ANNOTATED BIBLIOGRAPHY

Longitudinal measures of the pace of aging from the Dunedin Study:
The Pace of Aging, DunedinPoAm, DunedinPACE, and DunedinPACNI
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BACKGROUND

HISTORY OF RESEARCH FROM PACE OF AGING TO DUNEDINPACE

Our work aimed to develop a measure that operationalized the definition of aging as: functional decline that (1) simultaneously involves multiple organ systems across the whole body, and (2) is unidirectional, gradual, and progressive. Our Duke/Otago team first modelled the **Pace of**

Aging in 2014 using multiple waves of biomarkers assessed in the Dunedin Longitudinal Study when the cohort members were aged 26, 32, 38 (Belsky et al. PNAS 2015). We then updated and improved the Pace of Aging in 2021 by adding an additional wave of data, now covering ages 26, 32, 38, and 45 (Elliot et al. Nature Ageing 2021). The 19 biomarkers repeated every six years indexed variation among individuals in the function of six organ systems: the cardiovascular, metabolic, renal, immune, dental, and pulmonary systems. Dunedin Study participants with faster Pace of Aging scores had more cognitive difficulties, signs of advanced brain aging on MRI, diminished sensory–motor functions, older facial appearance and more pessimistic perceptions of aging.

Unfortunately, however, we realized that few other research teams could adopt our Pace of Aging approach at that time, because they lacked repeated waves of multiple biomarkers across the whole body. To meet this need to export the Pace of Aging to the research community, we translated the Pace of Aging into a DNA methylation version that can be derived from one blood draw. In 2020, we reported how to measure the Pace of Aging by using DNA methylation data, calling the measure **DunedinPoAm** (Belsky et al. eLife 2020).

DunedinPACE: In 2022 we updated the DNA methylation version of the Pace of Aging measure to cover the Pace of Aging assessed over ages 26, 32, 38, and 45, naming the new measure DunedinPACE for "**P**ace of **A**ging **C**alculated in the **E**pigenome." The details of DunedinPACE are published in a scientific article (Belsky et al. 2022 eLife, link here.). DunedinPACE is an epigenetic biomarker that measures the human pace of aging. It is designed to function as a speedometer for aging, offering a single-timepoint measurement of how fast a person is now aging. DunedinPACE is designed to function as a speedometer for aging, offering a single-timepoint measurement of how fast a person's body is now aging. We exported DunedinPACE to five additional cohorts, and found that it showed very high test-retest reliability, predicted morbidity, disability, and mortality, and indicated faster aging in adults who survived childhood adversity. The code to compute DunedinPACE in DNA methylation data from Illumina 450k or EPIC1 or EPIC2 arrays is available open-access on GitHub, here . The code is also included in BioLearn. (NOTE: because the Illumina company who provides DNA methylation arrays has updated from their 450k to EPIC1 to EPIC2, we have updated the Github algorithm. The algorithm automatically works with data created from any of those three Illumina platforms.)

DunedinPACNI: Despite the success of DunedinPACE in cohorts that have DNA methylation data, we realized that many important studies of aging have not invested in costly DNA methylation, but instead have invested in collecting brain MRI data. As a result, in 2024 we translated the Pace of Aging into a brain-MRI version that can be derived from one MRI brain scan image. We call this measure **DunedinPACNI** for "**P**ace of **A**ging **C**alculated in a **N**euro-**I**mage" (Whitman et al. 2025). It has very high test-retest reliability. We exported this measure to the Alzheimer's Disease Neuroimaging Initiative, the UK Biobank, and the BrainLAT neuroimaging datasets and found that faster DunedinPACNI predicted participants' cognitive impairment, accelerated brain atrophy, and conversion from mild cognitive impairment to diagnosed dementia. Underscoring close links between longitudinal aging of the body and brain, faster DunedinPACNI also predicted physical frailty, poor health, future chronic diseases, and mortality in older adults. The code to computer DunedinPACNI is here: XXXX.

Six Design Advantages Distinguish this Measure from Others

First, DunedinPACE was derived from 19 biomarkers ascertained longitudinally in a cohort over 2 decades at ages 26, 32, 38, and 45, comprising 69,715 data points. In this longitudinal design the participants were tested four times, each 5 years apart. This spacing allowed us to track unidirectional (i.e., downhill) decline that is correlated across organ systems as age advances gradually, long-term. This is an advantage because DunedinPACE has no *noise* from short term illnesses or infections, such as an infected tooth or a viral flu, that could temporarily elevate or spike a biomarker during a spell of illness. Most other bio-age measures are derived from only one cross-section of data and thus they inadvertently contain some noise from spells of sickness.

Second, DunedinPACE was derived on a cohort of research participants who were all born the same year. To date this design feature is unique in the world of aging measures. This is an advantage because DunedinPACE has no *noise* from historical differences in exposure to factors that in multi-age samples alter the participants' body or their epigenome. For example, older generations had more exposure to toxins like cigarette smoke and leaded gasoline, fewer years of education, and few fruits and vegetables in winter. Younger generations had childhood vaccinations, antibiotics, air-conditioning, anti-inflammatory medications, and oral contraception. These generational factors can alter the epigenome, but they are not relevant to aging. Other bio-age measures that were derived on people who have different birth years inadvertantly contain some of this exposure noise.

Third, DunedinPACE was derived in a cohort of adults studied up to age 45. This is an advantage because DunedinPACE has no *noise* from late-life diseases such as diabetes, heart disease, kidney disease, dementia, or cancers, because these diseases had not yet onset in the Dunedin cohort when we constructed DunedinPACE. We aimed for a measure that reflects aging and is not merely a repackaging of disease. Other bio-age measures are typically derived from samples in which many of the older members already have chronic diseases that have altered their body and epigenome, so those measures can reflect disease more than aging itself.

Fourth, DunedinPACE was derived in a midlife cohort for which attrition by death has been minimal; under 3%. This is an advantage because of so-called "survivor bias." Other bio-age measures are typically derived from samples in which the many of the older members are missing due to death. In multi-age samples, the youngest participants can be sampled from all people born their year, but the older participants can only be sampled from the subset of those left alive. In mixed-age samples with attrition and in studies that begin in midlife or later, the fastest agers will have already died, which reduces the potential range of scores on any bio-age measure. Thus, in other bio-age measures, the young versus old participants differed from each other on some noise factors that are not relevant for aging.

Fifth, DunedinPACE was derived from a pool of DNA methylation probe sites across the epigenome that were pre-selected to have strong test-retest reliability. Unreliable probe sites were excluded from DunedinPACE. We showed that most methylation probe sites are indeed unreliable, which adds noise to DNAm bio-age measures. Other bio-age measures sometimes offset this noise after the fact using statistical procedures, such as deriving principal factors. DunedinPACE builds reliability in.

Sixth, because we intended it to be responsive to anti-aging interventions, unlike other aging clocks DunedinPACE was trained on change over time. Evidence is accumulating that

DunedinPACE Among aging clocks, DunedinPACE is the most sensitive to intervention to date, slows most in intervention trials, and is the most consistently responsive across studies.

THE THIRD GENERATION IS LONGITUDINAL. DunedinPACE is sometimes referred to as a "third generation" among clocks, because earlier generations of clocks were not derived in longitudinal data to assess actual physiological decline in healthy people who are all the same chronological age.

PROGRESS TO DATE

More than 65 large epidemiological cohort studies in 17 countries are now using DunedinPACE, including, in alphabetical order: Add Health, The Alzheimer disease neuroimaging initiative (ADNI), AHAB, ALSPAC UK, ASPREE Study Australia, Austrian Stroke Prevention Study, BeCOME Germany, Baltimore Longitudinal Study of Aging (BLSA), BrainLAT, CALERIE, Canadian longitudinal study on aging (CLSA), CARDIA, Cebu Longitudinal Health and Nutrition Survey (CLHNS Philippines), Child Health and Development Study, Chinese National Twin Registry, COBRA, Dutch Hunger Winter Study, E-Risk Longitudinal Twin Study UK, FACHS (African American families), FinnTwin, Finnish Twin Study on Aging FITSA, Young Finn Study, Framingham Offspring Cohort, Fragile Families, Future of Families and Child Wellbeing (FFCW), Generation Scotland, German Socioeconomic Panel Study (SOEP-Gene), Grady Trauma Project, HANDLE (NIA's intramural study), Health and Retirement Study (HRS), InChianti, the Korean Genome and Epidemiology Study (KoGES), KORA (Cooperative Health Research in the Region Augsburg study), Leiden Longevity Study, Lothian Birth cohort, Melbourne Collaborative Cohort Study Australia, MESA, MIDUS, United States National Health and Nutrition Examination Survey (NHANESIII), National Heart, Lung, and Blood Institute Growth and Health Study (NGHS, 1992), Netherlands Twin Registry, NHANES, Normative Aging Study, Northern Finland 1966 birth cohort, Norwegian MOBA, Penn State Child Health Study, REWARD Study in Wisconsin, Rotterdam Study, Saint Jude Lifetime Cohort of Cancer Survivors (SJLIFE), Sister Study, SOL-INCA and HCHS-SOL, SOEPG Germany, Strong Heart Study of Native American Indians, Swedish Adoption/Twin Study of Aging, Swiss Family Study, Taiwanese Biobank, The Texas Twin Study, TILDA Ireland, Thinking and Living with Cancer (TLC) Study, TwinLife, UK Household Longitudinal Study, UK Understanding Society, Veterans Administration Post-Deployment Mental Health Study (PDMH), Women and their Children's Health in Southeast Louisiana, Women's Health Initiative, plus others.

STUDIES VALIDATING DUNEDINPACE AS A USEFUL MEASURE OF WHOLE-BODY AGING:

Other research teams have published more than 300 reports of new findings from these data sets since the measure became available to them in 2021 (these reports are not from our team).

We ourselves have published more than 35 reports with findings using our measures of the pace of aging. As an example, we reported that faster DunedinPACE distinguishes long-term cannabis users from recreational users (Meier et al. 2022, *Lancet Healthy Longevity*). As another example, people with more education have slower DunedinPACE, even after controls for genetic confounds (Sugden et al. 2023, *J of Gerontology*).

Replication and robustness. Our publications have generally replicated findings by using one or more of the data sets that now include DunedinPACE; we often report multi-study publications. Our paper announcing DunedinPACE reported validation in 5 data sets (Belsky et al 2022, *eLife*). As another example, we reported that faster DunedinPACE distinguishes Alzheimer dementia patients in **2 cohorts,** ADNI and the Framingham Heart Study (Sugden et al. 2022, *Neurology).* We also reported that people with more education have slower DunedinPACE, after controls for genetic confounds, in **5 cohorts** (Sugden et al. 2023, *J of Gerontology*). Other replication papers show that DunedinPACE relates to MRI-measured brain structure in **3 cohorts** (Whitman et al. 2023 *Neurobiology of Aging*) and to schizophrenia in **5 case-control studies** (Caspi et al. 2023 *Biological Psychiatry*).

Comparison to clocks: Overall, findings have shown that DunedinPACE routinely outperforms the Horvath, Hannum, and PhenoAGE clocks, and typically performs on par with the GrimAGE clock. DunedinPACE outperformed the GrimAGE clock on some analyses of cognitive decline and Alzheimers dementia, and in studies of responsiveness to interventions, but these comparisons are early days yet.

Ethnic ancestry: DunedinPACE was originally trained on white New Zealanders, but it is being reported on groups of other ancestries. Yin (2023) reported in the Taiwanese Biobank that DunedinPACE is faster in **Han Chinese** who have multimorbid physical illnesses. Kim (2024) reported that DunedinPACE is faster in the Korean Genome and Epidemiology Study (KoGES) in **Korean** participants who have chronic disease and poor health lifestyle factors. Boyer et al (2023) reported DunedinPACE findings in **Native American Indians**. Overall, published findings in **African Americans** are looking similar to those in Whites, as shown in the FACHS, HANDLE, Future of Families and Child Wellbeing (FFCW), MIDUS, National Heart, Lung, and Blood Institute Growth and Health Study (NGHS 1992), and HRS data sets, as well as the Veterans Administration Post-Deployment Mental Health Study (PDMH), which is 50% African American. Krieger et al. studied DunedinPoAm in Black, Hispanic, and white ethnicity with respect to racialized and economic injustice in MESA. Gibbs et al. reported on DunedinPACE in **Pasifika**

Age, children: DunedinPACE was originally trained on people age 45, but overall, published findings show DunedinPACE is relevant for studies of children and adolescents: E-Risk, ALSPAC, FinnTwin, PennState Child Health Study, Texas Twins, Quebec Longitudinal Study of Child Development, Future of Families and Child Wellbeing (FFCW), and others. More data are needed to understand interpretation of scores in children. (NOTE: some child studies used buccal/saliva as the source of DNA, whereas DunedinPACE was trained on venous blood as the source of DNA. The implications of this are not yet clear.)

Age, older adults: DunedinPACE was trained on people age 45, but it is proving relevant for studies of older adults: The Alzheimer disease neuroimaging initiative (ADNI), NIA's Baltimore Longitudinal Study on Aging (BLSA), Berlin Study of Aging, Framingham Heart Study, Healthy Aging in Neighborhoods of Diversity Across the Life Span study (HANDLE), Health and Retirement Study (HRS), Multi-Ethnic Study of Atherosclerosis (MESA), Normative Aging Study, The Irish Longitudinal Study on Ageing (TILDA), UK Understanding Society, Finnish Twin Study on Aging (FITSA), Canadian longitudinal study on aging (CLSA), 1958 British Birth cohort, Scottish Lothian birth Cohorts, Swedish Adoption/Twin Study of Aging (SATSA), Rotterdam Aging Study, Leiden Longevity Study, Women's Health Initiative, and others.

Predicting clinical endpoints: Faster DunedinPACE has been shown to statistically predict endpoint indicators including functional and cognitive decline, frailty, disease multimorbidity, and mortality (age at death) in AHAB, BLSA, Framingham, Generation Scotland, HANDLE, HRS, Melbourne Collaborative Cohort Study, MESA, NAM, UK Understanding Society, FITSA, Taiwanese Biobank, Rotterdam Study, Leiden Longevity Study, Lothian birth cohort, Women's Health Initiative, and others.

Predicting diagnosed dementia: Faster DunedinPACE has been reported to predict ADRD and Mild Cognitive Impairment in ADNI and Framingham (Savin et al, 2024; Sugden et al, 2022), and cognitive decline (Elliot et al. 2021; Reed et al. 2022). Another study did not find this (Schäfer Hackenhaar, et al. 2023).

Risk factors that predict fast aging: DunedinPACE has been shown to be accelerated in people who experienced psycho-social adversity and deprivation in AddHealth, ALSPAC, BLSA (Baltimore Longitudinal Study of Aging), E-Risk Longitudinal Twin Study UK, FACHS (African American families study), Future of Families and Child Wellbeing (FFCW), Generation Scotland, HANDLE (NIA's intramural study), HRS, MESA, NHANES, Normative Aging Study, the Sister Study, Swiss Family Study, The Texas Twin Study, TILDA (Ireland), UK Understanding Society, Canadian longitudinal study on aging (CLSA), Grady Trauma Project, Taiwanese Biobank, Veterans Administration Post-Deployment Mental Health Study, and five schizophrenia case-control studies, plus other studies.

Responsiveness, the sensitivity of DunedinPACE to detecting change in interventions: Sehgal et al (in press) re-analysed 51 longevity intervention trials in humans, and systematically tested 16 DNAm clocks across the 51 intervention trials. They concluded that among 16 aging clocks, DunedinPACE had the most evidence for sensitivity to intervention so far. DunedinPACE had the largest mean decrease in anti-aging intervention trails, and DunedinPACE was the most consistently responsive, significantly decreasing in 16 interventions and increasing in only 1. As an example, DunedinPACE was reported to slow in response to caloric restriction in the CALERIE trial. DunedinPACE has also slowed after successful treatment for insomnia. DunedinPACE was sensitive to a drug intervention in ClockBASE. It has also been shown to accelerate under conditions of psychological stress in FACHS, under conditions of bio-stress in three clinical datasets reported by Poganik et al. 2023, and in patients who are badly burned (Sullivan et al. 2025). This set of findings is potentially important because it shows that DunedinPACE can be sensitive to detecting change, and that it can be repeated as a before-after measure on a time-span of weeks to quantify short-term change in the pace of biological aging. But this is early days and much more research is needed to confirm.

GROUPED REFERENCE LISTS:

Literature reporting findings up to the date of this report for the DunedinPACE Pace of Aging measure (or prior version, PoAm):

The name of the dataset is below each citation.

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Biological Classification of Mental Disorders study in Germany (BeCOME)

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AUTHOR Remarkable concordance in associations between epigenetic clocks and health behaviors across three countries. Dec 2024.
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SUPPLEMENTAL MATERIALS:

Idan Shalev reports that most epigenetic sites are unstable, except those in Dunedin PACE (and in PC clocks). https://www.tandfonline.com/doi/full/10.1080/15592294.2023.2230686

Clockbase: Many studies that have derived DunedinPACE have joined ClockBASE, an open access computational biology data repository: http://gladyshevlab.org:3838/ClockBase/.

Ying, K., Tyshkovskiy, A., Trapp, A., Liu, H., Moqri, M., Kerepesi, C., & Gladyshev, V. N. (2023). ClockBase: a comprehensive platform for biological age profiling in human and mouse. *bioRxiv*, 2023-02.

Biolearn **https://bio-learn.github.io/**, is an open source library to help with calculating any composite biomarker of aging on any molecular data. This library will standardize all the public biomarker formulations, including the collection of epigenetic clocks standardized by Morgan Levine/Albert Higgins-Chen's (methylCIPHER) and Vadim Gladyshev's (ClockBase) groups as well as the blood biomarkers standardized by Daniel Belsky's group (BioAge). At the same time, Biolearn will harmonize public and private molecular datasets commonly used for biomarker validation. Biolearn is scientifically and financially supported by Biomarkers of Aging Consortium and Methuselah Foundation.

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Bryan Johnson's Blueprint for slowing aging and **Rejuvenation Olympics** lederboard features DunedinPACE.
https://www.lifespan.io/news/bryan-johnsons-race-against-time/
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TIME magazine feature story on Bryan Johnson.
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***Men's Health* tested three aging tests, including DunedinPACE.**
https://www.aol.com/lifestyle/fast-am-really-aging-took-191900851.html

Youtube: LifeNoggin video: *Body secrets*

https://www.youtube.com/watch?v=kcxdSt-wYsM

Application in geroprotection clinical trials:
https://www.newsobserver.com/press-releases/article277627968.html
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UNUSUAL papers:

[PDF] **DNA methylation clocks struggle to distinguish inflammaging from healthy aging, but feature rectification improves coherence and enhances detection of inflammaging**
CM Skinner, MJ Conboy, IM Conboy - bioRxiv, 2024

This paper completely describes PACE incorrectly, as trained on people age 53 who had heart attacks and strokes. Hmmmmm…..

[PDF] **Methylation Clocks Do Not Predict Age or Alzheimer's Disease Risk Across Genetically Admixed Individuals**
S Cruz-González, E Gu, L Gomez, M Mews, JM Vance… - bioRxiv, 2024

This paper claims that Clocks haven't been studied in blacks and Hispanics and don't work in non-white groups. Hmmmmm…..