younger and older healthy individuals. A lower lymphocyte percentage was significantly associated with less physical activity and reduced muscle strength in a study of institutionalized women. A lower lymphocyte count coupled with a higher neutrophil count increases the neutrophil:lymphocyte ratio, a marker of inflammation and possible indicator of frailty. Inflammation is associated with free radicals and oxidative stress which can reduce lymphocytes and damage muscle tissue (Navarro-Martínez, Rut 2021). An absolute lymphocyte count below 1.54 k/cumm, was associated with increased all-cause mortality in older community-dwelling women aged 65-101 (Leng 2005).

The proliferation of lymphocytes can be considered a marker of health, biological age, and remaining lifespan as observed in a study of 140 subjects between 30 and 103 years old. Evaluating lymphocyte proliferation over time may help to track changes that occur with nutrition and lifestyle improvements designed to strengthen immunity and slow the aging process (Martínez de Toda 2016).

Biological Age Biomarkers: White blood cell (WBC) count reflects immune function and inflammation

Physiological changes associated with total white blood cell count

White blood cells (WBCs), also called leukocytes, are immune cells produced in the bone marrow. They are used to fight infections, promote an inflammatory immune response to pathogens, and regulate the cellular response to injury (Tigner 2021).

Aging is associated with dysregulation of the immune system which progresses over time and is often marked by a low-grade chronic inflammatory state called “inflammaging” (Desai 2010). An elevated total WBC count (leukocytosis) is a non-specific marker of systemic inflammation that can occur in response to pathogens and toxins, including cigarette smoke (Kabat 2017).

Leukocytosis may reflect immune activation, infection, and physical or emotional stress. However, a low WBC count may indicate bone marrow failure, severe infection, autoimmunity, or malnutrition (Pagana 2022).

Total white blood cell count and cognitive decline

Total white blood cell counts, likely due to increased inflammation, can be associated with cognitive decline, including in neurodegenerative diseases such as Alzheimer's and Parkinson's. Peripheral immune activation, characterized by elevated WBCs, is thought to trigger central nervous system immune activation, reactive oxygen species, cytokines, etc. In a large retrospective study of 4,417 Alzheimer's and 2,995 Parkinson's patients, leukocytosis was the most prevalent anomaly observed. It was associated with worsening neurocognitive decline and is considered an independent risk factor for Parkinson's dementia (Unda 2021).

A higher total WBC, even within the normal range, was associated with cognitive decline in an analysis of NHANES data for 1,670 older adults. DSST cognitive test scores steadily decreased as WBC counts increased (Kao 2011).

A meta-analysis of 36 studies found that subjects with mild cognitive impairment, as well as Alzheimer's, had significantly increased total WBCs, neutrophils, and neutrophil:lymphocyte ratio than healthy controls (Huang 2022).

Total white blood cell count and biological age

Increased WBCs are associated with age-related factors, including all-cause mortality, hypertension, cardiovascular risk, ischemic stroke (Leng 2005), inflammation (Gkrania-Klotsas 2010), and atherosclerosis, as well as myocardial infarction and mortality with WBCs above 9.4 k/cumm (Horne 2005).

White blood cell counts helped predict mortality in the Women's Health and Aging Studies cohort study. A significantly increased mortality risk was associated with WBCs above 7.0 versus the lowest mortality risk in those with the lowest WBCs, i.e., below 5.6 k/cumm (Leng 2005).

Elevated total WBCs are independently associated with mortality. In population-based studies of subjects with and without a history of CVD, an increased WBC count doubled total mortality and increased CVD incidence and mortality. The association between total WBCs and mortality parallels or exceeds that of total cholesterol, LDL cholesterol, and hypertension (Kabat 2017).

In a large prospective study of 29,526 coronary angiography subjects, the lowest incidence of mortality was observed with a total WBC of 6.0 k/cumm or below. An elevated WBC count may independently predict cardiovascular events, even in healthy individuals (Anderson 2007).

The lowest mortality risk observed was associated with a WBC count of 3.5-6 in the 44-year Baltimore Longitudinal Study of Aging. Individuals with WBCs above 10 had a 2-fold increased mortality, while those with WBCS below 3.5 had a 3-fold increased mortality. Those who died were more apt to smoke, be less physically active, and have a worse cardiovascular health profile than survivors (Ruggiero 2007).

Early research of 2,011 healthy men in the longitudinal Normative Aging Study concluded that an elevated WBC count was an independent predictor of all-cause mortality. A total WBC above 9000/mm3 was associated with a 1.8-2.5 times greater mortality risk (de Labry 1990).

Biological Age Optimal Takeaways

- ✓ Biological age reflects the health and function of one's cells, tissues, and organs.
- ✓ Biological age can differ from chronological age depending on lifestyle habits, nutrition, physical activity, sleep, stress, and toxin exposure.
- ✓ Undesirable lifestyle habits and exposures are associated with inflammation, mitochondrial dysfunction, altered energy metabolism, and a biological age older than chronological age.
- ✓ A pro-inflammatory diet can contribute to immature aging or "inflammaging"
    - Pro-inflammatory foods include red meats, processed meats, organ meats, non-oily fish, eggs, sugar-sweetened beverages, tomatoes, and refined grains (especially if these foods and beverages are consumed frequently).
    - Anti-inflammatory foods include leafy green vegetables, dark yellow vegetables, fruit juice, oily fish, coffee, tea, wine, and beer or other alcohol beverages.
- ✓ Adopting beneficial habits, managing stress, and minimizing exposure to toxins can reduce one's biological age as well as chronic disease risk.
- ✓ Biological age evaluation may be based on
    - Common blood biomarkers
    - DNA methylation
    - Telomere length
- ✓ The nine primary blood biomarkers used to calculate biological age are
    - Albumin, alkaline phosphatase, creatinine, C-reactive protein, fasting glucose, lymphocyte %, MCV, RDW, and white blood cell count
- ✓ Biological age can be lowered by optimizing Life's Essential 8 (LE8)
    - Diet
    - Physical activity
    - Smoking abstinence
    - Sleep health
    - Body mass index (BMI)
    - Blood lipids
    - Blood glucose
    - Blood pressure
- ✓ Every step taken toward a healthy habit is a step toward a lower biological age

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