Quantification of Biological Aging

Daniel W. Belsky, PhD
Assistant Professor
Department of Epidemiology & Robert N Butler Columbia Aging Center
Columbia University Mailman School of Public Health
daniel.belsky@columbia.edu
@danbelsky

COLUMBIA UNIVERSITY | MAILMAN SCHOOL of PUBLIC HEALTH

COLUMBIA|AGING CENTER
THE ROBERT N. BUTLER COLUMBIA AGING CENTER

Disclosures

DWB is listed as an inventor on a Duke University and University of Otago invention (DunedinPACE) that has been licensed to a commercial entity

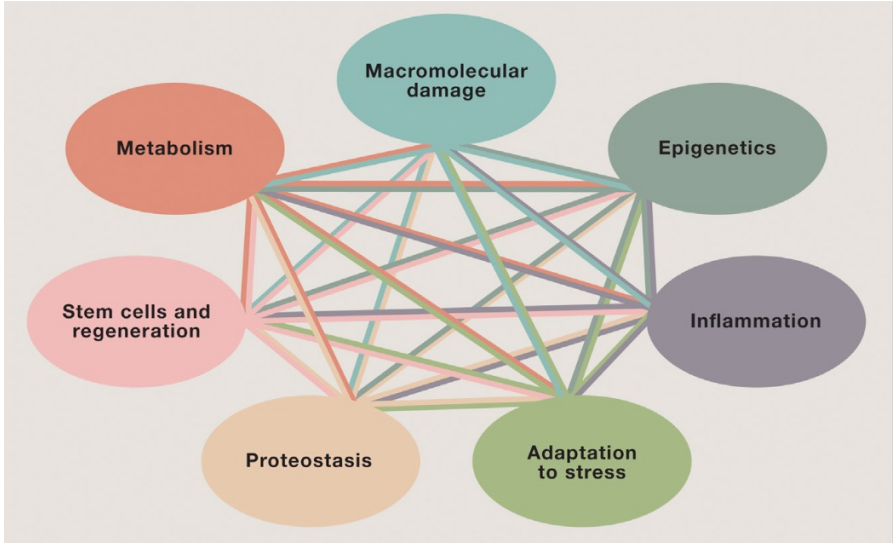
Aging is a biological process

a gradual and progressive decline in system integrity

Kirkwood 2005

Lopez-Otin et al.
2013 Cell

Kennedy et al.
2014 Cell



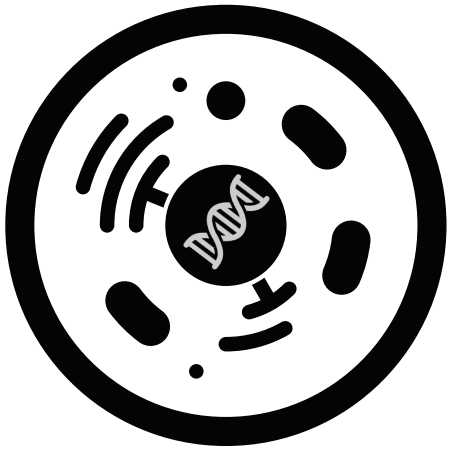
A geroscience model of aging-related burden of disease

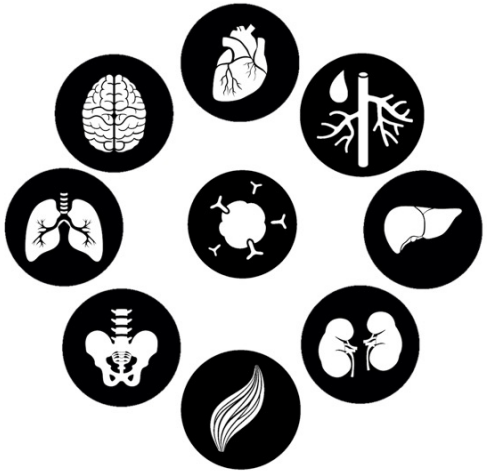
Molecular Changes

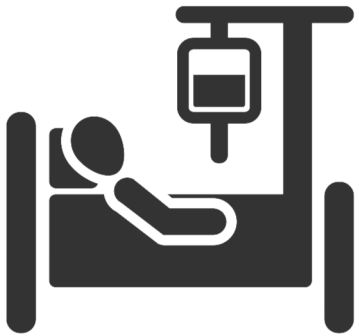
Decline in System Integrity

Functional Decline

Disease
Disability
Mortality

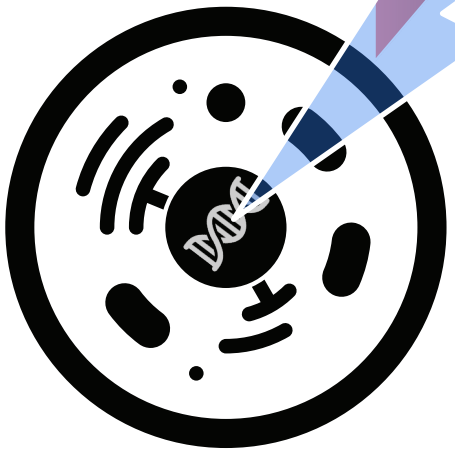




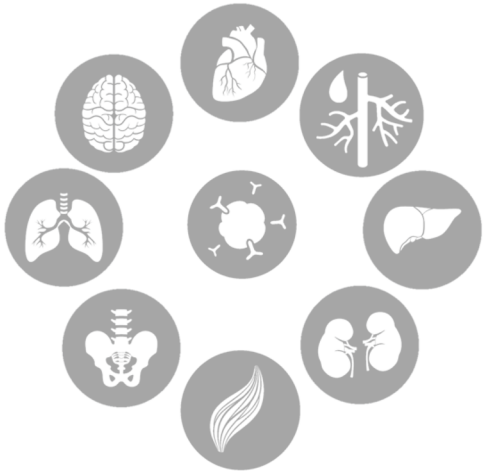


Geroprotective intervention

Molecular Changes

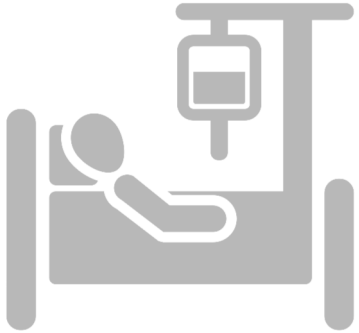


Decline in System Integrity



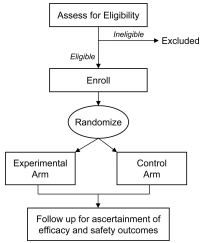
Functional Decline

Disease Disability Mortality



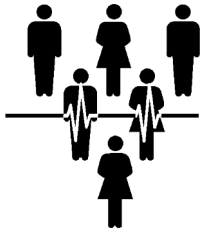
Why do we need measures of biological aging?

1. Testing effects of geroprotective interventions
   Decades of follow-up are needed to test effects on healthspan. Changes in biological aging could be measured in years.



2. Clinical risk assessment and prognosis
   Chronological age is a crude measure. We can improve precision for timing screening, monitoring health, and forecasting potential outcomes of intervention

3. Population surveillance and program/policy evaluation
   Monitoring changes in population health from data on lifespan, disease burden, or healthcare utilization gets us answer too late. Sensitive measures enable faster action and aid calculation of benefit/cost ratios



What is a biological age?

The age at which a person's biology would be "normal" in a reference population

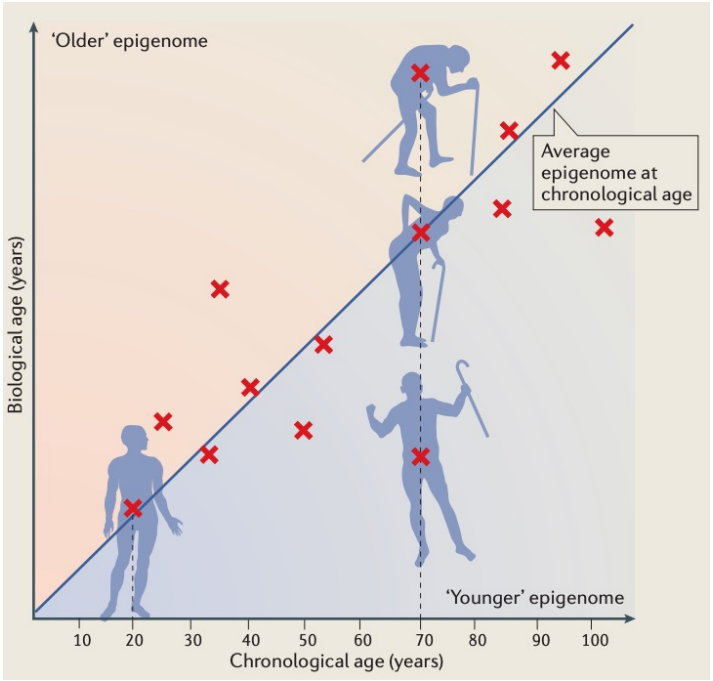


Figure – Benayoun et al. 2015 Nat Rev Mol Biol

What is a biological age?

The age at which a person's biology would be "normal" in a reference population

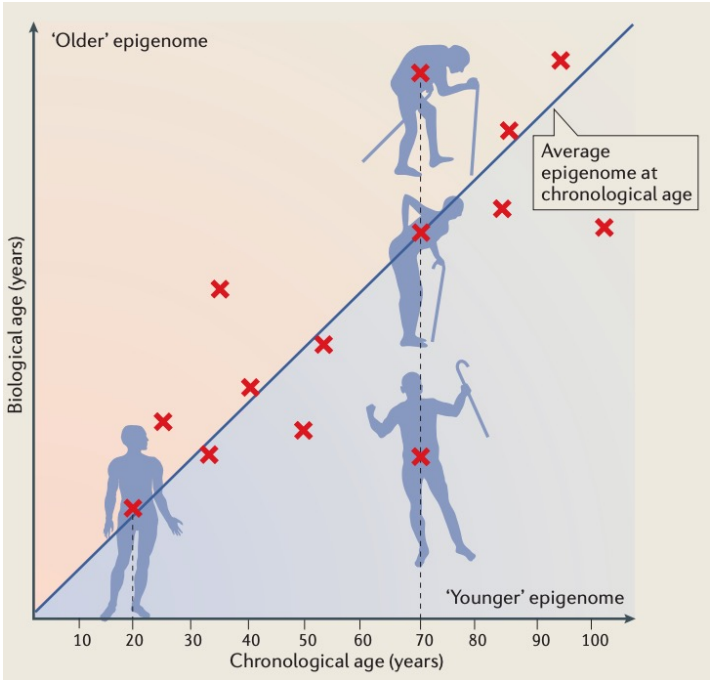


Figure – Benayoun et al. 2015 Nat Rev Mol Biol

What is a biological age?

The age at which a person's biology would be "normal" in a reference population

Healthspans and lifespans vary across places, populations, historical periods

Metrics of biological aging are scaled relative to the sample in which they are developed

The sample used for development should reflect the distribution of causes and features of aging in the population in which the measurement will be used

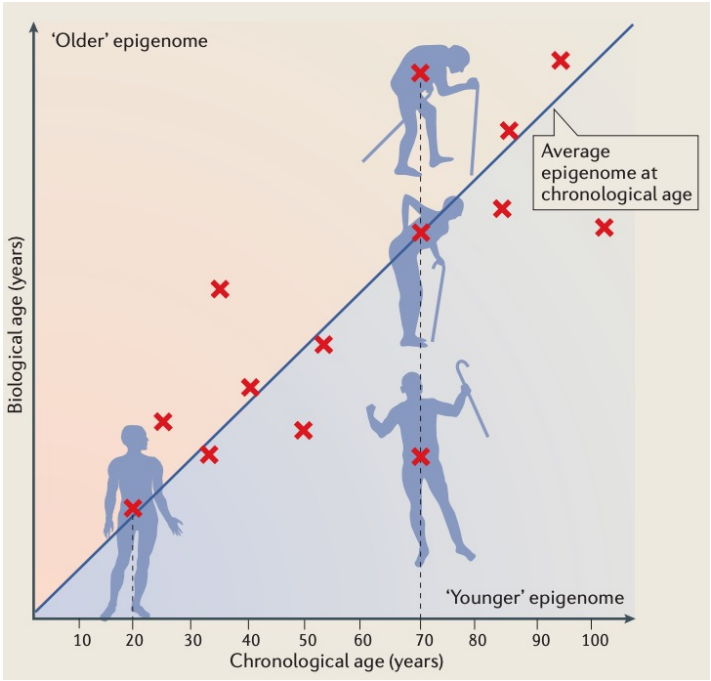


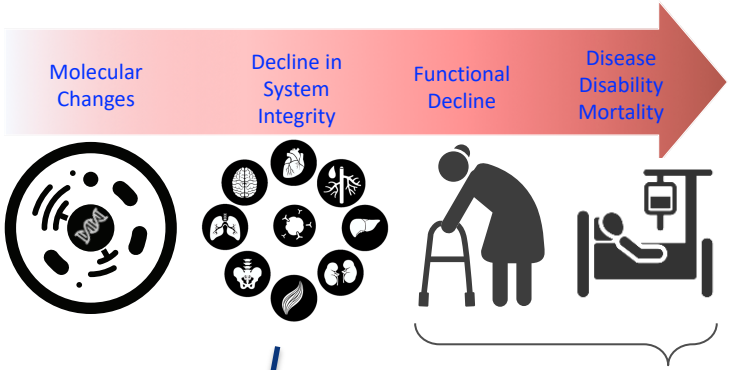
Figure – Benayoun et al. 2015 Nat Rev Mol Biol

What is a biological age?

The age at which a person's biology would be "normal" in a reference population

Biology can be observed at multiple levels of analysis/ organization

There are reciprocal interactions across levels



Cellular Level
- Epigenetics
- Transcriptomics
- Proteomics
- Metabolomics
- Etc.

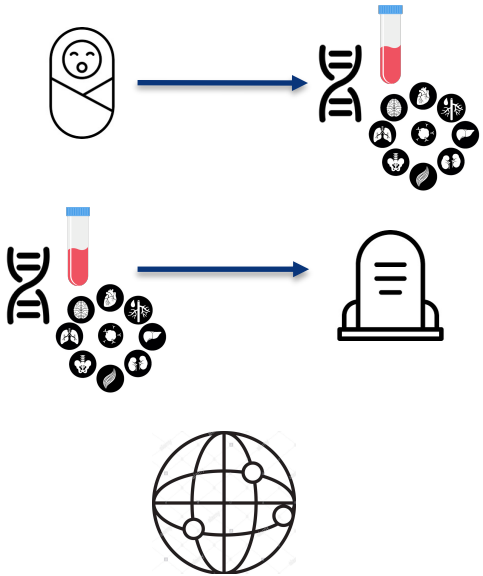
Organs/ Organ-systems
- Organ function testing
- Body composition
- Blood chemistries

Deficit Accumulation
- Functional testing
- Disease counts

What is a biological age?

The age at which a person's biology would be "normal" in a reference population

- **Time since birth**
  BA = differences between older and younger people
- **Time until death**
  BA = differences in mortality risk
- **Coordination across biological systems**
  BA = system integrity



Klemera-Doubal method BA
First-gen DNAm clocks
(Horvath, Hannum, many others)

PhenoAge (Levine),
GrimAge (Lu)

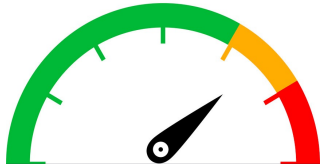
PCA-based methods, homeostatic dysregulation (Cohen)

Limitations of Biological Age

- **Survival Bias**
  Older people necessarily represent slower agers (because the faster agers have died)
- **Cohort Effects**
  People born at different times in history grow up under different exposure regimes (pathogens, toxicants, healthcare technology, health behavior norms)
- **Uncertain Timing**
  Uncertain if older/younger biological age reflect ongoing processes of aging or were established early in life

Belsky et al. 2018 Am J Epid, Belsky et al. 2022 eLife

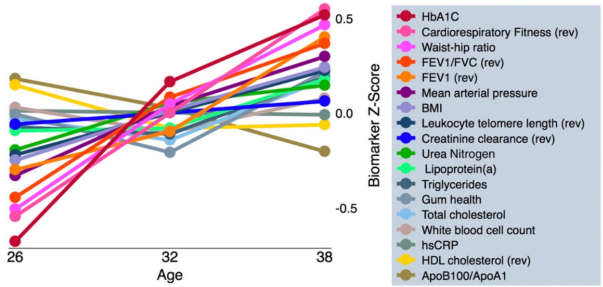
An alternative: Pace of Aging



Pace of Aging is the rate of decline in system integrity

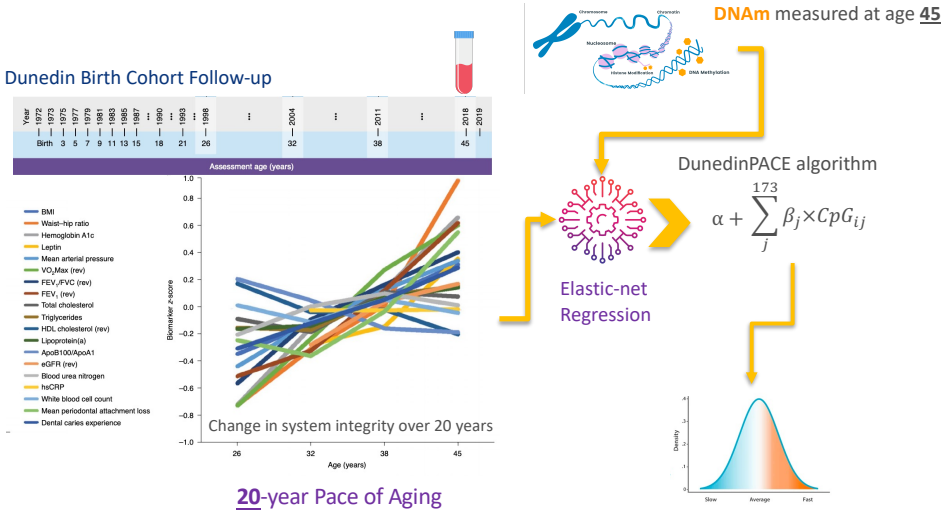
- Aging = *changes* within individuals' bodies/cells

In our work, we measure Pace of Aging from declines in integrity across multiple organ systems



Belsky et al. 2015 PNAS
Elliott et al. 2021 Nat Aging

DunedinPACE:
A DNAm blood test for the Pace of Aging



- Young adulthood-midlife follow-up excludes dropout from morbidity / mortality
- Single birth cohort excludes cohort effects
- Repeated measures to quantify change over 2 decades of follow-up

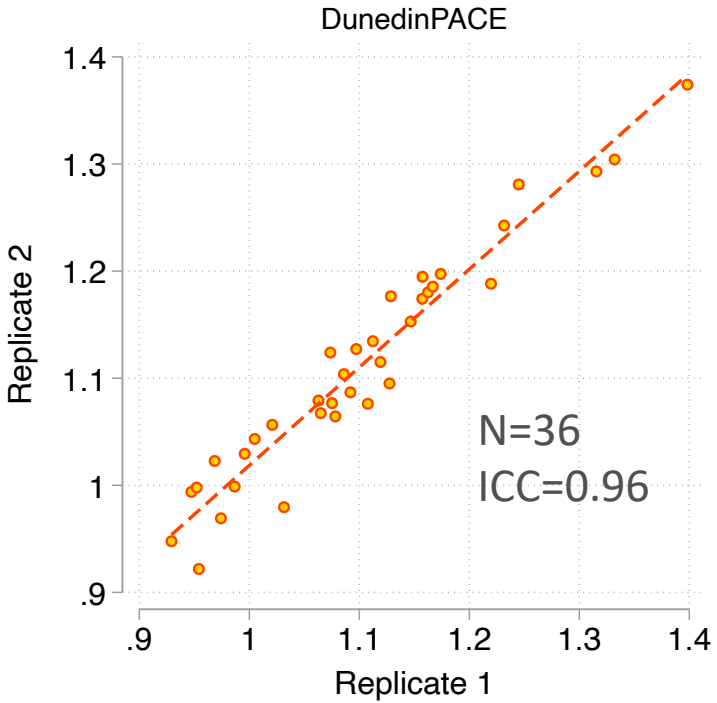
Technical improvements to the DNA methylation algorithm

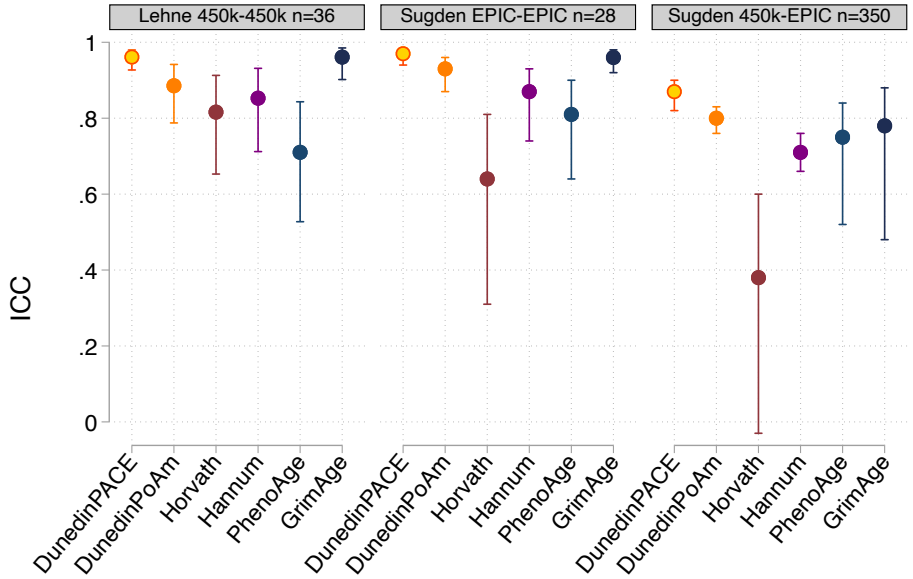
- Use of higher-reliability probes on Illumina arrays to improve test-retest reliability
- Internal normalization to enable single-sample analysis

Belsky et al. 2022 eLife

DunedinPACE has excellent test-retest reliability

Essential for testing within-individual change from pre-treatment baseline to follow-up

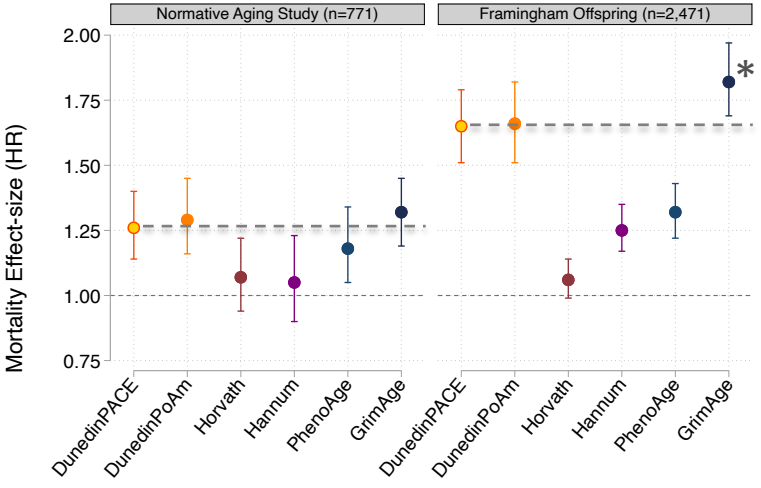




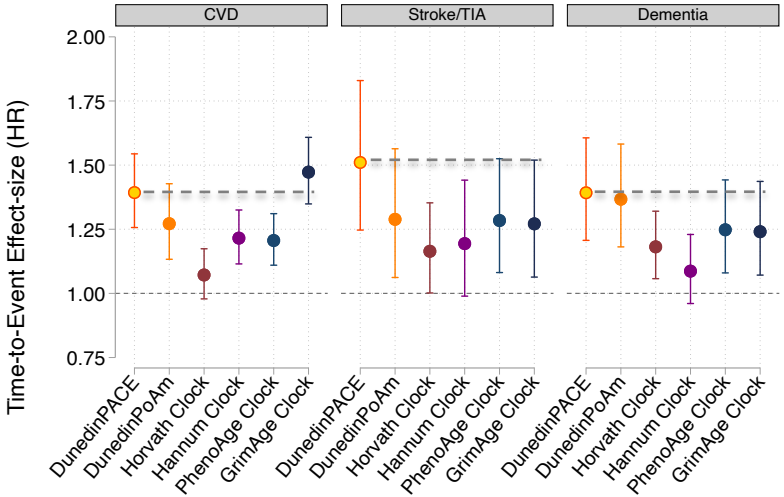
Data from GSE55763

Belsky et al. 2022 eLife

DunedinPACE shows similar prediction of mortality morbidity, and disability to GrimAge



Mortality in the Normative Aging Study & Framingham Heart Study

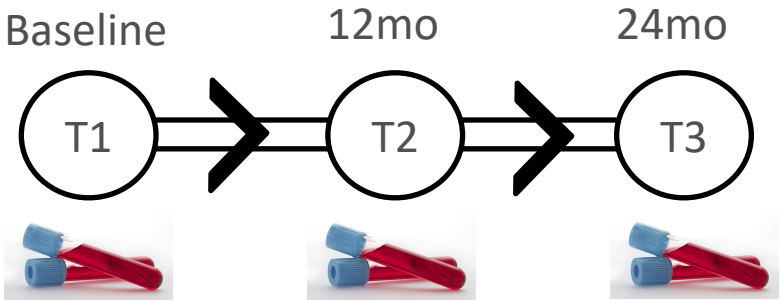


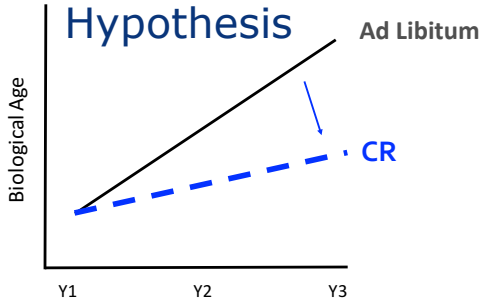
Incident Cardiovascular Disease, Stroke/TIA, and Dementia in the Framingham Heart Study

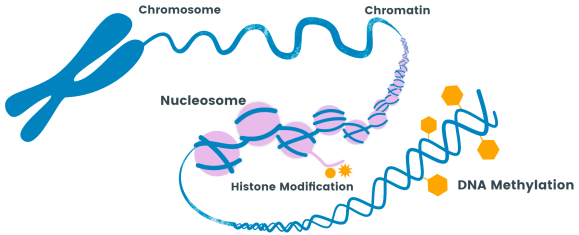
*GrimAge was developed to predict mortality within this dataset

Belsky et al. 2022 eLife

Testing geroprotection in the CALERIE RCT







DNAm measured from blood at baseline, 12mo, 24mo (n=197)

National Institute on Aging
R01AG061378

CALERIE intervention slows aging as measured by blood-chemistry biological age measures

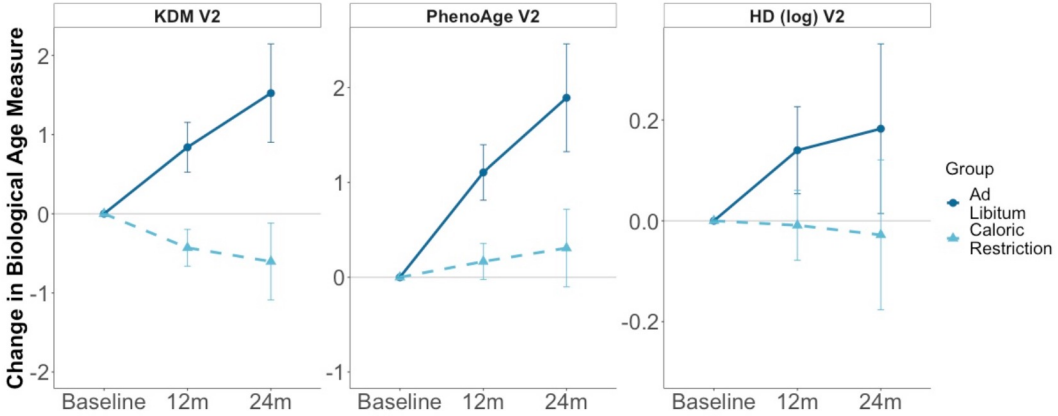


Fig. 3 Change in Klemera-Doubal method (KDM) Biological Age, PhenoAge, and homeostatic dysregulation (HD) from Baseline to 12- and 24-month follow-ups in the ad libitum (dark blue dots) and caloric-restriction (light blue triangles) groups of the CALERIE trial. The figure plots predicted values and 95% confidence intervals estimated from mixed-effects growth models for participants in the ad libitum control group (dark blue circles, solid line) and caloric restriction intervention group (light blue triangles, dashed line). Values of KDM Biological Age and PhenoAge are denominated in years. Values of HD are denominated in log units

Kwon et al. 2021 *Geroscience*

See also Belsky et al. 2017 J Geron A

1st Gen. Clocks	Developed to predict chronological age in mixed-age samples

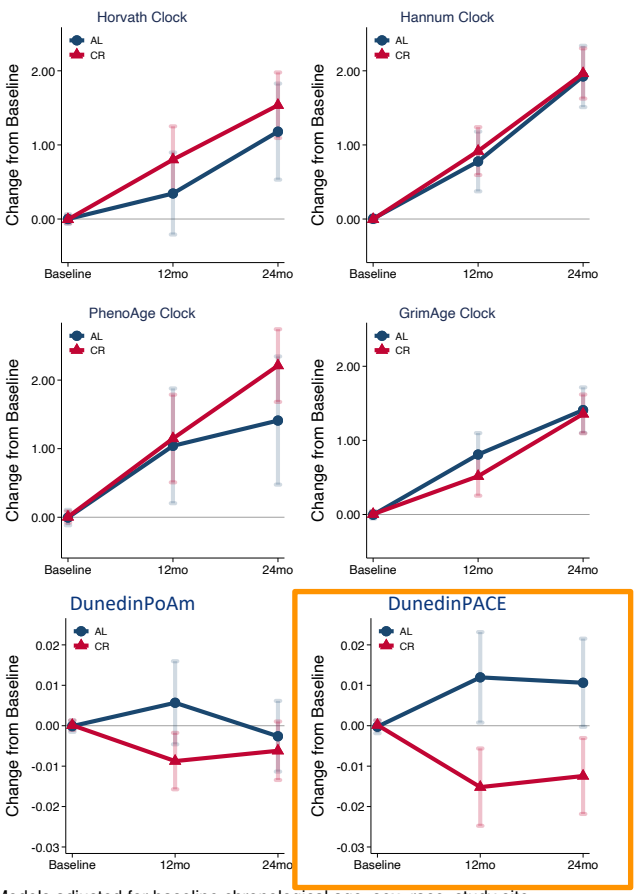
Expected change is 1y per 12mo

2nd Gen. Clocks	Developed to predict mortality risk in mixed-age samples

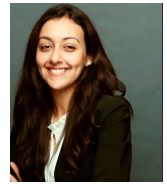
Expected change is 1y per 12mo

Pace of Aging	Developed to predict decline in system integrity in a birth cohort followed over time

Expected change is ~0



Models adjusted for baseline chronological age, sex, race, study site, and baseline BMI stratum



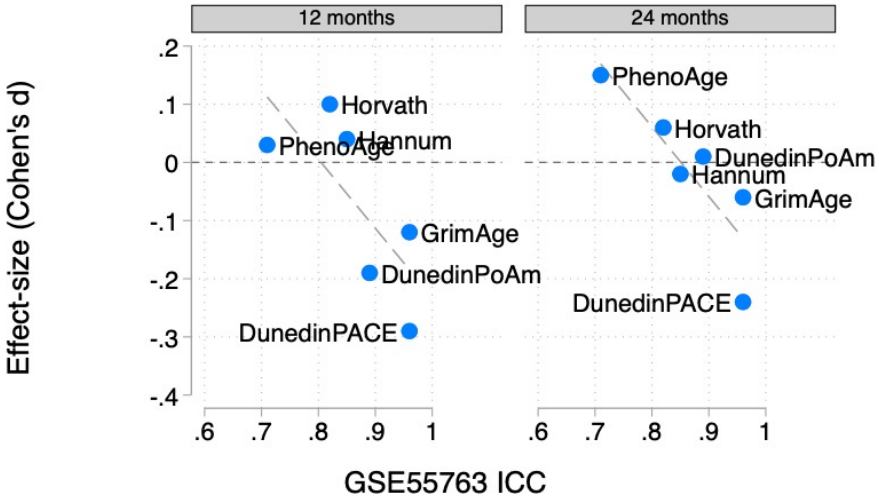
Waziry et al. 2021 MedRxiv

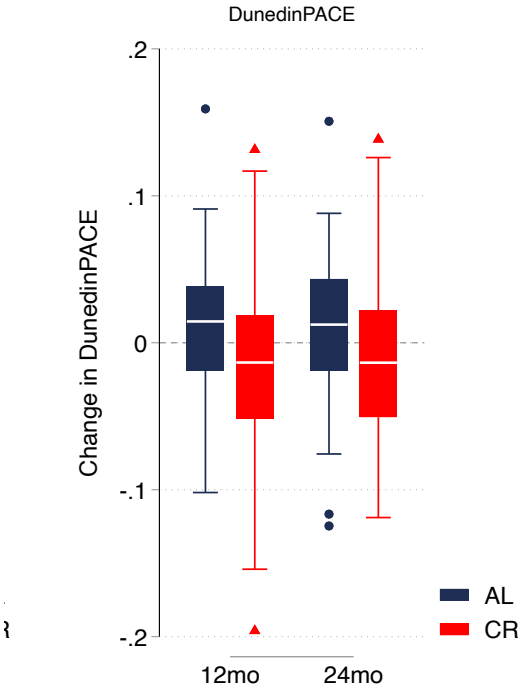
CALERIE RCT Treatment Effects

Only DunedinPACE showed consistent, statistically significant effects of treatment

Why?

- Superior test-retest reliability (less error in measurement)





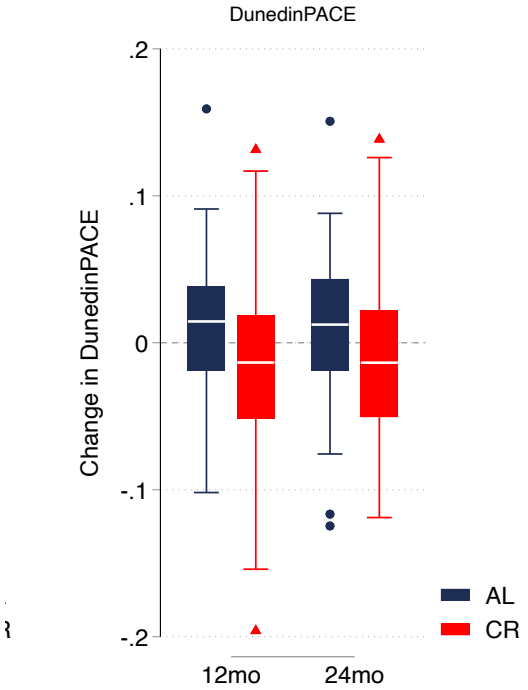
Waziry et al. 2021 MedRxiv

CALERIE RCT Treatment Effects

Only DunedinPACE showed consistent, statistically significant effects of treatment

Why?

- Superior reliability
- Pace of Aging method may be more sensitive to treatment effects

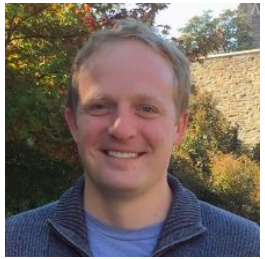


Waziry et al. 2021 MedRxiv

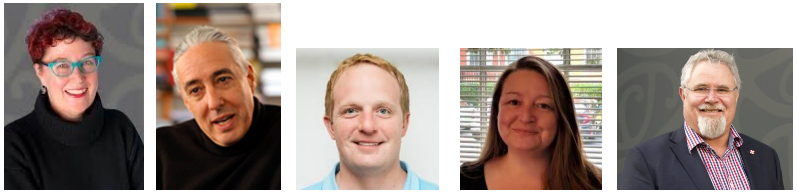
Conclusions and next steps

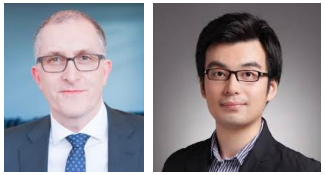
- Methods to quantify processes of biological aging represent new tools for aging science
- DunedinPACE is a new addition to this toolkit that is conceptually distinct from “clock” methods and may offer value added, especially for intervention studies
- Measures of biological aging open new frontiers to study interventions in young and midlife populations, esp. on health behavior, environmental toxicants, and social determinants
- Measures of biological aging may also provide new clinical tools for risk assessment and prognosis

Code to compute DunedinPACE from Illumina 450k and EPIC Array data is available on GitHub



Code to implement DunedinPoAm in Illumina 450k or EPIC array data at https://github.com/danbelsky/DunedinPACE





Thank you!

NIH National Institute on Aging

CIFAR

RUSSELL SAGE FOUNDATION

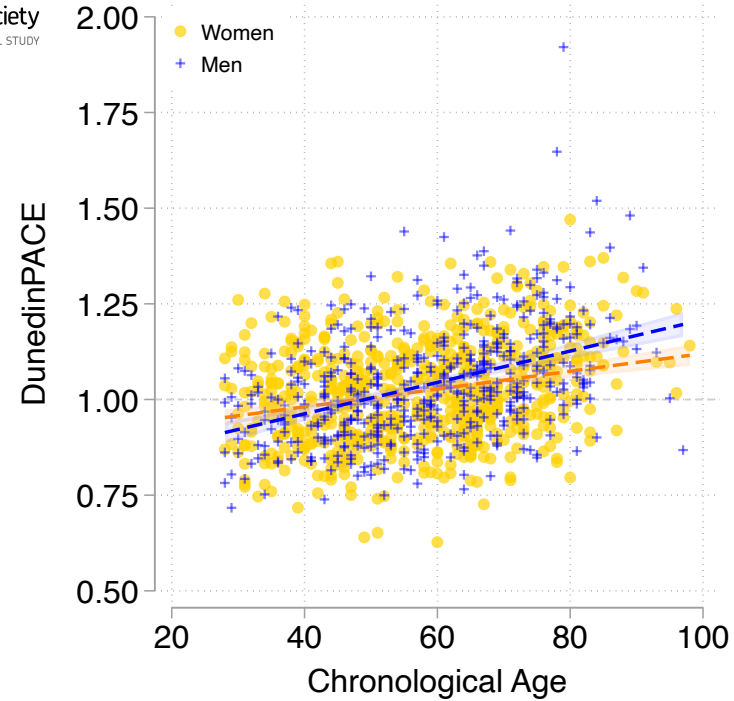
JACOBS FOUNDATION

Our Promise to Youth

DunedinPACE indicates faster Pace of Aging in individuals with older chronological and biological age

Understanding Society
THE UK HOUSEHOLD LONGITUDINAL STUDY

N=1,175



We expect the rate of aging to accelerate at older chronological ages parallel to acceleration in mortality risk

This hypothesis is not testable with standard DNAm clocks because their measure of “age acceleration” is uncorrelated with chronological age by design

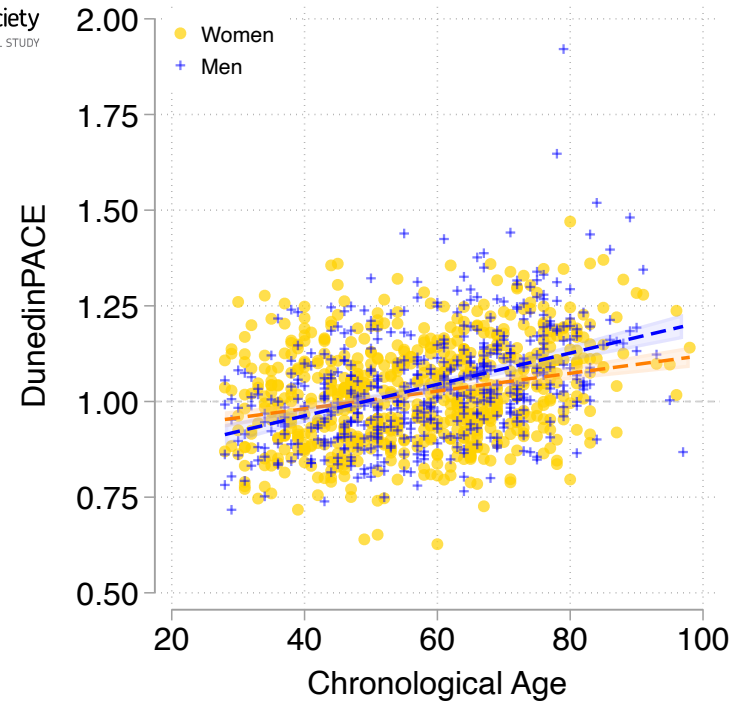
e.g. Finch & Crimmins 2016 PNAS

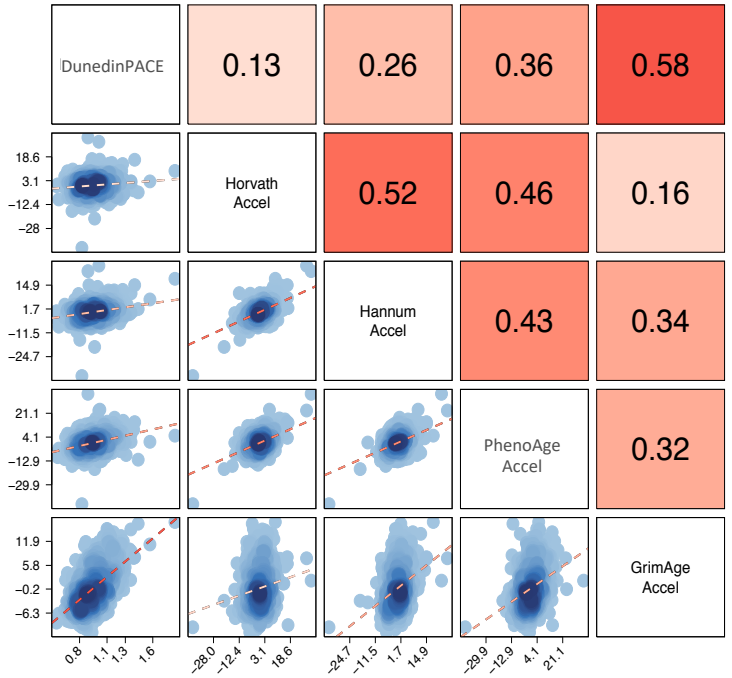
Belsky et al. 2022 eLife

DunedinPACE indicates faster Pace of Aging in individuals with older chronological and biological age

Understanding Society
THE UK HOUSEHOLD LONGITUDINAL STUDY

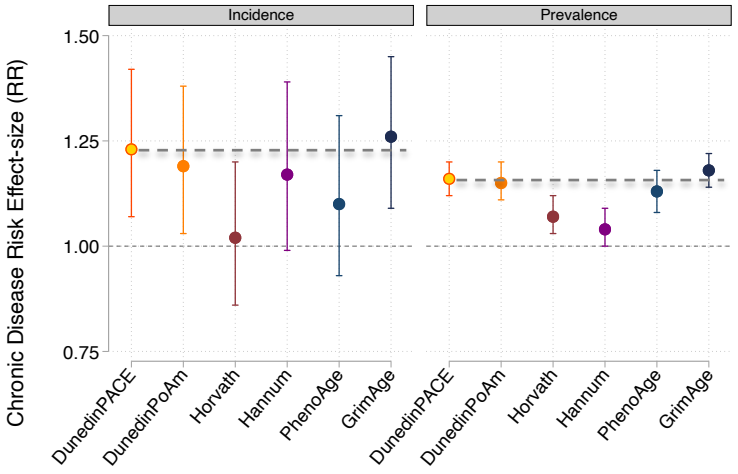
N=1,175



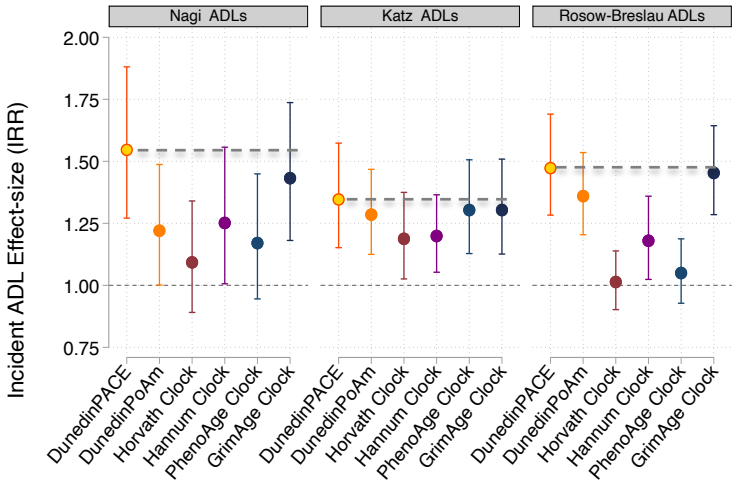


Belsky et al. 2022 eLife

DunedinPACE shows similar prediction of mortality morbidity, and disability to GrimAge



Incident and Prevalent Chronic Disease
in the Normative Aging Study



Incident Disability
in the Framingham Heart Study

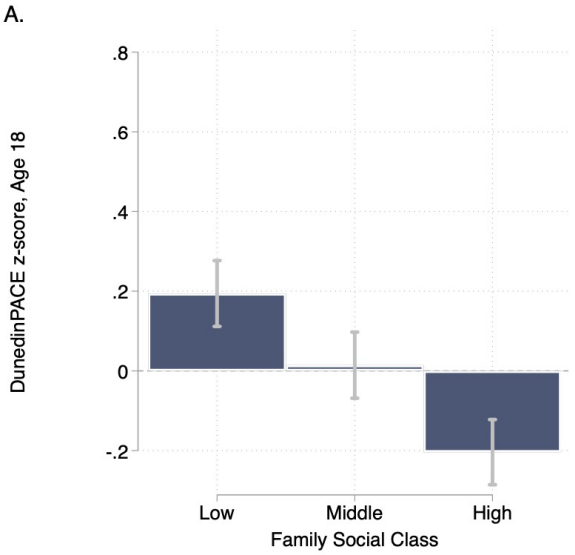
Belsky et al. 2022 eLife

DunedinPACE is faster in adolescents with histories of childhood adversity

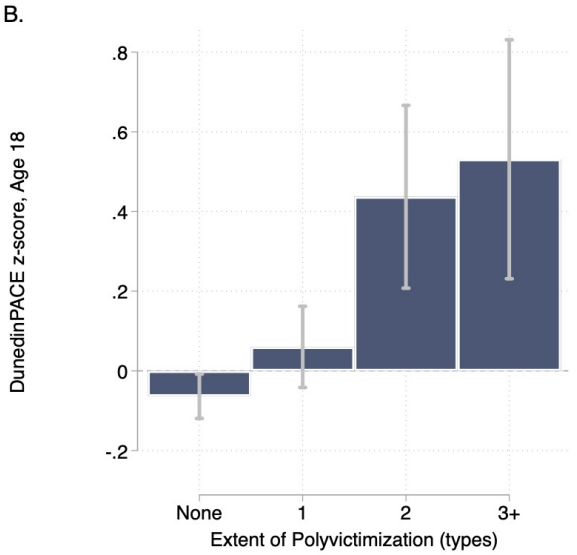
E-RiSK Study

N=1,116
Age 18 blood samples

Family Social Class (age 5)



Childhood Victimization (through age 12)



Belsky et al. 2022 eLife

https://calerie.duke.edu/

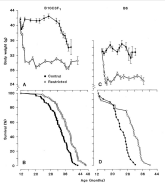
Calorie restriction (CR), macronutrient restriction with maintenance of micronutrient sufficiency, is the best-established geroprotective intervention in animals

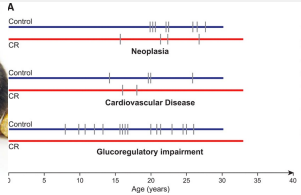
CALERIE is the first-ever RCT of long-term CR in healthy, non-obese humans

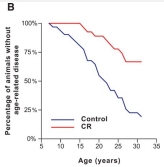
Duke University School of Medicine

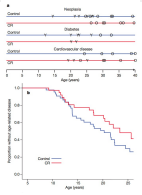
NIH National Institute on Aging

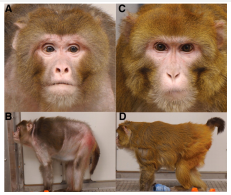
NIH National Institute of Diabetes and Digestive and Kidney Diseases





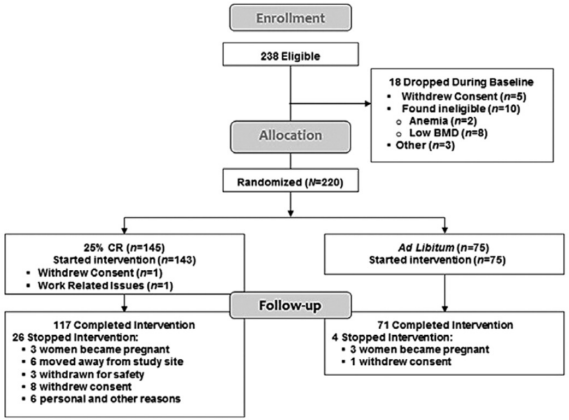


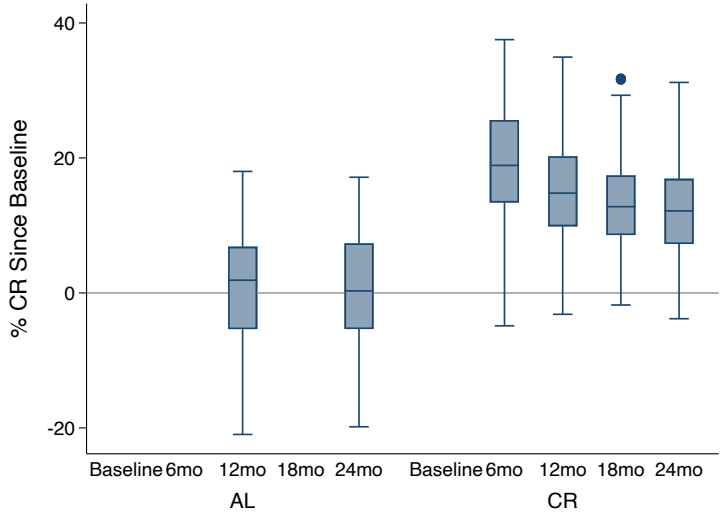


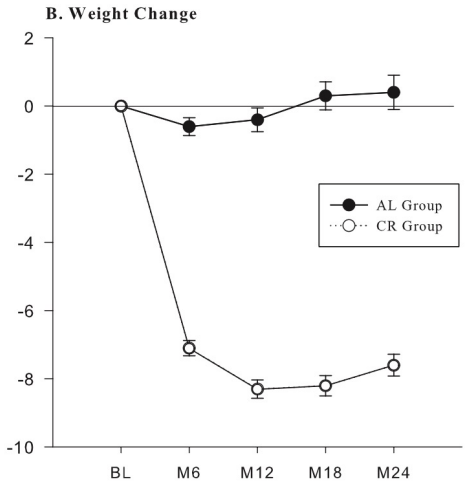


Weindruch & Walford 1982 Science
Colman et al. 2009 Science
Mattison et al. 2014 Nature
Colman et al. 2014 Nat Comm

Ravussin et al. 2015 *JGMS*







CALERIE randomized n=220 non-obese adults to 25% CR for 24 months

n=145 CR Treatment

n=75 AL Control

Adherence was imperfect

Average CR was 12% in the treatment group and ~2% in the control group

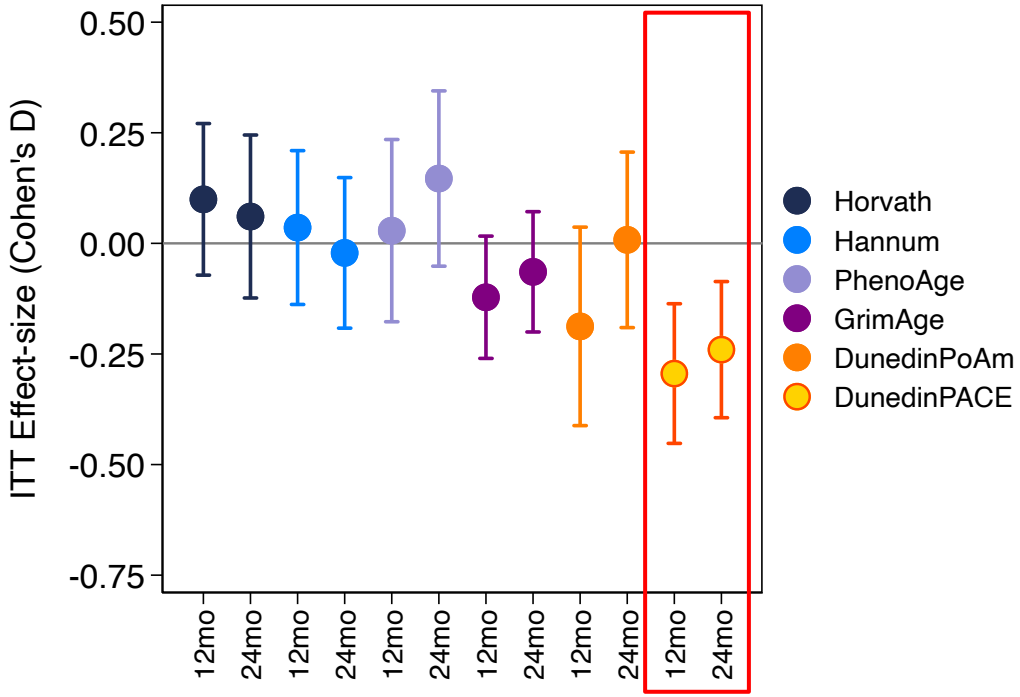
CALERIE RCT Treatment Effects

The GrimAge clock and both Pace of Aging measures indicate evidence of slowed aging in the CR treatment group

Only DunedinPACE showed consistent, statistically significant treatment effects

Why DunedinPACE?

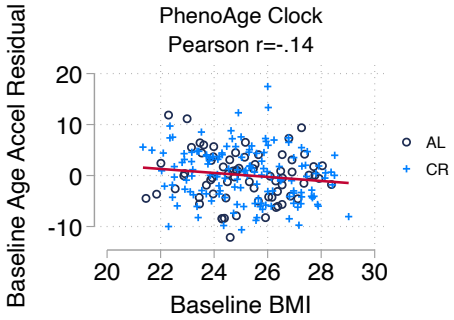
- Superior reliability
- Pace of Aging method may be more sensitive to treatment effects

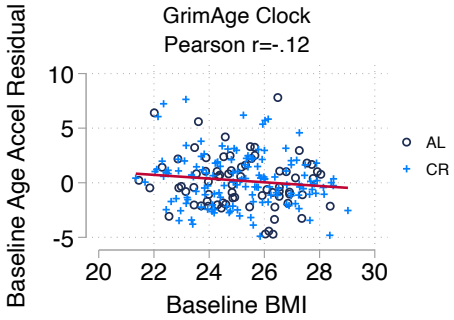


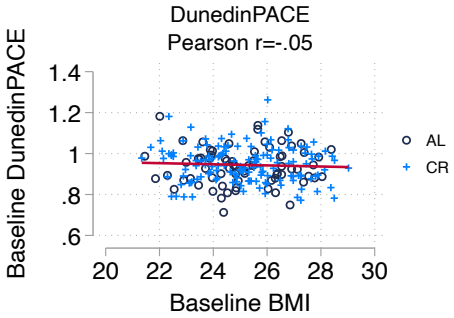
Intent-to-Treat (ITT) effect-sizes scaled in baseline standard-deviation units, estimated from repeated measures ANCOVA

Waziry et al. 2021 MedRxiv

CALERIE Treatment Effects are not explained by special sensitivity of DunedinPACE to weight loss







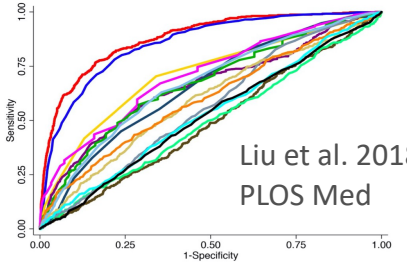
Code to compute blood-chemistry biological age measures from custom biomarker sets is available on GitHub

Code to implement KDM BA, PhenoAge, and Homeostatic Dysregulation methods
https://github.com/dayoonkwon/BioAge

Kwon & Belsky 2021 Geroscience

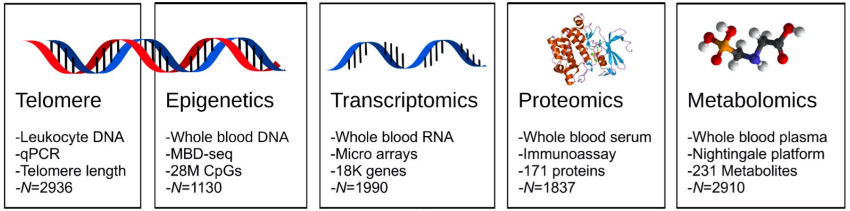
Progress in development of biological age indices (in humans)

- Improved measurement of sub-clinical health states
  (better prediction of morbidity and mortality)
- Increasing sophistication of methods for data mining
  (deep learning, neural networks)
- Increasing diversity of molecular data incorporated into studies of aging
  (proteomics, metabolomics)
- Emerging multi-omics datasets and analysis methods



Liu et al. 2018
PLOS Med

Galkin et al. 2021
Aging & Disease



Jansen et al. 2021 eLife

Frontiers

- Biology is still unknown
  Algorithms remain black boxes. But in-vitro studies are advancing knowledge.

Horvath & Raj 2018 Nat Rev Genet
Bell et al. 2019 Genom Biol
Sturm et al. 2019 Epigenetics
Liu et al. 2020 Aging Cell

- Reporting is uneven
  Many studies still argue proof of concept from prediction of chronological age without reference to other validation metrics

Belsky et al. 2017 Aging Cell
Belsky et al. 2017 J Geron A
Belsky et al. 2018 AJE
Hastings et al. 2019 PNE
Parker et al. 2019 J Geron A
Belsky & Kothari 2021 eLife
Graf et al. 2021 AJE

- External validity is unproven
  Only recently have studies moved beyond the well-off, well-educated, White, and “bio-curious” volunteers

- Modifiability is uncertain
  Intervention studies testing change are just beginning

- Significance of change is unclear
  Longitudinal data are needed to establish whether changes in measures of aging correspond to changes in healthspan

Limitations of Biological Age as a surrogate for geroprotector trials

- **Mortality Selection -** Older and younger individuals represent different populations

  Biological Age measures may under-estimate true aging because older participants necessarily represent slower agers

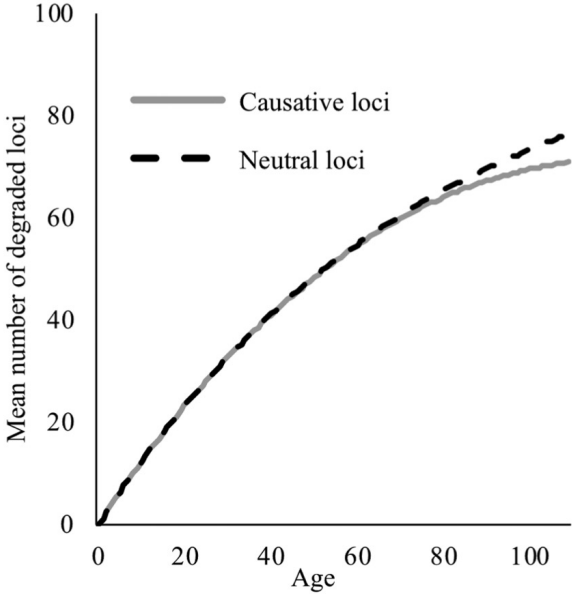


Figure 2. The mean number of degraded loci per individual when loci cause aging (grey), or are neutral (dashed black) out of 301 total loci. We use the largest effect size shown in Figure 3, where the degradation of one causative locus results in a 2.3% increase in mortality rates in an otherwise non-degraded individual, and all loci have an expected age of degradation of 75 years.

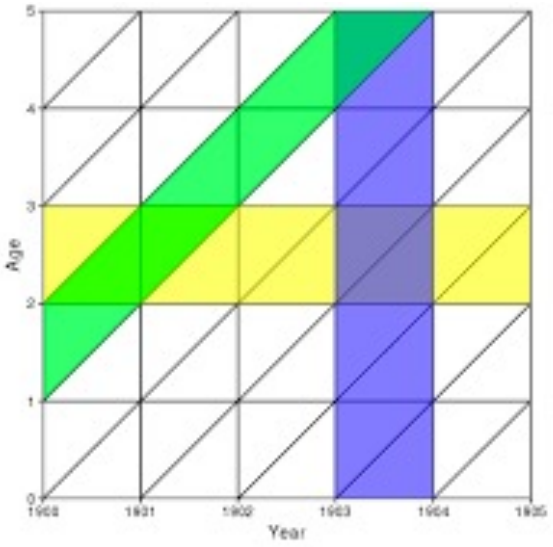
Nelson et al. 2020 J Geron A

Belsky et al. 2018 Am J Epid

Limitations of Biological Age as a surrogate for geroprotector trials

- **Cohort Effects -** Between-individual comparisons do not distinguish aging from cohort exposure history

  Biological Age measures may over-estimate true aging because older participants carry excess burden of early-life exposure to environmental toxicants, pathogens, poor nutrition, smoking, etc.



Moffitt et al. 2016 J Geron A

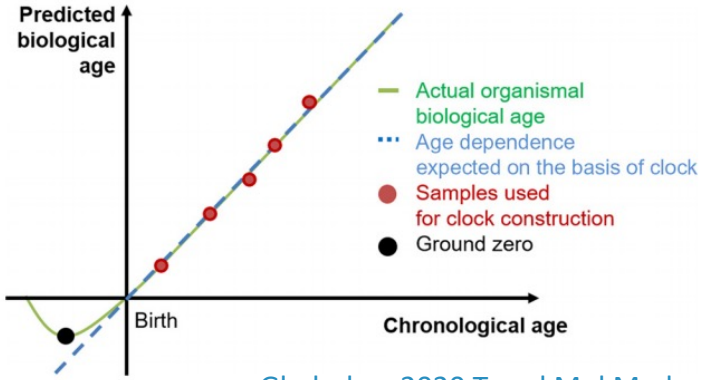
Belsky et al. 2018 Am J Epid

Limitations of Biological Age as a surrogate for geroprotector trials

- **Uncertain Timing -** Unclear when "age acceleration" occurs

  Biological Age measures summarize total aging over the lifespan and do not distinguish differences established early in development from ongoing processes of aging

  May result in lower sensitivity to effects of intervention



Gladyshev 2020 Trend Mol Med

Belsky et al. 2018 Am J Epid

Measuring Pace of Aging: Theory

Aging is characterized by a gradual and progressive decline in system integrity

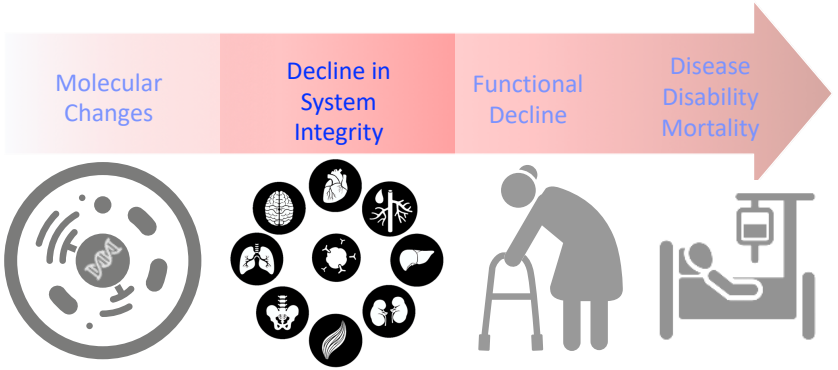
The rate of aging can be inferred from the rate of decline in integrity across multiple organ systems

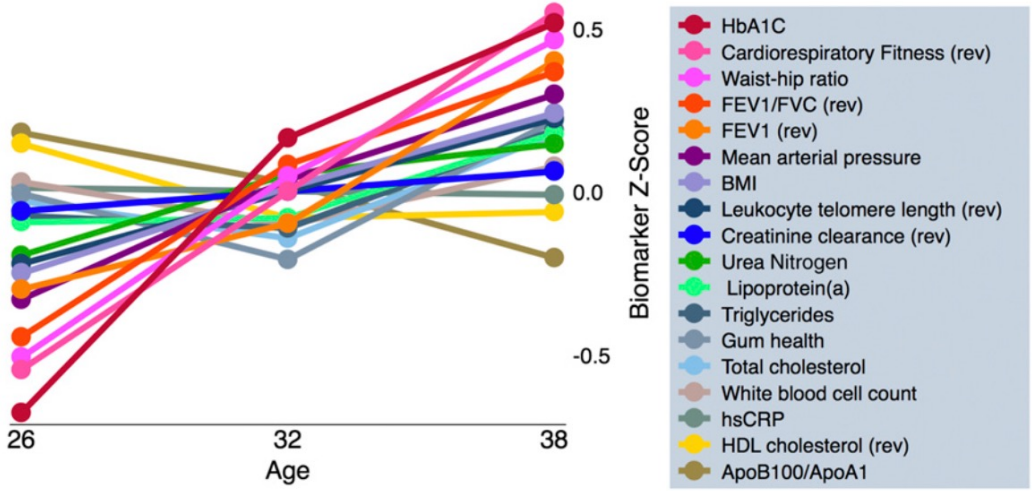
This decline should be observable already by young adulthood

Belsky et al. 2015 PNAS

Pace of Aging is a longitudinal measure of the *rate* of decline in system integrity

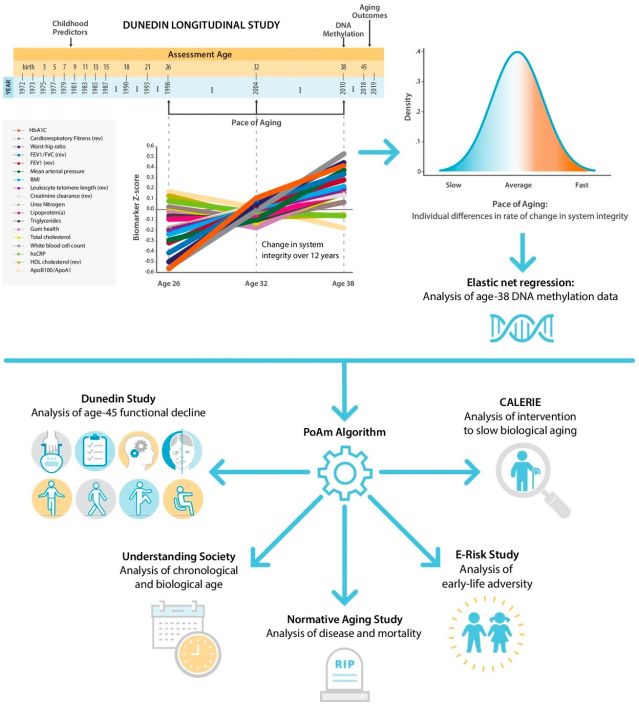
- Young Adulthood- Midlife follow-up excludes dropout from morbidity / mortality
- Single birth cohort excludes cohort effects
- Repeated measures to quantify change

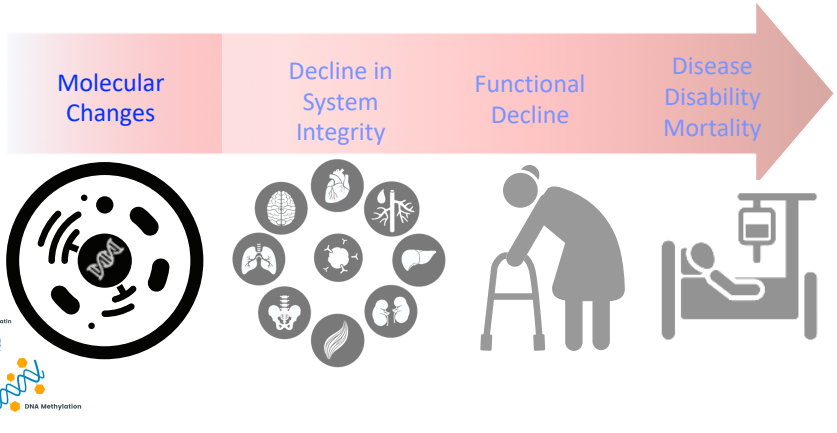




Belsky et al. 2015 PNAS

A DNAm surrogate for Pace of Aging: DunedinPoAm





eLife RESEARCH ARTICLE

Quantification of the pace of biological aging in humans through a blood test, the DunedinPoAm DNA methylation algorithm

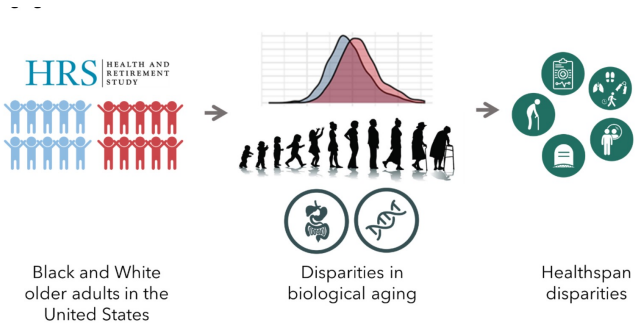
Daniel W Belsky[1,2]*, Avshalom Caspi[3,4,5,6], Louise Arseneault[3], Andrea Baccarelli[7], David L Corcoran[6], Xu Gao[7], Eiliss Hannon[8], Hona Lee Harrington[4], Line JH Rasmussen[4,9], Renate Houts[4], Kim Huffman[10,11], William E Kraus[10,11], Dayoon Kwon[2], Jonathan Mill[8], Carl F Pieper[11,12], Joseph A Prinz[6], Richie Poulton[13], Joel Schwartz[14], Karen Sugden[4], Pantel Vokonas[15], Benjamin S Williams[4], Terrie E Moffitt[3,4,5,6]

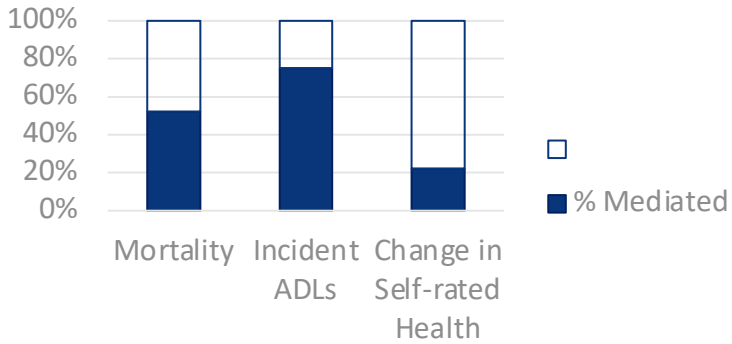
Belsky et al. 2020 eLife

Testing Black–White disparities in biological aging in older adults in the United States: analysis of DNA-methylation and blood-chemistry methods

Gloria H Graf, Christopher L Crowe, Meeraj Kothari, Dayoon Kwon, Jennifer J Manly, Indira C Turney, Linda Valeri, Daniel W Belsky ✉

American Journal of Epidemiology, kwab281,
https://doi.org/10.1093/aje/kwab281
Published: 01 December 2021 **Article history** ▾





HRS HEALTH AND RETIREMENT STUDY

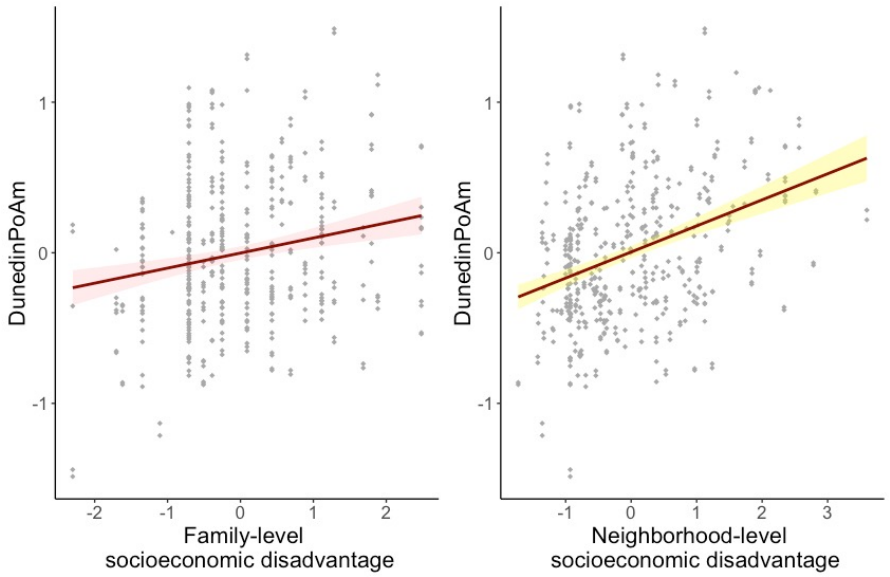
N=3,491
Mean Age=70
Blood DNAm

Graf et al. 2021 *Am J Epid*

Socioeconomic Disadvantage and the Pace of Biological Aging in Children

Laurel Raffington, PhD,[a,b] Daniel W. Belsky, PhD,[c,d] Meeraj Kothari, MPH,[d] Margherita Malanchini, PhD,[a,b,e]
Elliot M. Tucker-Drob, PhD,[a,b,*] K. Paige Harden, PhD[a,b,*]

PEDIATRICS®



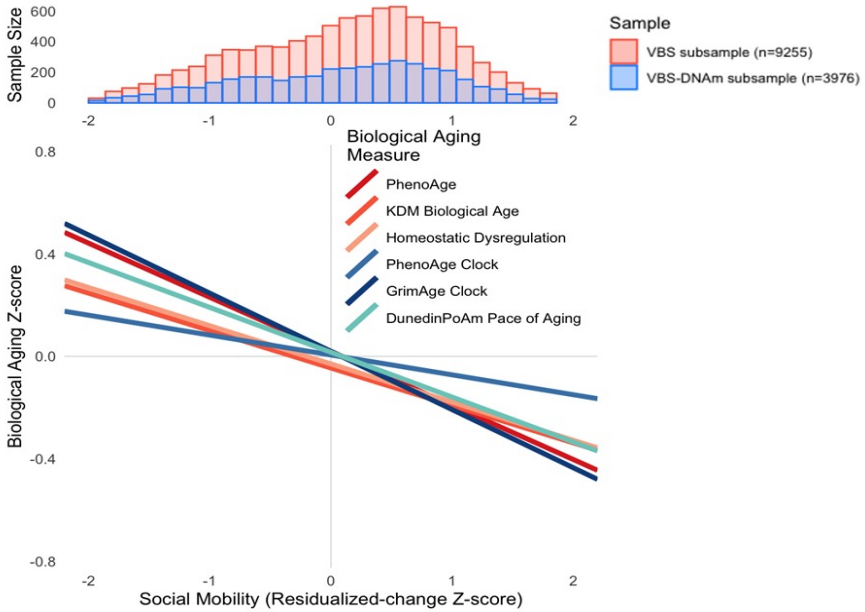
N=600
Mean Age=13
Saliva DNAm

Raffington et al. 2021 *Pediatrics*

Social mobility and biological aging among older adults in the United States

GH Graf, Y Zhang, BW Domingue, KM Harris, M Kothari, D Kwon, P Muennig, DW Belsky

doi: https://doi.org/10.1101/2021.10.19.465042



HRS HEALTH AND RETIREMENT STUDY

Graf et al. 2021 BioRxiv