WHAT IS YOUR INTRINSIC AND EXTRINSIC EPIGENETIC AGE?

Immune Report

TruDiagnostic™
The Epigenetic Company

A Recap on TruAge

Methylation is an epigenetic mechanism responsible for turning genes on and off. This phenomenon gives your body an internal clock that can be measured via methylation-specific epigenetic testing. We have reported this age output to you in our TruAge test. However, did you also know that we can break this down even further?

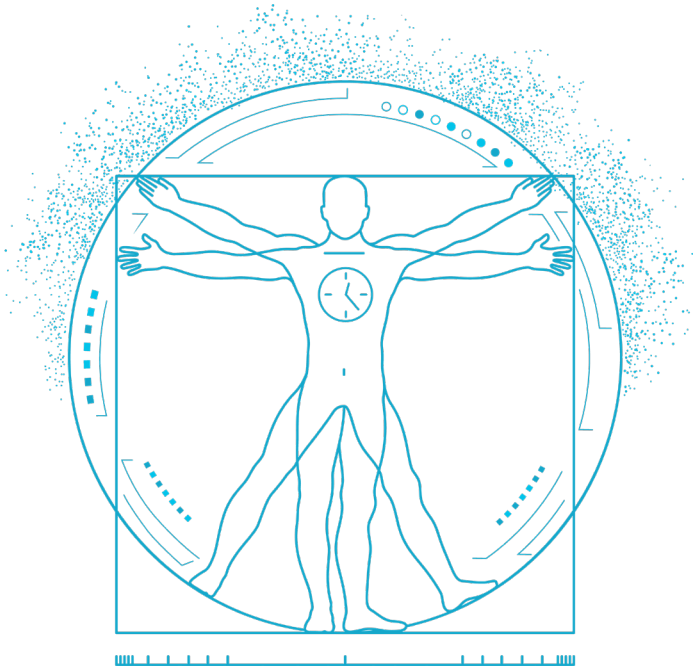
While biological age clocks are a good measure of age for your body, we can look at the age of particular systems in your body as well.

In this expanded report, we will discuss two metrics that give you more information beyond biological age. These metrics are the intrinsic and extrinsic ages of your body.

Our Clocks Tick in Different Ways, Thus Cell Type is Important

If every cell in your body has the same DNA, how do your heart cells become heart cells and your hair cells become hair cells?

The answer is epigenetics. Epigenetics controls cell development and function by switching certain genes on and off, which determines your phenotype and how your cells behave.



It makes sense that the epigenetic regulation of each cell would depend on its cell type. You wouldn't want your heart to make the proteins found in your hair and vice versa; thus, each cell has a different epigenetic signature.

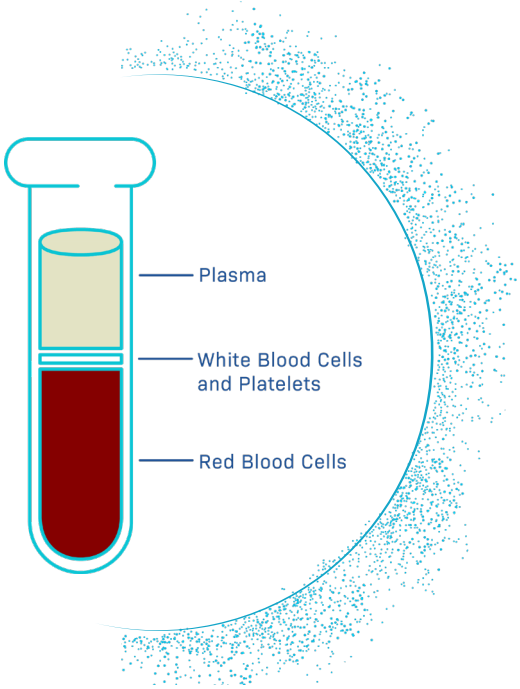
When measuring methylation, moreover, different tissues biologically age at different rates. For example, our cerebellum and brain age slower than the rest of the body. We also see that breast tissue in women can age faster than other tissues across the rest of our body.

Therefore, the rate of aging we calculate is dependent on what cell types we measure. So if we are using blood a the sample type, what cells are we looking at?

What Cells are Found in Your Blood?

The average human adult has more than 5 liters (6 quarts) of blood in his or her body. Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug within damaged blood vessels to prevent blood loss.

Thus, our blood has many different functions. For our circulatory system to function properly, our blood must contain these parts:



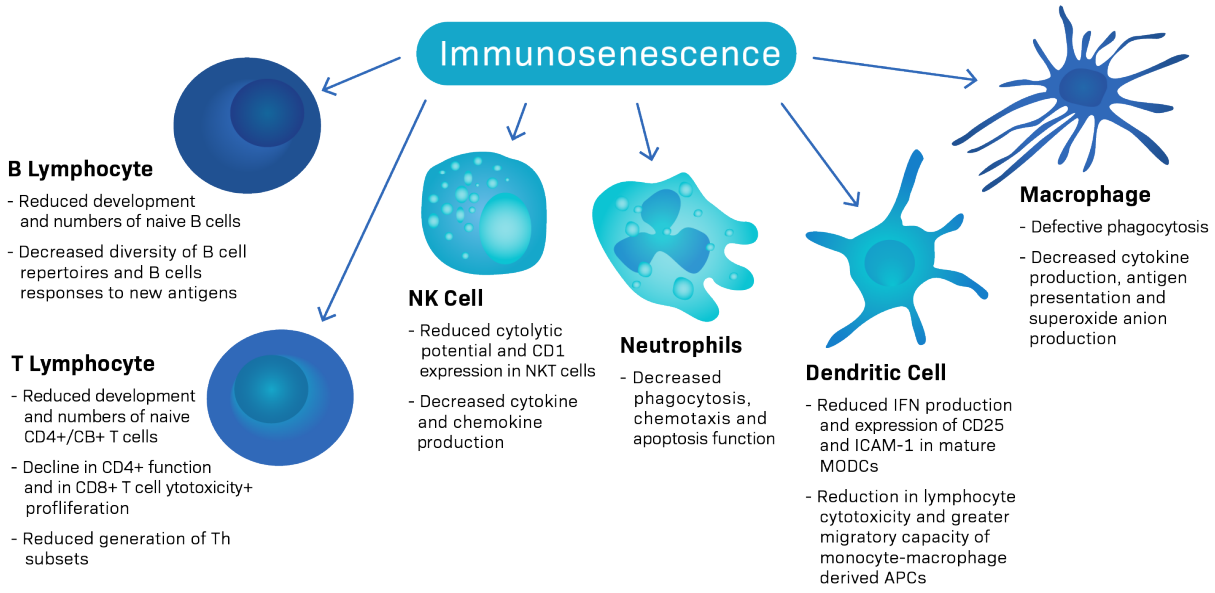
- **Red Blood Cells:** These cells contain hemoglobin and work to carry oxygen throughout the body.

- **Plasma:** The straw-colored fluid that forms the top layer and makes up about 60% of blood. Plasma is mainly water, but it also contains many important substances such as proteins (albumin, clotting factors, antibodies, enzymes, and hormones), sugars (glucose), and fat particles.

- **Platelets:** These are irregularly shaped fragments of cells that circulate in the blood until they are either activated to form a blood clot or are removed by the spleen. Platelets are in the blood so that if we get a tiny cut, we don't bleed out.

- **White Blood Cells (WBCs):** WBCs are an essential part of your immune system for fighting infections. They come in many different shapes and sizes. Some cells have nuclei with multiple lobes, whereas others contain one large, round nucleus. Some contain packets of granules in their cytoplasm, known as granulocytes.

It is important to note that the amounts of white blood cells greatly change with age as shown in the graphic below.



Do you ever wonder why older people are more likely to have negative outcomes with things like COVID-19 and the regular flu? It is because the cells needed to mount an effective response tend to decrease in the blood as we age. **This is called Immunosenescence.**

Immunosenescence: How it relates to Health, Aging, and Biological Age

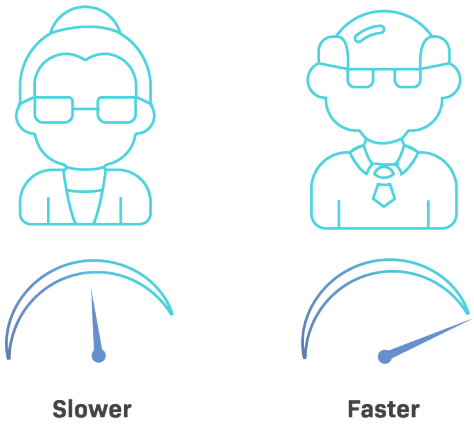
In humans, as well as in many other species, the immune system declines with age. This is known as immunosenescence. The process of immunosenescence leads to a higher incidence of infections, cancer, and autoimmune diseases in the population (6).

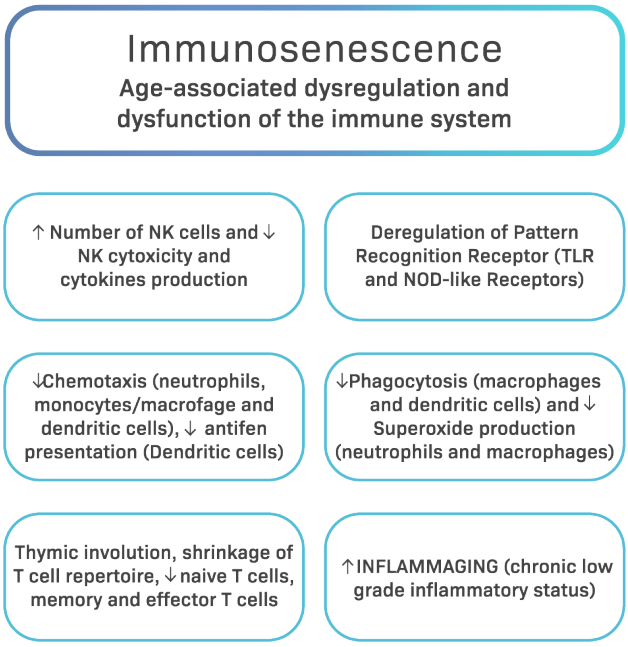
As you can see on the right, the figure displays that the progression to immunosenescence is faster in men than in women and is characterized by age-related changes in immune cells and inflammatory mediators. Immunosenescence also changes the number of immune cells in our blood. As we age we have fewer Naive T Cells, Natural Killer Cells, Macrophages, Dendritic cells, and others.

Since your immune cells change concentrations as we age, this means that our reading of biological age can be affected.

For Intrinsic Epigenetic Age, we control those immune cell changes so that your immune cell subsets are not affecting your reading of age.

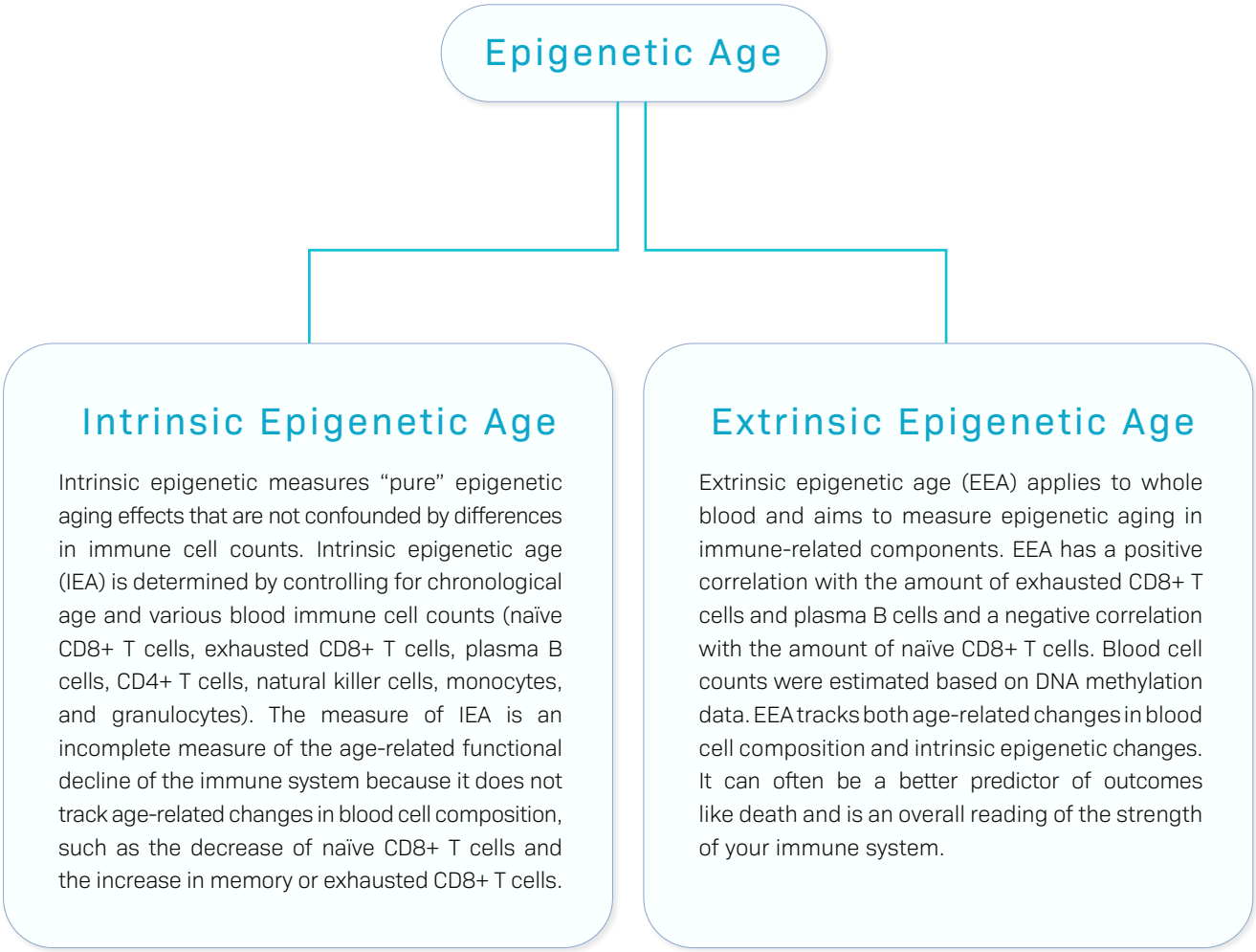
We also report out an Extrinsic Epigenetic age measurement, which does not control for those immune cell subsets. Hence, your extrinsic epigenetic age is a surrogate marker for the age of your immune system.





The Difference Between Intrinsic and Extrinsic Epigenetic Aging

If we break down epigenetic age, it can be split into two important categories: ***intrinsic*** and ***extrinsic*** epigenetic age.

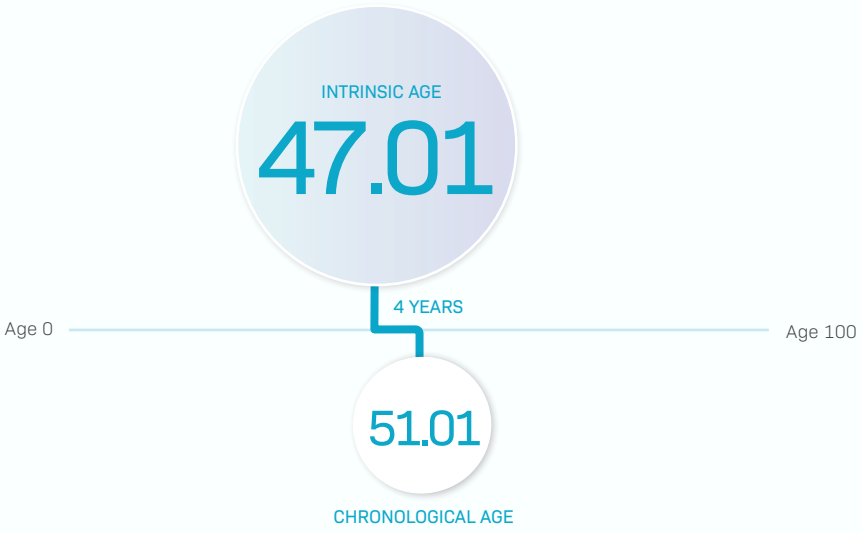


Definitions:

IEAA (Intrinsic Epigenetic Age Acceleration):
A measure that describes age acceleration of the body, independent of age-related changes associated with immune aging.

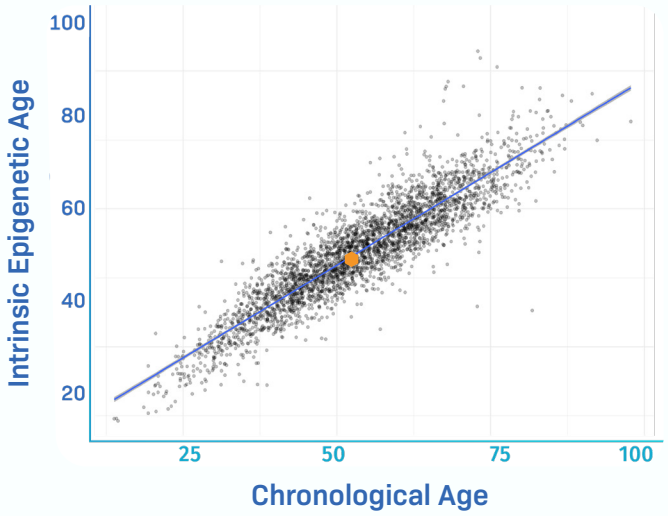
EEAA (Extrinsic Epigenetic Age Acceleration):
Calculates age acceleration and includes the immune system, thus capturing immune aging.

YOUR INTRINSIC
Epigenetic Age



TruAge IEAA=3.45

You versus the Population



49.88

This is your Intrinsic Epigenetic Age using our previous (not Principle Component Analysis corrected) algorithm.

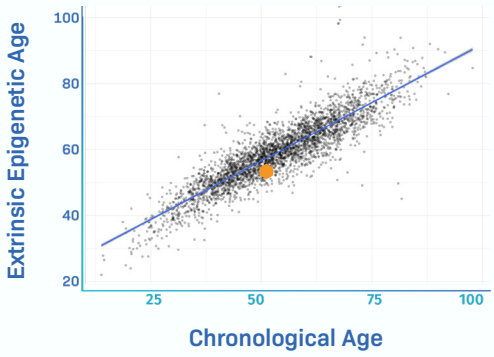
YOUR EXTRINSIC
Epigenetic Age

TruAge IEAA=3.45 | TruAge EEAA=1.25

Parameters	Reference Range	Percentage Values (%)
Bcell	20% to 40%	0%
CD4T		17.47%
CD8T		4.60%
NK		2%
Lymphocyte Total		24.07%
Neutrophils	40% to 60%	66.85%
Monocytes	2% to 8%	12.71%
Eosinophils	1% to 4%	0.93%
CD4T/CD8T Cell Ratio	1 to 4	4.25

This is a functional measurement of your immune system. This measures and predicts many of the cells which change in concentration as we age.

You versus the Population



Disclaimer: The immune cell estimation algorithm is based on a reference-free protocol that uses only your epigenetic profile to calculate the values. This makes the algorithm extremely sensitive to any changes in the methylation values of your DNA. Therefore, the number generated in this report may not be a true reflection of your immune cell counts and you might see some abnormal values as this continues to improve.

What Might have Played into my Score?

The Two Main Studies

Two studies have been done which have looked at correlations between these biological ages. One is the ***Bogalusa study*** and the other is the ***Women's Health Initiative Study*** (WHI). We have included tables of their associations below and summarized many results in the text.

Bogalusa Study Multivariate model that regresses epigenetic age acceleration on participant characteristics in the Bogalusa Study. Coefficients and p-values from regressing measures of intrinsic and extrinsic epigenetic age acceleration on participant characteristics from dataset 1.

Multivariate linear regression		Intrinsic EAA			Extrinsic EAA		
		Estimate (SE)	Z	*p*	Estimate (SE)	Z	*p*
Race	Caucasian vs. African American	-0.013 (0.316)	-0.04	0.97	0.843 (0.316)	2.67	0.0076
Gender	Female vs. Male	-0.622 (0.278)	-2.24	0.025	-0.718 (0.277)	-2.60	0.0093
Education	Grade 8-9 vs. < Grade 8	1.583 (1.468)	1.08	0.28	2.177 (1.465)	1.49	0.14
	Grade 10-12 vs. < Grade 8	1.285 (1.27)	1.01	0.31	2.267 (1.267)	1.79	0.074
	Vocat/Tech vs. < Grade 8	0.307 (1.299)	0.24	0.81	1.921 (1.295)	1.48	0.14
	College vs. < Grade 8	0.85 (1.281)	0.66	0.51	2.375 (1.277)	1.86	0.062
	Graduate vs. < Grade 8	0.147 (1.336)	0.11	0.91	1.53 (1.332)	1.15	0.25
Diabetes (II)		0.173 (0.485)	0.36	0.72	0.012 (0.483)	0.03	0.98
Hypertension		0.539 (0.291)	1.86	0.064	1.247 (0.29)	4.30	$1.7x10^{-5}$
R-squared		0.025			0.043		

WHI Study Multivariate model that regresses epigenetic age acceleration on participant characteristics in the WHI Study. Coefficients and p-values from regressing measures of intrinsic and extrinsic epigenetic age acceleration on participant characteristics from dataset 2.

Multivariate linear regression		Intrinsic EAA		Extrinsic EAA	
		Estimate (SE)	*p*	Estimate (SE)	*p*
Race/Ethnicity	Hispanic vs. African American	-0.94 (0.35)	0.007	3.363 (0.439)	$<10^{-15}$
	White vs. African American	0.71 (0.295)	0.016	1.94 (0.37)	$1.6x10^{-7}$
HDL-cholesterol		0.006 (0.01)	0.558	-0.003 (0.013)	0.799
Triglyceride		0.003 (0.002)	0.059	0.004 (0.002)	0.04
Insulin		0 (0.001)	0.664	0.001 (0.001)	0.337
Glucose		0.003 (0.004)	0.486	0.007 (0.005)	0.112
CRP		0.023 (0.018)	0.215	0.052 (0.023)	0.023
Creatinine		0.703 (0.594)	0.237	1.985 (0.745)	0.008
BMI		0.035 (0.021)	0.103	0.045 (0.027)	.093
Education	High School (HS) vs. no HS	0.357 (0.426)	0.403	-0.784 (0.534)	0.142
	Some College vs. no HS	0.469 (0.381)	0.219	-1.172 (0.478)	0.014
	College vs. no HS	0.486 (0.519)	0.349	-2.253 (0.65)	0.001
	Grad School vs. no HS	0.36 (0.424)	0.396	-1.648 (0.531)	0.002
Alcohol	Past Drinker vs. Never	1.668 (1.1)	0.13	-0.598 (1.379)	0.665
	Light Drinker vs. Never	-0.101 (0.536)	0.85	-0.751 (0.672)	0.264
	Moderate vs. Never	-0.416 (0.748)	0.578	-0.401 (0.937)	0.669
	Heavy vs. Never	-0.354 (0.88)	0.687	-0.833 (1.103)	0.45
Smoking	Former vs. Current	-0.573 (1.039)	0.581	-0.104 (1.302)	0.936
	Never vs. Current	-0.376 (1.039)	0.718	-0.122 (1.303)	0.925
Diabetes		0.216 (0.43)	0.616	-0.061 (0.539)	0.909
Hypertension		0.364 (0.241)	0.131	0.262 (0.302)	0.386
R-squared		0.029		0.069	

Contributing Factors

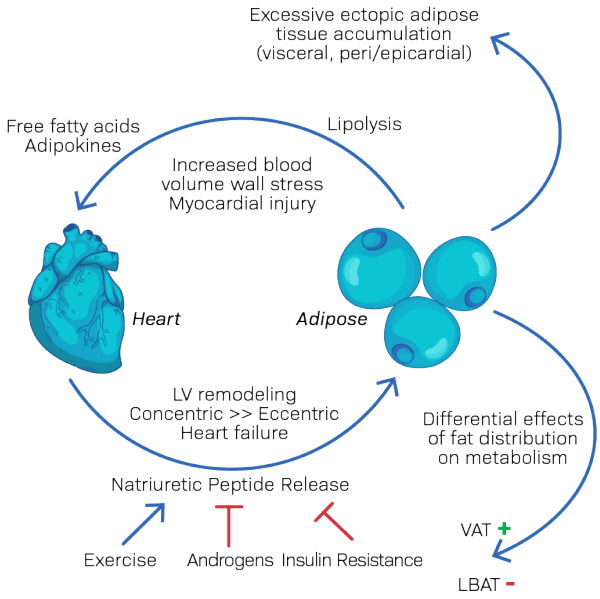
Cardiometabolic Disease, Metabolic Syndrome, and BMI

The health of your metabolic system and your cardiovascular system are intimately related. In fact, because these account for a large proportion of all disease risk, it is no wonder that these metrics can have effects on aging. Cardiometabolic disease can also have an affect on your extrinsic age.

EEA is linked more closely with risk factors for cardiometabolic disease than intrinsic aging according to one study.

EEA is also generally higher in individuals with higher triglyceride levels, higher C-Reactive protein, and higher creatinine.

Neither the intrinsic nor extrinsic epigenetic age of blood tissue are predictive of coronary heart disease (CHD) in the Women's Health Initiative study (WHI) even though EEA is weakly associated with several cardiometabolic risk factors of CHD (such as hypertension, triglycerides, and CRP) (1).



Dietary Intake

Extrinsic epigenetic age acceleration (EEAA) exhibits significant associations with fish intake, moderate alcohol consumption, and blood carotenoid levels (p=1x10-5), an indicator of fruit and vegetable consumption (7).

Race/Ethnicity

Race, ethnicity, and other underlying genetic features also have a significant effect on extrinsic epigenetic age. One study looked at race and found the correlations below.

Hispanics and Tsimane have a higher EEA than Caucasians

Hispanics have a significantly older extrinsic epigenetic age than Caucasians and fewer naïve CD4+ T cells, based on cytometric data from several studies. This pattern of fewer naïve CD4+ T cells is even more pronounced for Tsimane, who experience repeated acute infections and elevated, often chronic, inflammatory loads.

African Americans have lower EEA than Caucasians

African Americans have lower EEA than Caucasians in the WHI and the Bogalusa Study. A study found that African Americans have indications of a significantly younger immune system age than Caucasians ($p = 0.0076$) after controlling for gender, educational level, diabetes status, and hypertension.

In the Bogalusa study, we find three significant predictors of EEA: race/ethnicity, hypertension, and gender ($p = 0.0093$). A marginal analysis in the Bogalusa study identifies a significant association between EEA and hypertension ($p = 8.0 \times 10–5$), type II diabetes status in Caucasians ($p = 0.0085$), but not in African Americans (1).

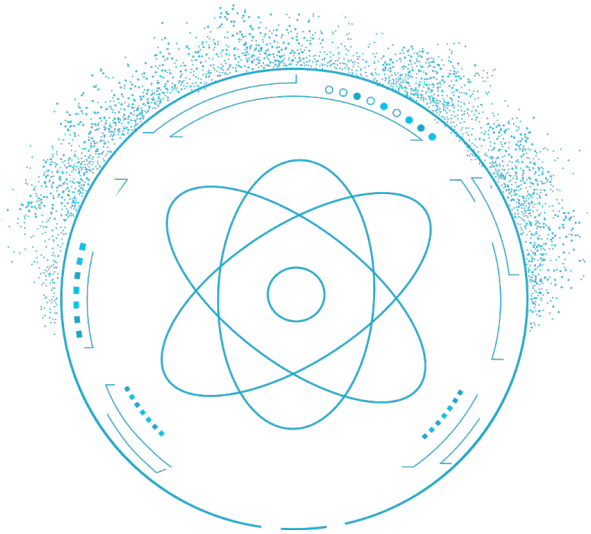
Contributing Factors

Education

Often, education is linked to changes in aging because it is correlated to other lifestyle metrics.

In the WHI study, extrinsic epigenetic age was lower with higher levels of education in all ethnic groups. For each racial/ethnic group, we find that women who did not finish high school exhibit the highest levels of EEA.

However, contrary to the findings in the WHI, no significant association can be observed between EEA and educational level in other studies. More studies are needed to correlate education with extrinsic epigenetic aging.



Mood Stabilizers

Are you currently taking mood stabilizers? Compared with controls, there was a decrease in EEA and IEA in patients with Bipolar Disorder (BD). Further, there was a significant decrease in EEA and IEA in patients with BD taking medication combinations of mood stabilizers (including lithium carbonate, sodium valproate, and carbamazepine) than in those taking no medication/monotherapy (5).

Smoking

Nominally, significant genetic correlations between EEA and lifestyle factors (including smoking behaviors and education) support the hypothesis that the extrinsic epigenetic age is sensitive to variations in the environment.

What are my concerns if my reading is high?

Your Immune System

Since extrinsic epigenetic age is also able to predict the amount of several of your immune values, it is also considered a surrogate marker of the immune system. As a result, a high score may signify that your immune system is not functioning at its highest potential.

When the immune system isn't functioning correctly, your risk of some diseases and disease complications increases. Some of these things include higher cancer risk, higher inflammation (often called inflammaging), higher burdens of senescence, higher risk of autoimmune disease, and much more. If you are worried about your score in this regard, please contact your healthcare provider to learn more.

Your Longevity

Unfortunately, a higher extrinsic epigenetic age is also correlated with shorter lifespans (1). 2,734 deaths were included in a study and it showed that higher extrinsic epigenetic age correlated to a higher hazard ratio for death.

Thus, the high predictive significance of EEA for all-cause mortality probably reflects the fact that it assesses multiple aspects of the biological age of the immune system including both changes in immune cell composition and cell-intrinsic epigenetic changes. It has been known for decades that poor T cell functioning is predictive of mortality (8).

How to Positively Affect this Metric and What Could have Affected your Metric

One of the best things about epigenetic measurements on aging is that proper interventions can lead to better health. While interventional trails on this topic are still in their infancy, we are working to find the best methods to change these metrics.

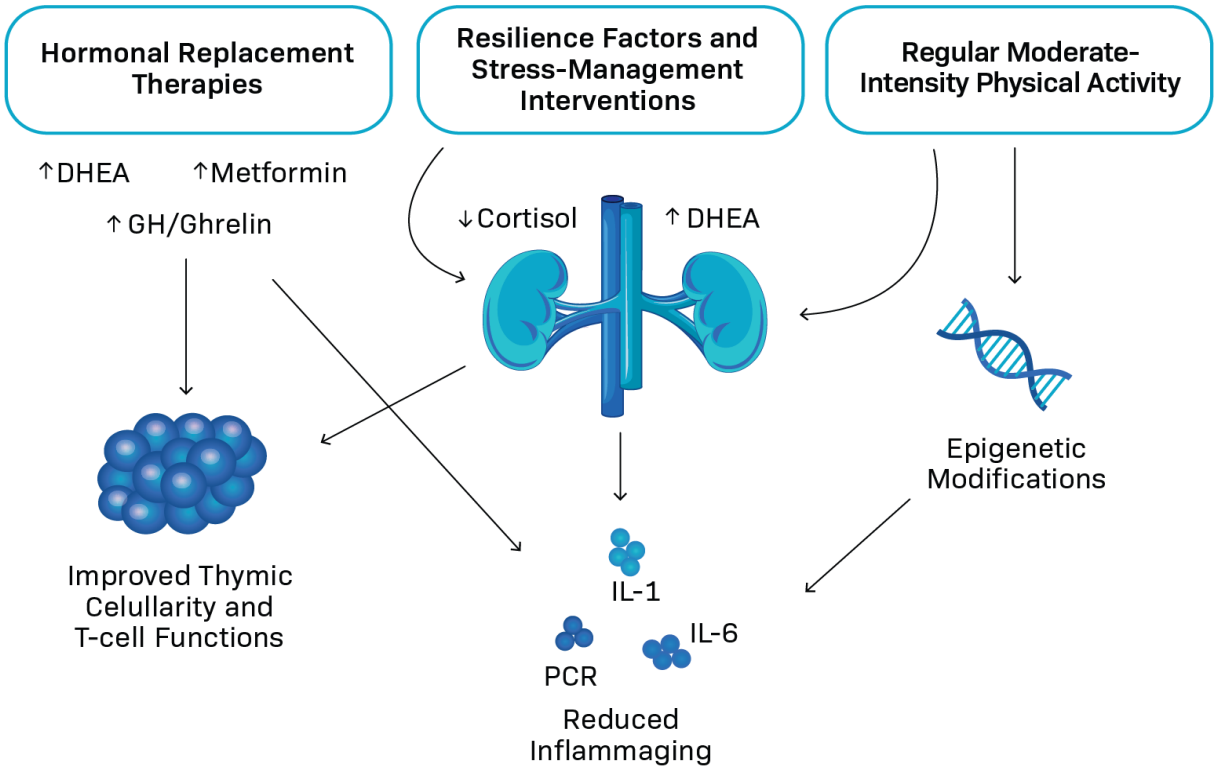
Currently, there is data on how to change this and ways to increase healthier outcomes furthermore.

First, extrinsic epigenetic age acceleration (EEAA) exhibits significant associations with fish intake (p=0.02), moderate alcohol consumption (p=0.01), BMI (p=0.01), and blood carotenoid levels (p=1x10-5), (an indicator of fruit and vegetable consumption) whereas intrinsic epigenetic age acceleration (IEAA) is associated with poultry intake (p=0.03) and BMI (p=0.05) (7).

This means that moderate consumption of alcohol (only validated at 1 drink per week) could help reduce this metric. The consumption of fish, fruits, and vegetables is correlated with an improved EEA.

Other interventions like reducing your BMI and body weight are also correlated with improved metrics.

It is plausible that therapies which prevent or delay the immune systems decline over time might be helpful as well. One validated intervention in this space revolves around the regeneration of the thymus. The thymus is one of our most important immune organs and gets smaller as we age. DHEA, Metformin, and GH-related therapies have all shown improvement in regenerating the thymus and changing the immune cells in our body. Please talk to your healthcare provider about therapies that can help benefit the immune system.



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