EDITORIAL

Which Epigenetic Clock Should I Use? A case study on clock limitations through the lens of DunedinPACE algorithm.

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ABSTRACT
Epigenetic clocks are considered the gold standard when measuring the biological age process. This hypothesis is supported by the evidence that these clocks have the greatest mortality hazards compared to other such predictors. (Jylhävä et al., 2017). Since 2013 (Horvath, 2013), a plethora of studies have been underway to develop new clocks for biological age prediction and have improved every year since (Levine et al., 2022). However, with all of these clocks available, it is important to know which is the best to quantify the aging process for personalized medicine. The most useful epigenetic clock should meet several criteria:

the measure should capture an instantaneous rate of aging (3rd generation clock) rather than an overall biological age (1st or 2nd generation clock);

1. it should be predictive of mortality and morbidity;
2. it should correlate to quality of life metrics;
3. it should be modifiable by interventions which we already know improve healthspan and lifespan;
4. it should be precise

There is already an epigenetic clock that meets thse criteria.
DunedinPACE: the best algorithm to use in personalized medicine. I will discuss each of these points and encourage anyone reading to ask similar questions to anyone else performing a biological age test.

1st, 2nd, and 3rd Generation Clocks: What Does the Generation Tell Us?

To understand why the first generation clocks left something to be desired, we have to explore the idea of "phenotypic variation". Why do we know people who are chronologically 50 years old who look like they're 30 years old chronologically and vice versa?

This difference isn't captured in their chronological age, it is captured in the biochemistry of their bodies.

The first clocks created by Dr. Horvath (Horvath, 2013) and Dr. Hannum (Hannum et al., 2013) in 2013 were a huge breakthrough in age research and science. At the time, there were many reasons this was exciting.

Mainly, the predictive capability of the clocks were amazing. We all know that age is the biggest risk factor for almost every chronic disease and death. It was immediately clear that these clocks were much better than chronological age at telling us how a patient was aging, and therefore their risk for almost every chronic disease was measurable.

The first clocks were trained to predict the chronological age of the patient it tested. This is the definition of a first generation clock (Bergsma and Rogaeva 2020). The problem with first generation clocks is that we don't necessarily care about the chronological age of a patient. Rather, we really care about the biochemistry of aging. So, how can we detect that better?

The answer is to measure and train these DNA methylation patterns to better measurements of aging rather than chronological age. This is how the second generation clocks were created. The three most popular second generation clocks are PhenoAge (Levine et al., 2018) which was trained to 10 blood measurements, GrimAge (Lu et al., 2019) which was trained to predict 12 protein measurements and time until death, and the Telomere Length Clock (Lu et al., 2019) which was trained to predict telomere length.

These second generation clocks were much better. How do we know? Accelerated aging scores were even more predictive of negative health outcomes,

and decelerated aging scores were even more positive health outcomes (Bergsma and Rogaeva 2020).

Beyond this, the second generation clocks were also associated more highly with diseases (Bergsma and Rogaeva 2020). Even then, however, there was still room for improvement. This is because these second generation clocks were created with samples from many people over different timepoints in their life. To get the best aging signal, it would be best to follow the same individuals across their own life at various time points.

That's exactly what the DunedinPACE did. Unlike previous clocks, the Dunedin Pace of Aging (DunedinPACE) was not trained on chronological age. It is the first clock to be trained entirely on phenotypes of aging in the same patients across their lifespan - all the way from age 3 to age 51.

This is helpful because we aren't picking up 'noise" in our measurements. By following the same individuals we can make sure that things like environmental exposures aren't included in these clocks. For example, 50 years ago many people were exposed to more lead through leaded gasoline, less antibiotics, and less microplastics. If we don't control for the time at which people lived, our algorithm might include markers associated with these exposures rather than just measuring aging.

No clock to date has done this.

The clock creation was led by Duke professors Terrie Moffitt and Avshalom Caspi (Belsky et al., 2022). Their team built a database of phenotypic expressions of age and designed the clock to quantify the rate of decline in system integrity experienced by an individual over the recent past. It is designed to track methylation markers of Age Acceleration - the speed at which your cellular functions fail throughout the body. It acts like a speedometer for the body's aging process.

The DunedinPACE is the Most Predictive Clock of Mortality and Morbidity

While the precision of clocks is driven by the reproducibility, the accuracy is often driven by how well it can predict health outcomes. Thus, the best clock would show increased aging for someone who

Table 1

Time-to-Event Analysis of Mortality, Cardiovascular Disease (CVD) Diagnosis, and Stroke or Transient Ischemic Attack (TIA)

	Mortality			CVD			Stroke/TIA		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
DunedinPACE	1.64	[1.48-1.82]	6.46E-22	1.39	[1.25-1.54]	8.08E-10	1.42	[1.18-1.70]	1.57E-04
Horvath Clock	1.02	[0.95-1.10]	0.584	1.04	[0.95-1.14]	0.429	1.00	[0.86-1.17]	0.998
DunedinPACE	1.60	[1.43-1.78]	8.59E-17	1.34	[1.21-1.49]	1.73E-08	1.35	[1.13-1.61]	0.001
Hannum Clock	1.09	[1.01-1.17]	0.019	1.12	[1.02-1.23]	0.020	1.17	[1.00-1.36]	0.052
DunedinPACE	1.57	[1.40-1.75]	2.11E-15	1.35	[1.21-1.50]	9.25E-08	1.33	[1.07-1.65]	0.011
PhenoAge Clock	1.14	[1.03-1.25]	0.009	1.10	[1.00-1.20]	0.053	1.20	[1.02-1.40]	0.025
DunedinPACE	1.24	[1.12-1.38]	6.89E-05	1.18	[1.05-1.34]	0.007	1.33	[1.05-1.69]	0.019
GrimAge Clock	1.61	[1.49-1.74]	1.30E-33	1.33	[1.19-1.49]	5.74E-07	1.11	[0.91-1.35]	0.295

Repeated Measures Analysis of Incident Limitation to Activities of Daily Living (ADLs)

	Nagi ADLs			Katz ADLs			Rosow-Breslau ADLs		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
DunedinPACE	1.39	[1.17-1.65]	1.49E-04	1.31	[1.14-1.50]	1.02E-04	1.40	[1.24-1.57]	2.36E-08
Horvath Clock	1.05	[0.88-1.26]	0.565	1.11	[0.98-1.26]	0.091	0.96	[0.89-1.05]	0.385
DunedinPACE	1.37	[1.14-1.64]	6.63E-04	1.30	[1.13-1.51]	3.07E-04	1.37	[1.20-1.57]	2.84E-06
Hannum Clock	1.08	[0.91-1.28]	0.381	1.06	[0.94-1.19]	0.367	1.04	[0.91-1.19]	0.562
DunedinPACE	1.40	[1.14-1.72]	0.001	1.26	[1.06-1.50]	0.007	1.43	[1.25-1.64]	3.54E-07
PhenoAge Clock	1.00	[0.77-1.30]	0.973	1.13	[0.95-1.35]	0.161	0.93	[0.81-1.07]	0.298
DunedinPACE	1.27	[1.02-1.58]	0.032	1.26	[1.02-1.54]	0.029	1.27	[1.08-1.50]	0.005
GrimAge Clock	1.18	[0.94-1.48]	0.158	1.10	[0.90-1.34]	0.357	1.15	[0.97-1.37]	0.098

is likely to become sick or to die and decreased aging for someone who is healthy and resistant to disease.

The DunedinPACE is excellent at this with high correlations to disease.

Even just being slightly above an aging rate of 1 biological year/chronological year can increase your risk of death by 56% in the next 7 years and increase your risk of a chronic disease diagnosis by 54% over the next 7 years.

This is why it's important to keep this as low as possible for as long as possible.

Research diving into the Dunedin Pace of Aging algorithm (Belsky et al,. 2020) verified that people the PACE algorithm identified as aging faster had a greater risk of poor health, developing chronic diseases or dying earlier. Faster-aging cohorts also displayed a higher long-term risk of cardiovascular diseases, Alzheimer's disease, along with MRIs that showed a reduction in cortical thickness.

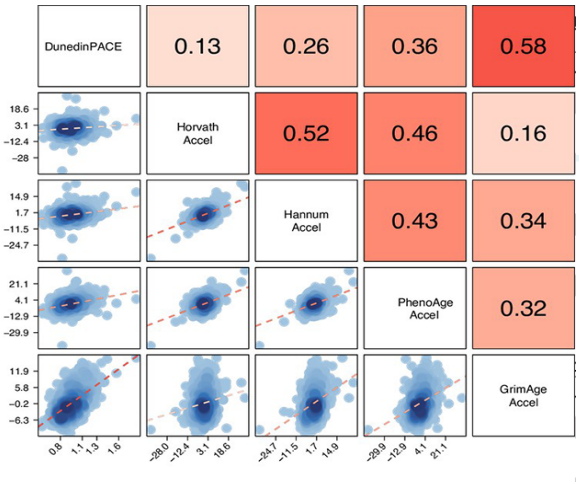
Conversely, those that the algorithm identified as aging more slowly performed better on tests of balance, strength, walking speed and mental ability, with greater muscle mass and a facial appearance that looked younger.

In fact, patients who were considered fast agers, were 16% more likely to die and 23% more likely to develop a chronic disease. That means they were 65% more likely to die in our cohorts than those at normal or slow aging.

You can even see that the DunedinPACE is moderately correlated with some DNA methylation clocks, in particular GrimAge (see Table 1). The Duke researchers therefore conducted an analysis to test if DunedinPACE contributed new information about health-span and lifespan over and above existing DNA methylation clocks. They tested if DunedinPACE associations with morbidity, disability, and mortality were statistically independent of each of the DNA methylation clocks within the Framingham Heart Study.

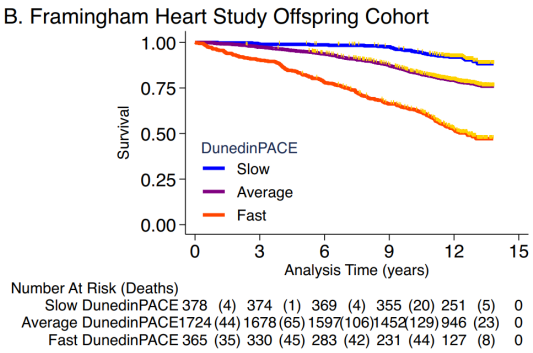
In the case of the GrimAge clock, which was developed to predict mortality using this Framingham dataset, this analysis provides an especially rigorous test.

Thus, Dunedin PACE adds incremental prediction over and above all clocks studied here.



The panel above shows a matrix of correlations and association plots among DunedinPACE and age-acceleration residuals of Horvath, Hannum, Levine-PhenoAge and Lu-GrimAge epigenetic clocks. The diagonal cells of the matrix list the DNA methylation measures. The half of the matrix below the diagonal shows scatter plots of associations.

For each scatter-plot cell, the y-axis corresponds to the variable named along the matrix diagonal to the right of the plot and the x-axis corresponds to the variable named along the matrix diagonal above the plot. The half of the matrix above the diagonal lists Pearson correlations between the DNA methylation measures. For each correlation cell, the value reflects the correlation of the variables named along the matrix diagonal to the left of the cell and below the cell.



The graph above shows mortality in the Framingham Heart Study Offspring Cohort. The figure plots Kaplan-Meier curves for three groups of participants in each of the two cohorts: those with DunedinPACE 1 SD or more below the mean ('slow' DunedinPACE, blue line); those with DunedinPACE within 1 SD of the mean ('average' DunedinPACE, purple line); and those with DunedinPACE 1 SD or more above the mean ('fast' DunedinPACE, red line).

Censoring of participants prior to death is indicated with gold hash marks. The table below the figure details the number of participants at risk per 3-year interval and, in parentheses, the number who died during the interval.

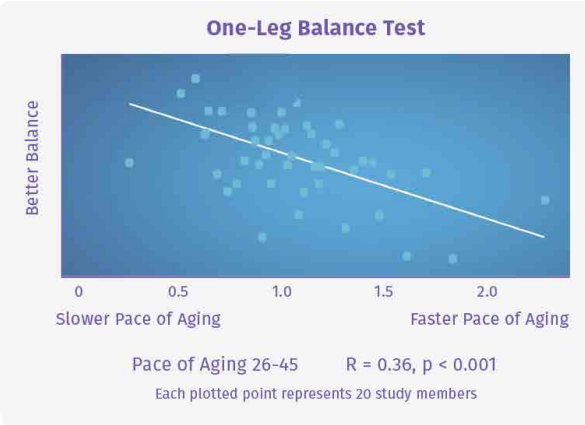
The DunedinPACE has the Highest Correlations to Quality of Life Metrics

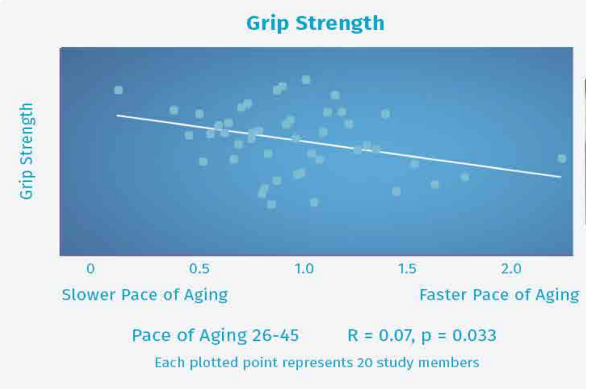
We also know that optimal aging is more than just trying to live longer and to avoid disease. We also want to thrive with a high quality of life. The good news is that we know the DunedinPACE is extremely correlated with these outcomes as well.

Take a look at some of the pictures below. In these pictures, Duke researchers analyzed the DunedinPACE cohort at the age of 45. Remember, that these patients have been studied since they were 3 years of age.

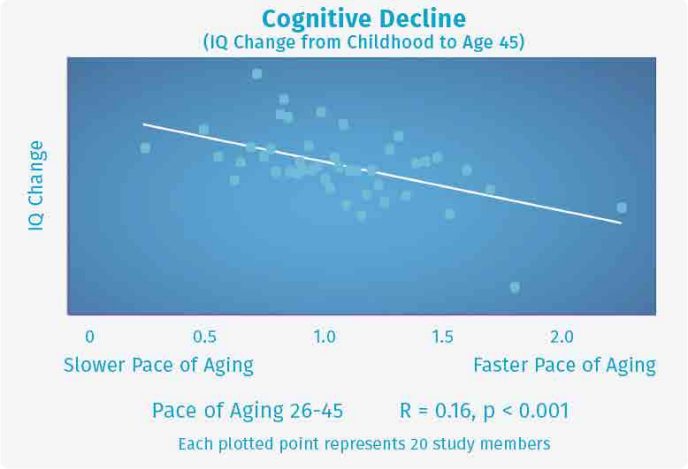
Researchers wanted to see how their rate of aging compared to quality of life outcomes such as Grip Strength (we lose muscle with age), Balance testing (we lose balance with age), Brain MRIs (our brains shrink with age), IQ (we lose intelligence as we age) and even Facial Aging (we look older as we age).

The fast agers on the right have poor balance as adults, and have weaker grip strength. Each data point represents 20/938 cohort members at age 45

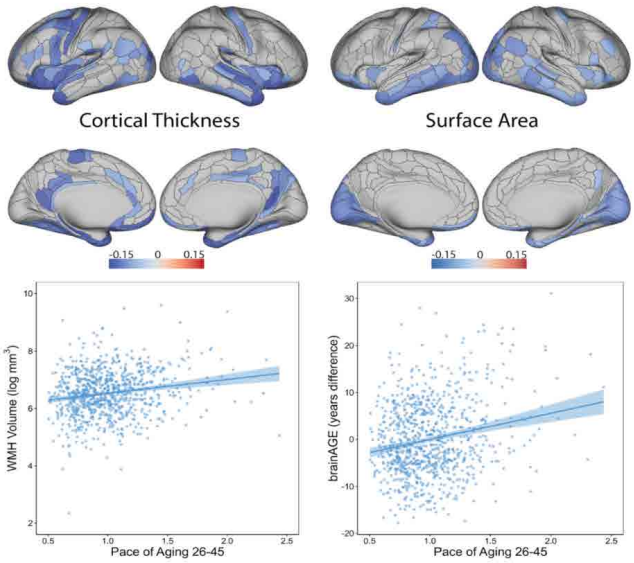




The fast agers had more decline on tested cognitive function from childhood to age 45.

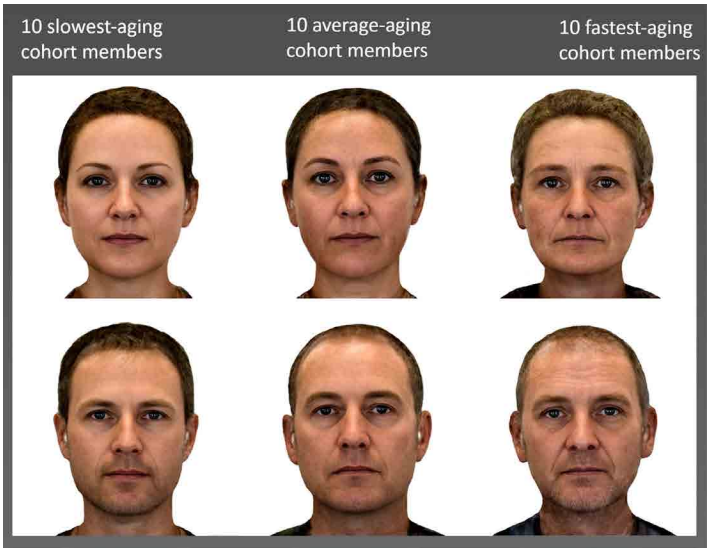


These changes also occurred inside the brain. A faster pace of aging was associated with a number of brain measures that are associated with advanced age.

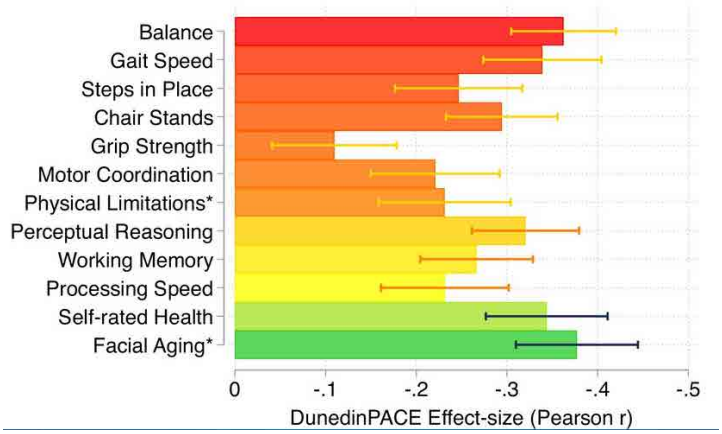


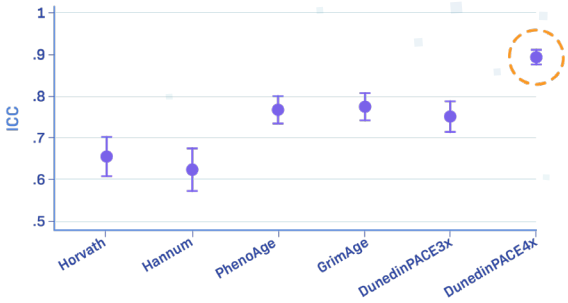
In the above image you see they were associated with thinner cortex and smaller surface area of the brain as assessed by MRI at age 45. Faster aging was also associated with more volume of white matter hyperintensities.

Everyone in the following image is 45 years old chronologically. You can see that the fast agers even had faces which trained researchers rated as older looking at age 45



In addition, as you can see in the pictures below, as the rate of aging increases, the performance of the DunedinPACE participants in all of these areas decreases (Belsky et al,. 2020). This means that with slower rates of aging, you can move about the world better, you can think better and remain sharp, and you will even look younger for longer. All of these outcomes are quality of life improvements as well. For DunedinPACE, these effect sizes are also bigger than any other age algorithm.





The figure above shows effect-sizes for associations of DunedinPACE, DunedinPoAm, and DNA methylation clocks with physiology-based measures of biological age and self-rated health. Effect-sizes were estimated from the Understanding Society data (n=1,175).

Effect-sizes are reported as standardized regression coefficients interpretable as Pearson r values. Error bars show 95% confidence intervals. DNA methylation clocks were residualized for chronological age prior to analysis. Models included covariates for chronological age and sex. Physiology-based measures of biological age were computed from Understanding Society biomarker data (albumin, alkaline phosphatase, creatinine, C-reactive protein, blood urea nitrogen, glycated hemoglobin, systolic blood pressure, and forced expiratory volume in 1second) based on algorithms derived in data from the US NHANES according to the methods developed by (Levine et al., 2018), (Klemera and Doubal, 2006), and (Cohen et al., 2013).

The DunedinPACE is Modifiable by Interventions which we already know Improve Healthspan and Lifespan

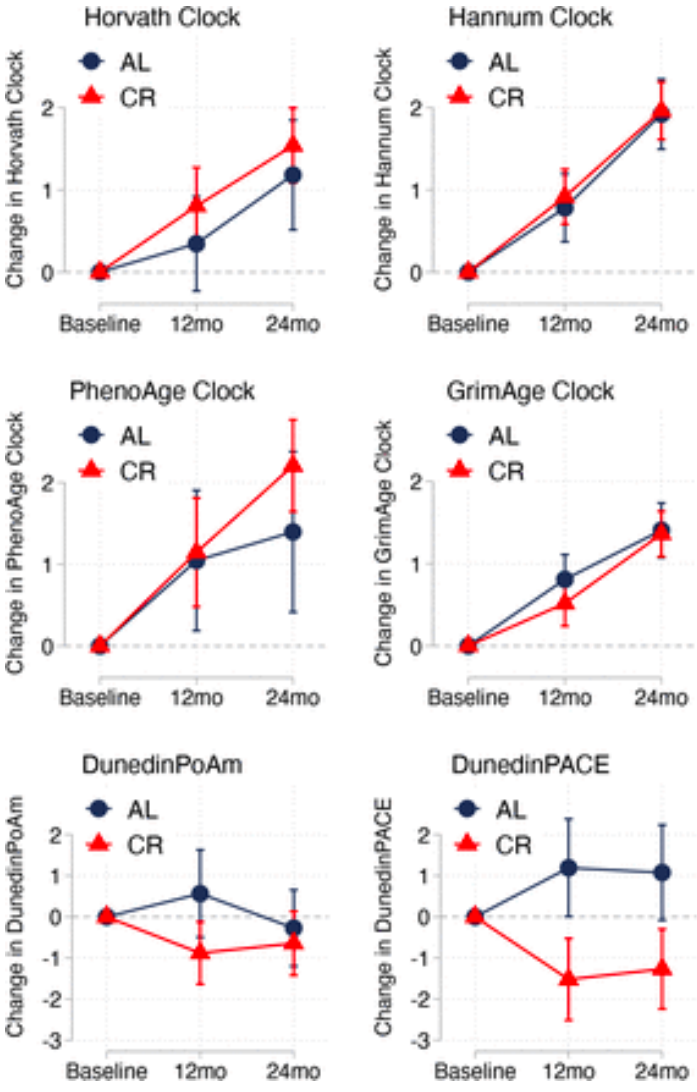
These clocks are the best ways to predict age related outcomes. However, we still don't know exactly why we see these patterns in our DNA.

In order to make sure that this is a reliable and useful measurement, we also need to make sure that these clocks respond to things we already know beneficially affect biology. An article from 2020 by Jamie Justice PhD, from Wake Forest, outlines the following criteria for an aging biomarker

Biomarker Criteria	Horvath epigenetic age	Hannum epigenetic age	GrimAge	PhenoAge	DunedinPoAm
DNA Methylation Biomarker Calibrated to Detect:	Chronologic Age	Chronologic Age	Biomarkers, Smoking, Death	Phenotypic Age	Pace of Aging (change)
Feasible for use in a clinical trial in older adults?	✓	✓	✓	✓	✓
Robustly associated with chronological age across independent cohorts?	✓	✓	✓	✓	✓
Predict age-related change in function, chronic disease, or death?	✓	✓	✓	✓	✓
Responsive to interventions that beneficially affect the biology of aging?	--	--	--	--	--

As you can see, none of the clocks have been able to fulfill this last criteria. However, this has changed. Now, the DunedinPace has satisfied all criteria. One of the cohorts used to validate to prove this consisted of middle-aged, non-obese adults enrolled in the CALERIE trial. This trial tested the effects of caloric restriction – an intervention that has beensuccessful in animal models – over a period of two years.

As you can see in the image below, just as expected, the DunedinPACE was able to show a decrease in the rate of aging in those groups who restricted calories by approximately 11% over 2 years.

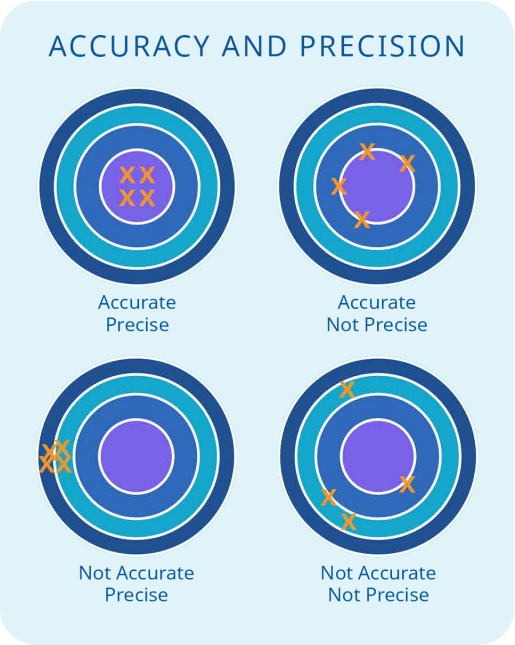


In this figure, we see change from baseline to 12- and 24-month follow-up in DunedinPACE (Pace of Aging) measures of aging in ad libitum (AL) and caloric restriction (CR) groups in CALERIE Trial. (Waziry, R., et al.) This means that this algorithm does respond to interventions which we know beneficially affect aging.

Most first and second-generation algorithms failed to predict the positive effects of caloric restriction in the same cohort. The CALERIE study found that, only GrimAge and DunedinPACE both were able to predict improvements in health, PACE was far more responsive to short-term changes.

The DunedinPACE is the Most Precise Clock

One of the biggest critiques of methylation based clocks are that they have too much variability. Some of the original clocks could vary up to 4 years even if you tested the same sample at the same exact time. This is a very valid concern. For instance, if you took a baseline test then implemented a new diet and exercise regime and measured it again after a few months you could see "false" increases or decreases which could give you incorrect information.



Thus, there is an extreme need for these tests to be highly accurate.

This precision is measured by the Intraclass Correlation (ICC) value. The intraclass correlation is a statistical number which describes how a number within a group compares to each other. In this type of testing, it is usually applied to the same samples tested twice. This depicts the margin of error between samples. All of the popular algorithms' ICC values are depicted in the graph below. As you can see, they have had some issues with precision. However, the DunedinPACE is extremely precise with an ICC value over 0.90 - the best of any clock!

Out of all aging clocks, DunedinPACE offers the highest ICC value, across the board, making it the most precise clock. If you see small changes happening, it is likely due to actual changes in biological age - not just statistical noise in the measurement.

The DunedinPACE is the Best for Personalized Medicine

One of the big issues that aging medicine has faced is the lack of an objective metric to measure changes in aging rate in individuals. Two individuals may not respond in the same way to the same intervention. For instance, individuals can have drastically different responses to the same medications and supplements.

PACE solves this problem, offering a personal look at a patient's own rate of aging. One person can try the same intervention as another, however, their impact on aging might differ.

The sensitivity, precision, and biological signal that DunedinPACE is measuring makes it perfect for individualized and personalized medicine.

I recommend three months between tests, to show actual epigenetic changes that come as a result of recent lifestyle changes and clinical interventions.

PACE is a great tool to add to your longevity-based analysis because, unlike earlier biological age clocks, it is able to tell you how your recent choices are affecting aging at the precise moment of the test, instead of just the overall age of your body.

Optimize Aging Early

Changes to pace of aging is especially effective when patients begin aging optimization while they are young. This means that people of all ages should pursue a lower PACE score, to have a longer life with fewer age-related diseases. You now have the ability to detect rapid aging at an early age, and head it off with preventative measures as a young adult, rather than trying to reverse the cumulative effects of aging that have begun to express outwardly.

Why start early:

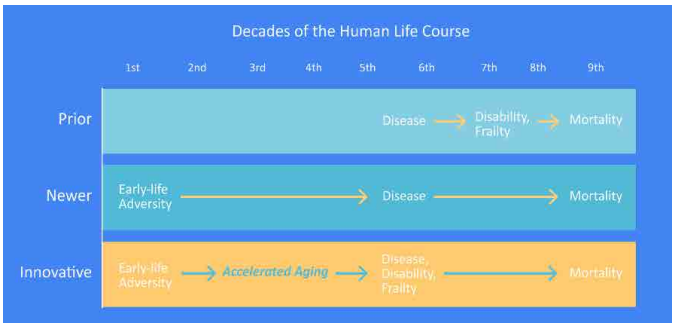
- Exposures begin accumulating early in life.
- Changes to physiology and aging biomarkers appeared many years before disease diagnosis.
- Organ damage is difficult to reverse fully
- Preventive interventions are more effective, the earlier you begin

For example, children who grow up in socioeconomic disadvantage face an increased burden of disease and disability throughout their lives. One hypothesized mechanism for this increased burden is that early-life disadvantage accelerates biological processes of aging, increasing vulnerability to subsequent disease. You can see this represented in the image above.

Summary

We are witnessing rapid progress in the intersection of quantifying the aging process, so it is important to understand which clock is the golden standard for personalized medicine.

The DunedinPACE is the best algorithm for epigenetic age quantification. It is the most precise, the most predictive, linked to outcomes which include quality of life metrics, changes in response to validated interventions, and is now available at a lower cost.



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