Quantification of Biological Aging

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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.

Kennedy et al. 2014 Cell

Lopez-Otin et al. 2013 Cell
A geroscience model of aging-related burden of disease

Molecular Changes

Decline in System Integrity

Functional Decline

Disease Disability Mortality
Geroprotection intervention

Molecular Changes

Decline in System Integrity

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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 an 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
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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.

Deficit Accumulation
- Functional testing
- Disease counts

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

There are reciprocal interactions across levels
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
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

Baseline 12mo 24mo

T1 T2 T3

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

Hypothesis

Ad Libitum

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

Fig. 3 Change in Klemera-Douhal 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)
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!
DunedinPACE indicates faster Pace of Aging in individuals with older chronological and biological age

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

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

N=1,116
Age 18 blood samples

Belsky et al. 2022 eLife
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.

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

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

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

• 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 underestimate 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.*

*Belsky et al. 2018 Am J Epid*  
*Nelson et al. 2020 J Geron A*
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.

Belsky et al. 2018 Am J Epid

Moffitt et al. 2016 J Geron A
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

Belsky et al. 2018 Am J Epid  
Gladyshev 2020 Trend Mol Med
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

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


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

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Black and White older adults in the United States
Disparities in biological aging
Healthspan disparities

HRS Health and retirement study

N=3,491
Mean Age=70
Blood DNAm

Mortality
Incident ADLs
Change in Self-rated Health

% Mediated
Socioeconomic Disadvantage and the Pace of Biological Aging in Children

Laurel Raffington, PhD, Daniel W. Belsky, PhD, Meeraj Koithari, MPH, Margherita Malanchini, PhD, Elliot M. Tucker-Drob, PhD, K. Paige Harden, PhD

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

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