Quantification of Biological Aging in Young Adults

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ABSTRACT

Model-organism research suggests aging can be slowed. Interventions to slow human aging need to be applied to still-young individuals. Most human aging research examines older adults, many with chronic disease. Little is known about aging in young humans. We studied aging in 954 young humans, the Dunedin Study birth-cohort, tracking multiple biomarkers across three time points spanning their third and fourth decades of life. Young individuals of the same chronological age varied in their "biological aging" (declining integrity of multiple organ systems). Already before midlife, individuals who were aging more rapidly were less physically able, showed cognitive decline and brain aging, self reported worse health, and looked older. Measured biological aging in young adults can be used to identify causes of aging and evaluate rejuvenation-therapies.

INTRODUCTION

By 2050, the world population aged 80 years and above will more than triple, approaching 400 million individuals (1, 2). As the population ages, the global burden of disease and disability is rising (3). From the 5th decade of life, advancing age is associated with an exponential increase in burden from many different chronic conditions (**Figure 1**). The most effective means to reduce disease burden and control costs is to delay this progression by extending healthspan, years of life lived free of disease and disability (4). A key to extending healthspan is addressing the problem of aging itself (5–8).

At present, much research on aging is being carried out with animals and older humans. Paradoxically, these seemingly sensible strategies pose translational difficulties. The difficulty with studying aging in old humans is that many of them already have age-related diseases (9–11). Age-related changes to physiology accumulate from early life, affecting organ systems years before disease diagnosis (12–15). Thus, intervention to reverse or delay the march toward age-related diseases must be scheduled while people are still young (16). Early interventions to slow aging can be tested in model organisms (17). The difficulty with these non-human models is that they do not typically capture the complex multifactorial risks and exposures that shape human aging. Moreover, whereas animals' brief lives make it feasible to study animal aging in the laboratory, humans' lives span many years. A novel solution is to study human aging in the first half of the life course, when individuals are starting to diverge in their aging trajectories, but most diseases (and regimens to manage them) have not yet onset. The main obstacle to studying aging prior to old age—and before the onset of age-related diseases—is the absence of methods to quantify the pace of aging in young humans.

We studied aging in a population-representative 1972-1973 birth cohort of 1,037 young adults followed from birth to age 38 years with 95% retention; the Dunedin Study (**Supplementary Methods**). When they were 38 years old we examined their physiologies to test whether this young population would show evidence of individual variation in aging despite remaining free of age-related disease. We next tested the hypothesis that cohort members with "older" physiologies at age 38 had actually been aging faster than their same chronologically-aged peers who retained "younger" physiologies —specifically, we tested

whether indicators of the integrity of their cardiovascular, metabolic, and immune systems, their kidneys, livers, gums, and lungs, and their DNA had deteriorated more rapidly according to measurements taken repeatedly since a baseline 12 years earlier at age 26. We further tested if, by midlife, young adults who were aging more rapidly already exhibited deficits in their physical functioning, showed signs of early cognitive decline, and looked older to independent observers.

RESULTS

Are young adults aging at different rates? Measuring the aging process is controversial. Candidate biomarkers of aging are numerous, but findings are mixed (18–21). Multi-biomarker algorithms have been suggested as a more reliable alternative to single-marker aging indicators (22, 23). A promising algorithm is the 10-biomarker US National Health and Nutrition Survey (NHANES)-based measure of "*Biological Age.*" In more than 9,000 NHANES participants aged 30-75 years at baseline, Biological Age outperformed chronological age in predicting mortality over a two-decade follow-up (24). We applied this algorithm to calculate the Biological Age of Dunedin Study members when they were all chronologically 38 years old (**Supplementary Methods**). Even though the cohort remained largely free of chronic disease, Biological Age took on a normal distribution, ranging from 28-61 years (M=38 years, SD=3.23; **Figure 2**). This distribution was consistent with the hypothesis that some same-aged cohort members were biologically older than others.

Biological Age is assumed to reflect ongoing longitudinal change within a person. But it is a cross-sectional measure taken at a single point in time. Therefore, we next tested the hypothesis that young adults with "older" Biological Age at age 38 years were actually aging faster. The Dunedin Study biobank contains longitudinal measures of 18 biomarkers that track the physiological integrity of Study members' cardiovascular, metabolic, and immune systems, their kidneys, livers and lungs, their dental health, and their DNA. We analyzed within-individual longitudinal change in these 18 biomarkers across chronological-ages 26, 32, and 38 years to quantify each Study member's personal rate of physiological deterioration, their "*Pace of Aging*."

The Pace of Aging was calculated from longitudinal analysis of the 18 biomarkers in three steps (**Supplementary Methods**). First, all biomarkers were standardized to have the same scale (mean=0, SD=1 based on their distributions when Study members were 26 years old) and coded so that higher values corresponded to "older" levels (i.e. scores were reversed for cardiorespiratory fitness, high-density lipoprotein cholesterol, lung function, and leukocyte telomere length, for which values are expected to decline with increasing chronological age). Even in our cohort of young adults, biomarkers showed a pattern of age-dependent decline in the functioning of multiple organ system over the 12-years follow-up period (**Figure 3**). Second, we used mixed-effects growth models to calculate each Study member's personal slope for each of the 18 biomarkers. 954 individuals with repeated measures of biomarkers contributed data to this analysis. Of the 51,516 potential observations (n=954 Study members x 18 biomarkers x 3 time points), 44,475 (86.3%) were present in the database and used to estimate longitudinal growth curves modeling the Pace of Aging. The models took the form $B_{it} = \gamma_0 + \gamma_1 Age_{it} + \boldsymbol{\mu_{0i}} + \boldsymbol{\mu_{1i}} Age_{it} + \epsilon_{it}$, where B_{it} is a biomarker measured for individual 'i' at time 't', γ_0 and γ_1 are the fixed intercept and slope estimated for the cohort, and μ_{0i} and μ_{1i} are the "random" intercepts and slopes estimated for each individual 'i'. Finally, we calculated each study member's Pace of Aging as the sum of these 18 slopes: $Pace\ of\ Aging_i = \sum_{B=1}^{18} \mu_{1iB}$. The resulting Pace of Aging measure was normally distributed in the cohort, consistent with the hypothesis that some cohort members were aging faster than others.

The Pace of Aging can be scaled to reflect physiological change relative to the passage of time. Because the intact birth cohort represents variation in the population, it provides its own norms. We scaled the Pace of Aging so that the central tendency in the cohort indicates one year of physiological change for every one chronological year. On this scale, cohort members ranged in their Pace of Aging from near zero years of physiological change per chronological year to nearly three years of physiological change per chronological year.

Study members with advanced Biological Age had experienced a more rapid Pace of Aging over the past 12 years as compared to their biologically younger age peers (r=0.38, p<0.001, **Figure 4**). Each year increase in Biological Age was associated with a 0.05-year increase in the Pace of Aging relative to the population norm. Thus, a 38-year-old with a

Biological Age of 40 years was estimated to have aged 1.2 years faster over the course of the 12-year follow-up period as compared to a peer whose chronological and Biological Age were 38. This estimate suggests that a substantial component of individual differences in Biological Age at midlife emerges during adulthood.

We next tested if individual variation in Biological Age and the Pace of Aging related to differences in the functioning of Study members' bodies and brains, measured with instruments commonly used in clinical settings (**Supplementary Methods**).

Does accelerated aging in young adults influence indicators of physical function? In gerontology, diminished physical capability is an important indication of aging-related health decline that cuts across disease categories (25, 26). Study members with advanced Biological Age performed less well on objective tests of physical functioning at age 38 than biologically "younger" peers (**Figure 5**). They had more difficulty with balance and motor tests (For Unipedal Stance Test of balance $r=-0.22$, $p<0.001$, for Grooved Pegboard Test of fine-motor coordination $r=-0.13$, $p<0.001$), and they were not as strong (Grip Strength test $r=-0.19$, $p<0.001$). Study members' Biological Age was also related to their subjective experience of physical limitation. Biologically older Study members reported having more difficulties with physical functioning than did biologically younger age-peers (SF-36 Physical Functioning Scale, $r=0.13$,$p<0.012$). We repeated these analyses using the Pace of Aging measure. Consistent with findings for Biological Age, Study members with a more rapid Pace of Aging exhibited diminished capacity on the 4 measures of physical functioning relative to more slowly aging age-peers.

Does accelerated aging in young adults influence indicators of brain aging? In neurology, cognitive testing is used to evaluate age-related decline in brain integrity. The Dunedin Study had conducted cognitive testing when Study members were children and repeated this testing at the age-38 assessment (27). Study members with older Biological Age had poorer cognitive functioning at midlife ($r=-0.17$, $p<0.001$). Moreover, this difference in cognitive functioning reflected actual cognitive decline over the years. When we compared age-38 IQ test scores to baseline test scores from childhood, Study members with older Biological

Age showed a decline in cognitive performance net of their baseline level (r=-0.09, p=0.010). Results were similar for the Pace of Aging (**Figure 6**).

Neurologists have also begun to use high-resolution two-dimensional photographs of the retina to evaluate age-related loss of integrity of blood vessels within the brain. Retinal and cerebral small vessels share embryological origin and physiological features, making retinal vasculature a noninvasive indicator of the state of the brain's microvasculature (28). We calculated the average caliber of Study members' retinal arterioles and venules from images taken at the age-38 assessment. Consistent with the cognitive testing findings, Study members with advanced Biological Age had "older" retinal vessels (narrower arterioles, r=-0.20, p<0.001; wider venules r=0.17, p<0.001). Results were similar for the Pace of Aging measure (**Figure 6**).

Do young adults who are aging faster feel and look older? Beyond clinical indicators, a person's experience of aging is structured by their own perceptions about their wellbeing and by the perceptions of others. Consistent with tests of aging indicators, Study members with older Biological Age perceived themselves to be in poorer health as compared to biologically younger peers (r=-0.22, p<0.001). In parallel, these biologically older Study members were perceived to be older by independent observers. We took a frontal photograph of each Study member's face at age 38, and showed these to a panel of Duke University undergraduates who were kept blind to all other information about the Study members, including their age. Based on the facial images alone, student raters scored Study members with advanced Biological Age as looking older than their biologically younger peers (r=0.21, p<0.001). Results for self-perceived wellbeing and facial age were similar when analyses were conducted using the Pace of Aging measure (**Figure 7**).

DISCUSSION

Aging is now understood as a gradual and progressive deterioration of integrity across multiple organ systems (7, 29). Here we show this process can be quantified already in young adults. We followed a birth cohort of young adults over 12 years, from ages 26-38, and observed systematic change in 18 biomarkers of risk for age-related chronic diseases that was consistent with age-dependent decline. We were able to measure these changes even though

the typical age of onset for the related diseases was still one to two decades in the future, and just 1.1% of the cohort members had been diagnosed with an age-related chronic disease.

Measuring aging remains controversial. We measured aging in two ways. First, we used a biomarker scoring algorithm previously calibrated on a large, mixed-age sample. We applied this algorithm to cross-sectional biomarker data collected when our Study members were all chronologically aged 38 years to calculate their Biological Age. Second, we conducted longitudinal analysis of 18 biomarkers in our population-representative birth cohort when they were aged 26, 32, and 38 years. We used this longitudinal panel dataset to model how each individual changed over the 12-year period to calculate their personal Pace of Aging.

Pace of Aging and Biological Age represent two different approaches to quantifying aging. Pace of Aging captures real-time longitudinal change in human physiology across multiple systems and is suitable for use in studies of within-individual change. For this analysis, we examined all 18 biomarkers with available longitudinal data in the Dunedin Study biobank. The other approach, Biological Age, provides a point-in-time snapshot of physiological integrity in cross-sectional samples. For this analysis, we used the published 10-biomarker set developed from the NHANES. These two approaches yielded consistent results. Study members with "older" Biological Age had evidenced "faster" Pace of Aging over the preceding 12 years. Based on Pace of Aging analysis, we estimate that roughly ½ of the difference in Biological Age observed at chronological age 38 had accumulated over the past 12 years. Our analysis shows that Biological Age can provide a summary of accumulated aging in cases where only cross-sectional data are available. For purposes of measuring the effects of risk exposures and anti-aging treatments on the aging process, Pace-of-Aging-type longitudinal measures provide a means to test within individual change.

Biological measures of Study members' aging were mirrored in their functional status, brain health, self-awareness of their own physical wellbeing, and their facial appearance. Study members who had older Biological Age and who experienced a faster Pace of Aging scored lower on tests of balance, strength, and motor coordination, and reported more physical limitations. Study members who had an older Biological Age and who experienced a faster Pace of Aging also scored lower on IQ tests when they were aged 38 years, showed actual decline in

full-scale IQ score from childhood to age-38 follow-up, and exhibited signs of elevated risk for stroke and for dementia measured from images of micro-vessels in their eyes. Further, Study members who had older Biological Age and who experienced a faster Pace of Aging reported feeling in worse health. Undergraduate student raters who did not know the study members beyond a facial photograph were able to perceive differences in the aging of their faces.

Together, these findings constitute proof of principle for the measures of Biological Age and Pace of Aging studied here to serve as technology to measure aging in young people. Further research is needed to refine and elaborate this technology. Our analysis was limited to a single cohort, and one that lacked ethnic minority populations. Data were right censored (follow-up extended only to age 38); aging trajectories may change at later ages. Future waves of data collection in the Dunedin cohort will capture these nonlinear patterns of change. Data were left censored (biomarker follow-up began at age 26); when and how aging trajectories began to diverge was not observed. Studies of children are needed. Measurements were taken only once every six years. Contiguous annual measurements would provide better resolution to measure aging, but neither our funders nor our research participants favored this approach. The biomarkers used to measure aging in our study were restricted to those scalable to a cohort based on technology available during the measurement period. They necessarily provide an incomplete picture of age-related changes to physiology.

Within the bounds of these limitations, the implication of the present study is that it is possible to quantify individual differences in aging in young humans. This development breaks through two blockades separating model organism research from human translational studies. One blockade is that animals age quickly enough that whole lifespans can be observed whereas in humans, lifespan studies outlast the researchers. A second blockade is that humans are subject to a range of complex social and genomic exposures impossible to completely simulate in animal experiments. If aging can be measured in free-living humans early in their lifespans, there are new scientific opportunities. These include: Testing the fetal programming of accelerated aging (e.g. does intrauterine growth restriction predispose to faster aging in young adulthood?); testing the effects of early-life-adversity (e.g. does child maltreatment accelerate aging in the decades before chronic diseases develop?); testing social gradients in health (e.g.

do children born into poor households age more rapidly than their age-peers born into rich ones? Can such accelerated aging be slowed by childhood interventions?); and searching for genetic regulators of aging processes (e.g. interrogating biological aging using high throughput genomics). There are also potential clinical applications. Early identification of accelerated aging before chronic disease becomes established may offer opportunities for prevention. Above all, measures of aging in young humans allow for testing the effectiveness of anti-aging therapies (e.g. caloric restriction) without waiting for participants to complete their lifespans.

MATERIALS & METHODS

A more detailed description of study measures, design, and analysis is provided in the **Supplementary Materials**.

Sample. Participants are members of the Dunedin Multidisciplinary Health and Development Study, which tracks the development of 1,037 individuals born in 1972–1973 in Dunedin, New Zealand.

Measuring Biological Age. We calculated each Dunedin study member's Biological Age at age 38 years using the Klemera-Doubal equation (22) and parameters estimated from the NHANES-III dataset (24) for ten biomarkers. Biological Age took on a normal distribution, ranging from 28-61 years (M=38 years, SD=3.23).

Measuring the Pace of Aging. We measured Pace of Aging from repeated assessments of a panel of 18 biomarkers, 7 of which overlapped with the Biological Age algorithm. We modeled change over time in each biomarker and composited results within each individual to calculate their pace of aging. Study members ranged in their Pace of Aging from near zero years of physiological change per chronological year to nearly three years of physiological change per chronological year.

Measuring diminished physical capacity. We measured physical capacity as balance, strength, motor coordination, and freedom from physical limitations when Study members were aged 38 years.

Measuring cognitive aging. We measured cognitive aging using neuropsychological tests in childhood and at age 38 years and images of retinal microvessels.

Measuring subjective perceptions of aging. We measured subjective perceptions of aging using Study members' self reports and evaluations of facial photographs of the Study members made by independent raters.

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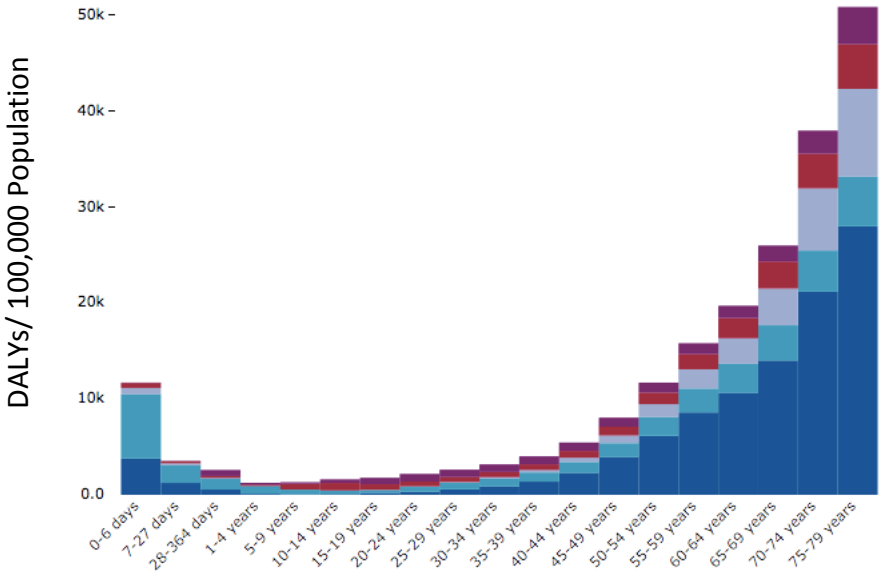
Figure 1. Burden of chronic disease rises exponentially with age. To examine the association between age and disease burden, we accessed data from the Institute for Health Metrics and Evaluation Global Burden of Disease database (http://www.healthdata.org/gbd).(30) Data graph (a) disability-adjusted life-years (DALYs) and (b) deaths per 100,000 population by age. Bars, from bottom to top, reflect the burden of cardiovascular disease (navy), type-2 diabetes (light blue), stroke (lavender), chronic respiratory disease (red) and neurological disorders (purple).

(a)



(b)

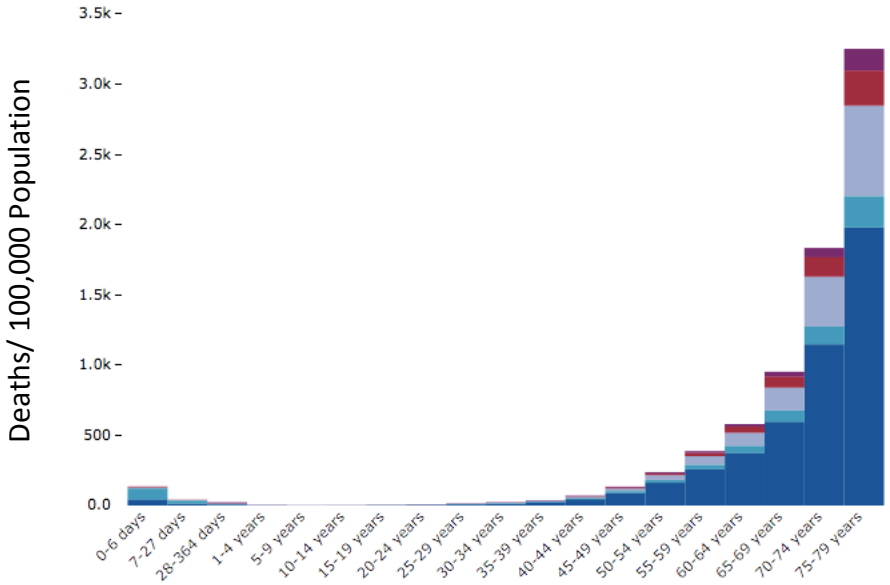


Figure 2. Biological age is normally distributed in a cohort of adults aged 38 years.

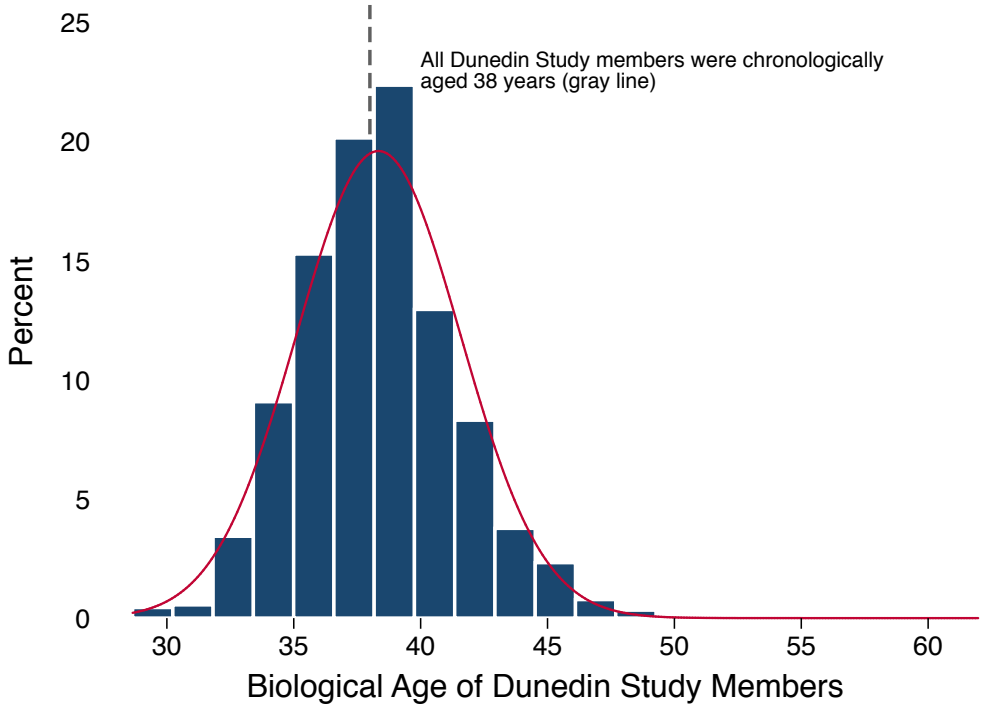


Figure 3. Healthy adults exhibit biological aging of multiple organ systems over 12 years of follow-up. Biomarker values were standardized to have mean=0 SD=1 across the 12 years of follow-up (Z-scores). Z-scores were coded so that higher values corresponded to "older" levels of the biomarkers; i.e. Z-scores for cardiorespiratory fitness, lung function (FEV_1 and FEV_1/FVC), leukocyte telomere length, creatinine clearance, and HDL cholesterol, which decline with age, were reverse coded so that higher Z-scores correspond to lower levels.

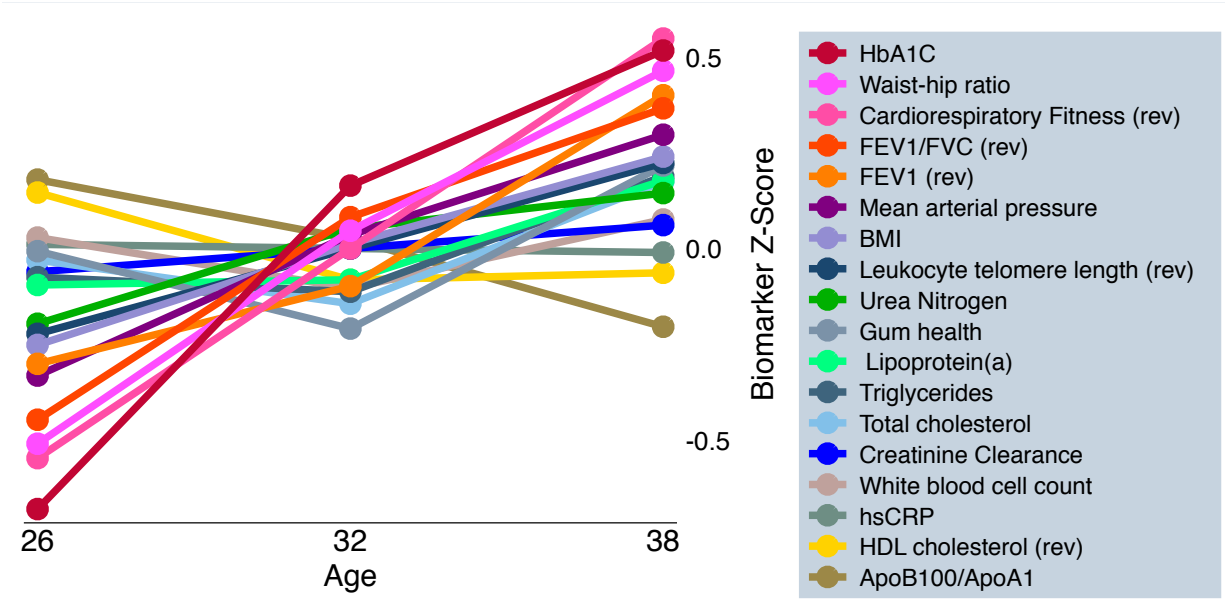


Figure 4. Dunedin Study members with older Biological Age at 38 years exhibited an accelerated Pace of Aging from age 26 to 38 years. The figure shows a binned scatterplot and regression line. Plotted points show means for "bins" of data from 20 Dunedin Study members. Effect-size and regression line were calculated from the raw data.

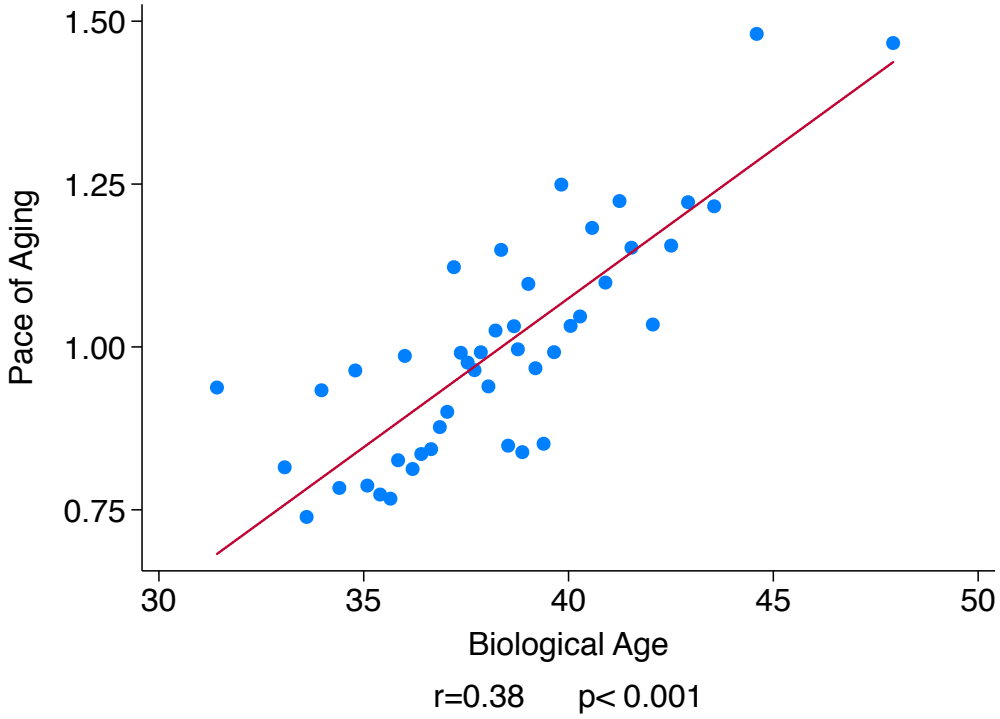


Figure 5. Healthy adults who were aging faster exhibited deficits in physical functioning relative to slower-aging peers. The figure shows binned scatter plots of the associations of Biological Age and Pace of Aging with tests of physical functioning (unipedal stance test, grooved pegboard test, grip strength) and Study members' reports of their physical limitations. In each graph, Biological Age associations are plotted on the left in blue (red regression line) and Pace of Aging associations are plotted on the right in green (navy regression line). Plotted points show means for "bins" of data from 20 Dunedin Study members. Effect-size and regression line were calculated from the raw data.

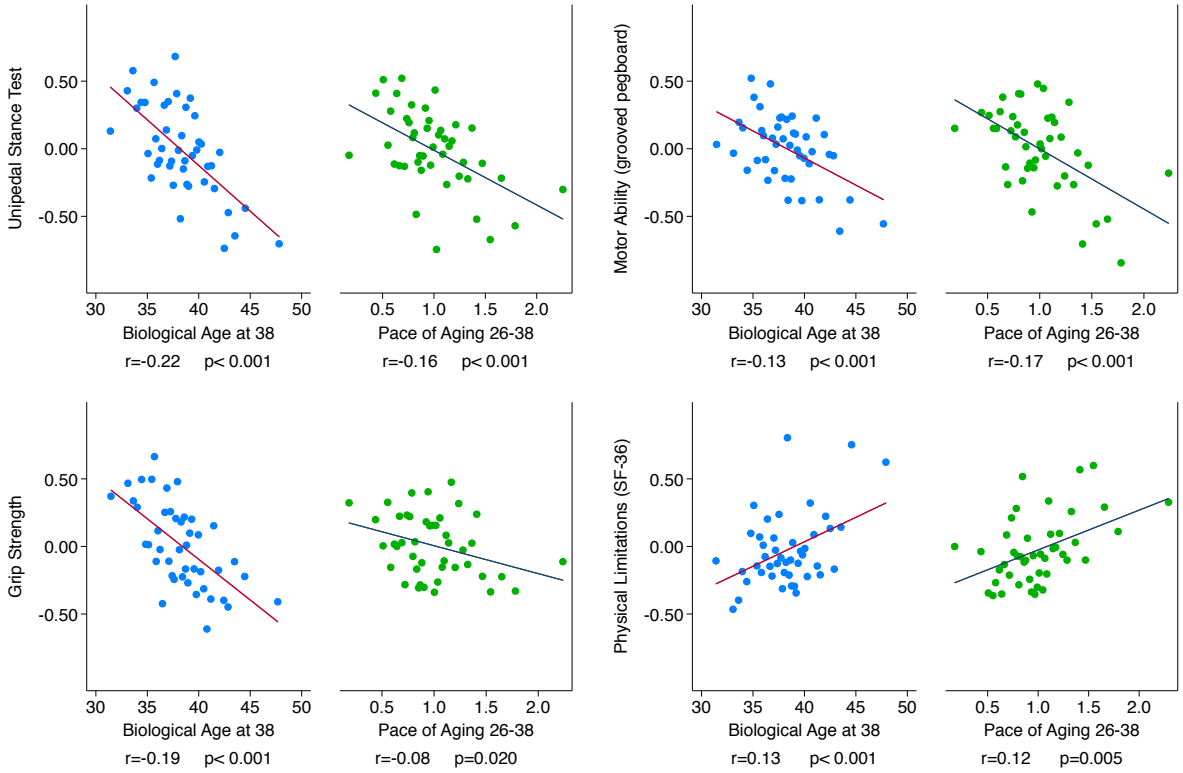


Figure 6. Healthy adults who were aging faster showed evidence of cognitive decline and increased risk for stroke and dementia relative to slower-aging peers. The figure shows binned scatter plots of the associations of Biological Age and Pace of Aging with cognitive functioning and cognitive decline (top row) and with the calibers of retinal arterioles and venules (bottom row). The Y-axes in the graphs of cognitive functioning and cognitive decline are denominated in IQ points. The Y-axes in the graphs of arteriolar and venular caliber are denominated in standard deviation units. In each graph, Biological Age associations are plotted on the left in blue (red regression line) and Pace of Aging associations are plotted on the right in green (navy regression line). Plotted points show means for "bins" of data from 20 Dunedin Study members. Effect-size and regression line were calculated from the raw data.

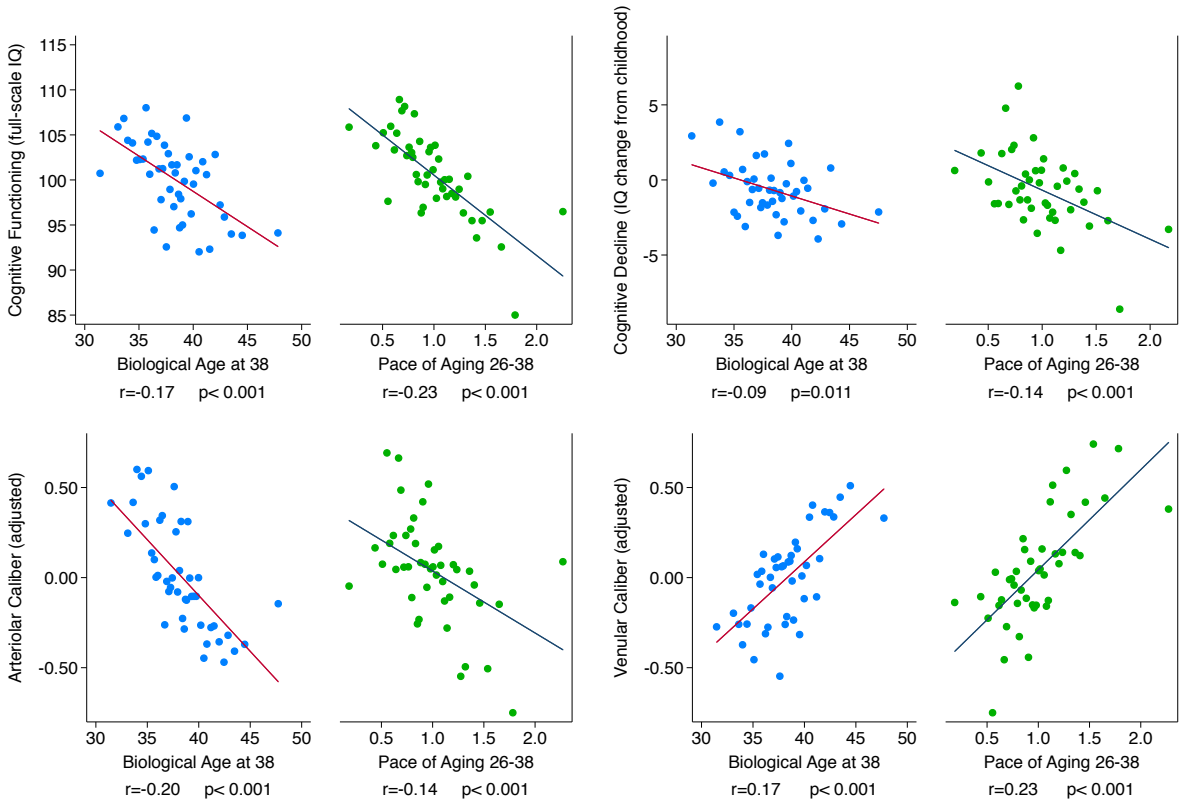
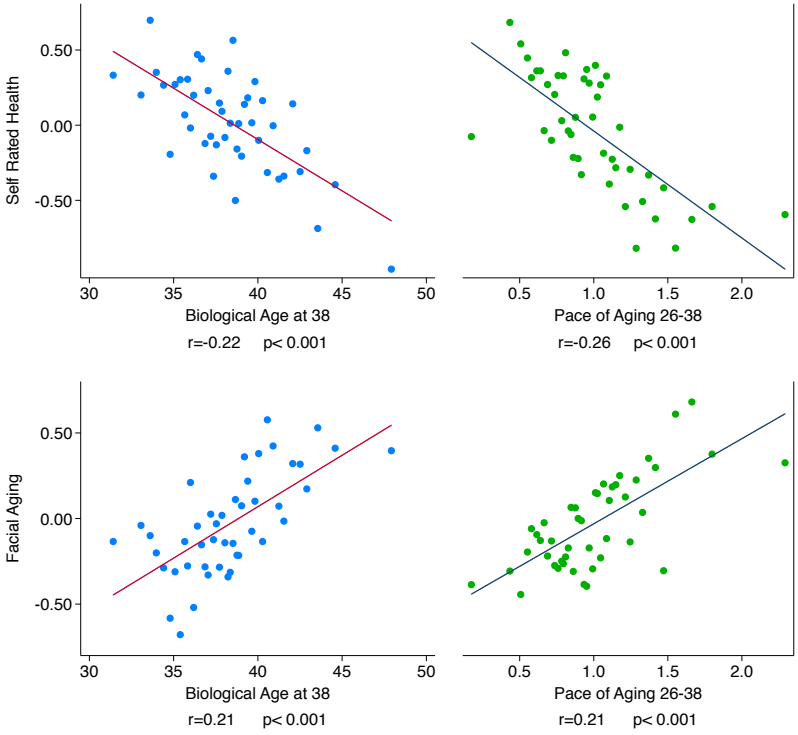


Figure 7. Healthy adults who were aging faster felt less healthy and were rated as looking older by independent observers. The figure shows binned scatter plots of the associations of biological age and the pace of aging with self-rated health (top) and with facial aging (bottom). Y-axes are denominated in standard deviation units. In each graph, biological age associations are plotted on the left in blue (red regression line) and pace of aging associations are plotted on the right in green (navy regression line). Plotted points show means for "bins" of data from 20 Dunedin Study members. Effect-size and regression line were calculated from the raw data.



SUPPLEMENTAL METHODS TO QUANTIFICATION OF BIOLOGICAL AGING IN YOUNG ADULTS

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Sample.

Participants are members of the Dunedin Study, a longitudinal investigation of health and behavior in a complete birth cohort. Study members (N=1,037; 91% of eligible births; 52% male) were all individuals born between April 1972 and March 1973 in Dunedin, New Zealand (NZ), who were eligible based on residence in the province and who participated in the first assessment at age 3. The cohort represents the full range of socioeconomic status on NZ's South Island and as adults matches the NZ National Health and Nutrition Survey on key health indicators (e.g., BMI, smoking, GP visits) (1). Cohort members are primarily white; fewer than 7% self-identify as having partial non-Caucasian ancestry, matching the South Island (2). Assessments were carried out at birth and ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, and, most recently, 38 years, when 95% of the 1,007 study members still alive took part. At each assessment, each study member is brought to the research unit for a full day of interviews and examinations. The Otago Ethics Committee approved each phase of the study and informed consent was obtained from all study members.

Measuring aging.

By age 38 years, only 11 Study members had developed an age-related chronic disease (diagnosed type-2 diabetes, myocardial infarction, or stroke).

We measured aging in two ways.

Biological Age Algorithm. Calculating human biological age is a relatively recent enterprise(3) and there is disagreement about methods (4, 5). Our goal was to borrow and implement the best-validated methods. Recently, data from NHANES-III participants aged 30 to 75 years were used to compare the ability of five Biological Age algorithms to predict mortality

when participants were followed-up 20 years later (6). Results showed that the Klemera-Doubal method (7) performed the best in NHANES-III (i.e., it predicted mortality, did so better than chronological age, and accounted for the association between chronological age and mortality). We calculated each Dunedin study member's Biological Age at age 38 years using the Klemera-Doubal equation (7) and parameters estimated from the NHANES-III dataset (6). The equation takes information from *m* number of regression lines of chronological age regressed on *m* number of biomarkers:

$BA_{EC} = \frac{\sum_{j=1}^{m}(x_j - q_j)\frac{k_j}{s_j^2} + \frac{CA}{s_{BA}^2}}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j}\right)^2 + \frac{1}{s_{BA}^2}}$, where *x* is the value of biomarker *j* measured for an individual in the Dunedin cohort. For each biomarker *j*, the parameters *k*, *q*, and *s* are estimated from a regression of chronological age on the biomarker in data from NHANES-III. *k*, *q*, and *s*, are the regression intercept, slope, and root mean squared error, respectively. s_{BA} is a scaling factor equal to the variance in chronological age explained by the biomarker panel in the NHANES database. CA is chronological age (38 for all Dunedin cohort members). Biomarkers used to calculate biological age in the Dunedin cohort at age 38 years are the same as those used in the NHANES analysis. (These ten biomarkers were selected for inclusion in the algorithm on the basis of their association with chronological age in NHANES-III.) The biomarkers are: Glycated hemoglobin, Forced expiratory volume in one second (FEV_1), Blood pressure (systolic), Total cholesterol, C-reactive protein, Creatinine, Urea nitrate, Albumin, Alkaline phosphatase, and Cytomegalovirus IgG. Details on biomarker measurements are provided in **Supplemental Table 1**. Biological Age took on a normal distribution, ranging from 28-61 years (M=38 years, SD=3.23).

Pace of Aging. We measured Pace of Aging from repeated assessments of a panel of 18 biomarkers. Seven biomarkers overlapped with the Biological Age algorithm: Glycated hemoglobin, Forced expiratory volume in one second (FEV_1), Blood pressure (mean arterial pressure), Total cholesterol, C-reactive protein, Creatinine clearance, and Urea nitrate. In addition, Pace of Aging included the following 11 additional biomarkers: Cardiorespiratory fitness (VO_2Max), Waist-hip ratio, Forced vital capacity ratio (FEV_1/FVC), Body mass index (BMI),

Leukocyte telomere length (LTL), Lipoprotein(a), Triglycerides, Periodontal disease, White blood cell count, High density lipoprotein (HDL), and Apolipoprotein B100/A1 ratio. Biomarkers were assayed at the age-26, -32, and -38 assessments. (Albumin, Alkaline phosphatase, and Cytomegalovirus IgG could not be included in the Pace of Aging because they were measured only at the age-38 assessment.) Details on biomarker measurements are provided in **Supplemental Table 1**.

We calculated each Study member's Pace of Aging in three steps. In the first step, we transformed the biomarker values to a standardized scale. For each biomarker, we standardized values according to the age-26 distribution, setting the mean to zero and the corresponding standard deviation to one. Standardization was conducted separately for men and women. Scores were reversed for VO_2Max, FEV_1/FVC, FEV_1, LTL, creatinine clearance, and HDL cholesterol, which are known to decline with age. Thus, standardized biomarker values greater than zero indicated levels that were "older" and values less than zero indicated levels "younger" as compared to the average 26-year-old. Over the 12 years of follow-up, the biomarker panel indicated a progressive deterioration of physiological integrity with advancing chronological age; i.e. values tended to increase from the age-26 assessment to the age-38 assessment.

In the second step, we calculated each Study member's personal slope for each of the 18 biomarkers—the average year-on-year change observed over the 12-year period. Slopes were estimated using a mixed effects growth model(8) that regressed the biomarker level on age. The models took the form $B_{it} = \gamma_0 + \gamma_1 Age_{it} + \boldsymbol{\mu_{0i}} + \boldsymbol{\mu_{1i}} Age_{it} + \epsilon_{it}$, where B_{it} is a biomarker measured for individual 'i' at time 't', γ_0 and γ_1 are the fixed intercept and slope estimated for the cohort, and μ_{0i} and μ_{1i} are the "random" intercepts and slopes estimated for each individual 'i'. (Only two measurement waves were available for LTL, high sensitivity CRP (hsCRP), and creatinine clearance. Slopes for these biomarkers were calculated as difference scores; between ages 32 and 38 for hsCRP and creatinine clearance and between ages 26 and 38 for LTL.) Within individuals, levels and slopes of the 18 biomarkers were positively correlated (averaged across all pairs of biomarkers, r=0.10 for intercepts and r=0.07 for slopes). A complete list of average biomarker slopes and pairwise correlations among biomarker slopes is

presented in **Supplemental Table 2**. For four of the biomarkers we examined, levels either did not change or changed in a direction counter to published associations with age-related chronic disease: White blood cell count and CRP levels did not change, HDL cholesterol increased modestly, and apolipoprotein B100/A1 ratio declined. However, slopes for these biomarkers did show the expected pattern of correlation with other biomarkers. For example, Study members whose apolipoprotein B100/A1 ratio increased during the follow-up period also showed increasing adiposity, declining lung function, and increasing systemic inflammation.

In the third step, we combined information from the slopes of the 18 biomarkers to calculate each Study member's personal "pace of aging." Because we did not have a priori basis for weighting differential contributions of the biomarkers to an overall pace of aging measure, we combined information using a unit-weighting scheme. (All biomarkers were standardized to have mean=0, SD=1 based on their age-26 distributions, so slopes were denominated in comparable units). We calculated each study member's Pace of Aging as the sum of age-dependent annual changes in biomarker Z-scores: $Pace\ of\ aging_i = \sum_{B=1}^{18} \mu_{1iB}$, where μ_{1iB} is the slope of biomarker 'B' for individual 'i'. Pace of Aging was normally distributed in the cohort (M=0.70 age-26 SD units, SD=0.29). Because the Dunedin birth cohort represents its population, its mean and distribution represent population norms. We used these norms to scale the Pace of Aging to reflect physiological change relative to the passage of time. We set the cohort mean Pace of Aging as a reference value equivalent to the physiological change expected during a single chronological year. Using this reference value, we rescaled Pace of Aging in terms of years of physiological change per chronological year. On this scale, cohort members ranged in their Pace of Aging from near zero years of physiological change per chronological year to nearly three years of physiological change per chronological year (**Supplemental Figure 1**).

Measuring diminished physical capacity at age 38 years.

Physical Functioning. We employed three measures. First, we measured balance as the maximum time achieved across three trials of the Unipedal Stance Test (with eyes closed) (9–11). Second, we measured grip strength with dominant hand (elbow held at 90°, upper arm held tight against the trunk) as the maximum value achieved across three trials using a Jamar

digital dynamometer (12, 13). Third, we measured motor functioning as the time to completion of the Grooved Pegboard Test with the non-dominant hand (14).

Physical Limitations. Study member responses ("limited a lot," "limited a little," "not limited at all") to the 10-item SF-36 physical functioning scale (15) assessed their difficulty with completing various activities, e.g., climbing several flights of stairs, walking more than 1 km, participating in strenuous sports.

Measuring cognitive functioning in childhood and at age 38 years.

Cognitive Testing. IQ is a highly reliable measure of general intellectual functioning that captures overall ability across differentiable cognitive functions. We measured IQ from the individually administered Wechsler Intelligence Scale for Children-Revised (WISC-R; averaged across ages 7, 9, 11, and 13)(16) and the Wechsler Adult Intelligence Scale-IV (WAIS-IV; age 38) (17), both with M=100 and SD=15. We measured IQ decline by comparing scores from the WISC-R and the WAIS-IV.

Retinal Imaging. Digital fundus photographs were taken at the Dunedin Research Unit after 5 min of dark adaptation. The same camera (Canon NMR-45 with a 20D single-lens reflex backing; Canon, Tokyo, Japan) was used for all photographs, to avoid artifactual variation from different cameras. Both the left and the right eyes were photographed, and we report analyses of the average for the two eyes. Retinal photographs were graded at the Singapore Eye Research Institute, National University of Singapore, using semi-automated computer software, Singapore I Vessel Assessment (SIVA) Version 3.0. Trained graders, blind to participants' characteristics, used the SIVA program to measure the retinal vessel diameters according to a standardized protocol with high inter-grader reliability (18). Diameter (or caliber) denotes the size of the lumen, which is the internal space of the vessel. Measurements were made for arterioles and venules where they passed through a region located 0.50 to 2.00 disk diameters from the optic disk margin (19). Vessel calibers were based on the six largest arterioles and venules passing though this region and were summarized as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) using the revised Knudtson-Parr-Hubbard formula (18, 20).

Arteriolar and venular calibers were normally distributed within our population-representative cohort. The mean arteriolar caliber was 137.33 measuring units (SD=10.86, median=137.30, range=105.66–179.47), and the mean venular caliber was 196.20 measuring units (SD=14.83, median=195.51, range=141.07–245.68). Before all analyses, arteriolar and venular caliber were each adjusted for the effect of the other vessel, as recommended (21, 22), in order to isolate the unique effects for each vessel and adjust for any potential effects of refractive errors (23).

Measuring self-perceptions of health and others' perceptions of aging at age 38 years.

Self Rated Health. Study members rated their health on a scale of 1-5 (poor, fair, good, very good, or excellent).

Facial Aging. We took two measurements of perceived age based on facial photographs. First, Age Range was assessed by an independent panel of 4 Duke University undergraduate raters. Raters were presented with standardized (non-smiling) facial photographs of Study members (taken with a Canon PowerShot G11 camera with an optical zoom, Canon Inc., Tokyo, Japan) and were kept blind to their actual age. Photos were divided into sex-segregated slideshow batches containing approximately 50 photos, viewed for 10s each. Raters were randomized to viewing the slideshow batches in either forward progression or backwards progression. They used a Likert scale to categorize each Study member into a 5-year age range (i.e., from 20-24 years old up to 65-70 years). Scores for each study member were averaged across all raters (α=0.71). The second measure, Relative Age, was assessed by a different panel of 4 Duke University undergraduate raters. The raters were told that all photos were of people aged 38 years old. Raters then used a 7-item Likert scale to assign a "relative age" to each study member (1="young looking", 7="old looking"). Scores for each study member were averaged across all raters (α=0.72). Age Range and Relative Age were highly correlated (r=0.73). To derive a measure of perceived age at 38 years, we standardized and averaged both Age Range and Relative Age scores to create Facial Age at 38 years.

Analysis

For analysis, validation measures were standardized to have mean=0, SD=1. For measures of physical functioning, physical activity, physical limitations, retinal vessel calibers, and self-rated health, standardization was conducted separately for men and women to account for different means/SDs between the sexes. Cognitive test scores and facial ages were similarly distributed in men and women. Regression analyses were adjusted for sex. Thus, effect-sizes reported for associations between aging measures and validation measures represent population average effects independent of sex. Effect-size estimates were calculated as standardized beta-coefficients from linear regressions (equivalent to Pearson's r).

To test whether associations between Pace of Aging and the validation metrics were dependent on any single biomarker, we conducted a leave-one-out sensitivity analysis. In the leave-one-out analysis, we re-calculated Pace of Aging leaving out each biomarker in turn. Full Pace of Aging and Leave-One-Out Pace of Aging effect-sizes from models predicting Biological Age and each validation metric are reported in **Supplemental Figure 2**.

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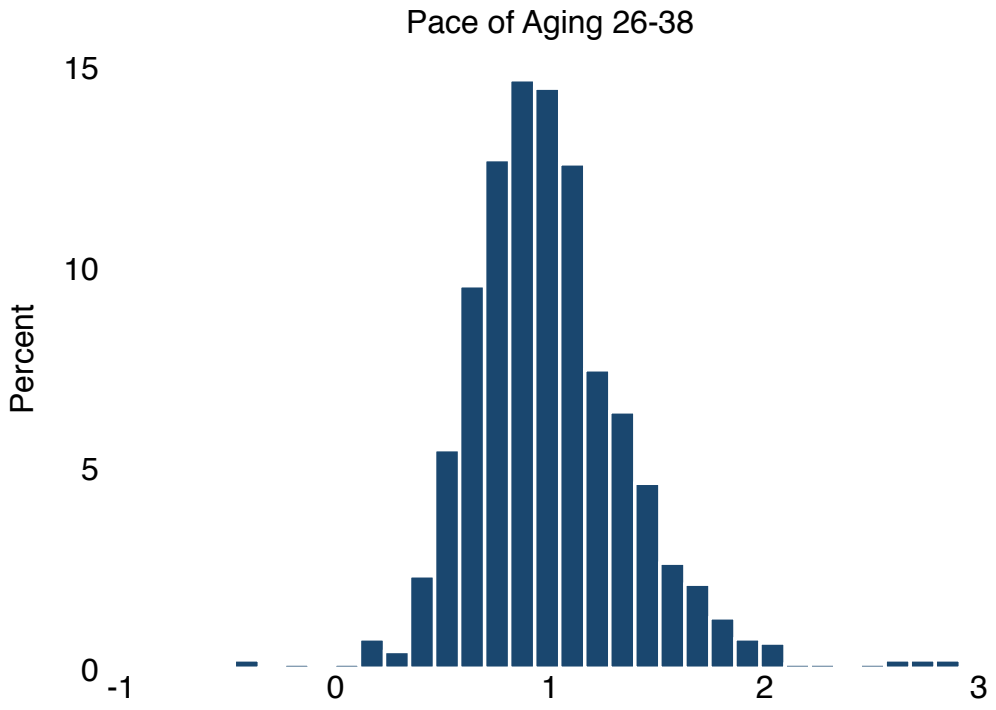
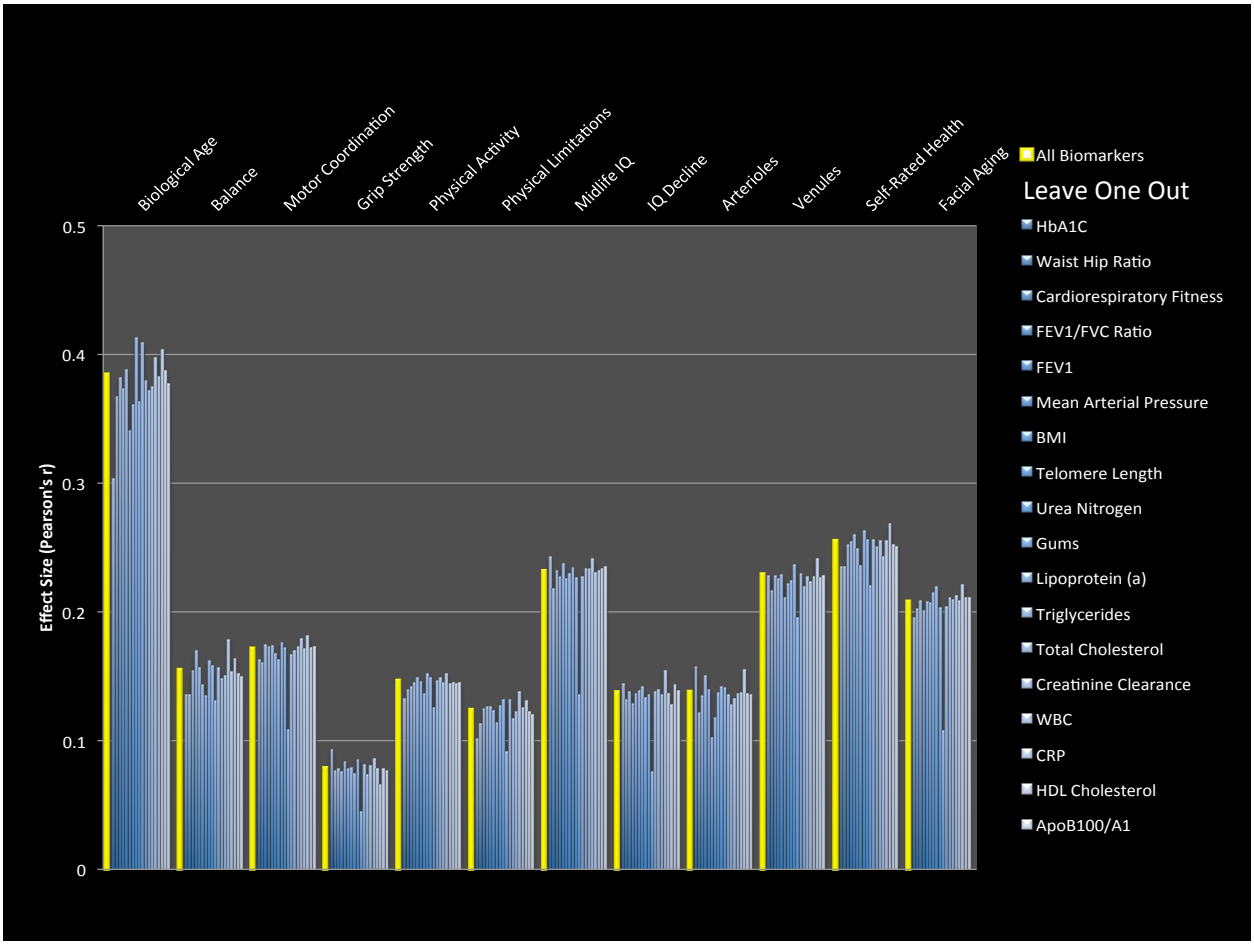
Supplemental Figure 1. Distribution of Pace of Aging in the Dunedin Cohort. Pace of Aging is denominated in years of physiological change per chronological year. Pace of Aging of 1 indicates a cohort member who experienced one year of physiological change per chronological year (the cohort average). Pace of Aging of two indicates a cohort member aging at a rate of two years of physiological change per chronological year, twice as fast as the population norm. Pace of Aging equal to zero indicates a cohort member whose physiology remained unchanged between ages 26 and 38.



Supplemental Figure 2. Pace of Aging and Leave-One-Out Pace of Aging effect sizes for models predicting Biological Age and validation metrics. The graph shows effect sizes for the full Pace of Aging (yellow bars) and for Leave-One-Out Pace of Aging measures calculated after omitting each biomarker in turn (blue bars). For example, the HbA1C Leave-One-Out Pace of Aging was calculated from the slopes of the other 17 biomarkers.



Supplemental Table 1. Measurement of biomarkers used to calculate Biological Age and Pace of Aging measures. Measures were taken in counterbalanced order across Study members with the exception of blood, which was drawn at the same time of day for all Study members at all three ages. Women who were pregnant at the time of a given assessment were excluded from that wave of data collection.

Glycated hemoglobin level (HbA1C)	Serum glycated hemoglobin concentration (expressed as a percentage of total hemoglobin) was measured by ion exchange high performance liquid chromatography (Variant II: BioRad, Hercultes, Calif.), a method certified by the US National Glycohemoglobin Standardization Program (http://www.ngsp.org/).
Cardiorespiratory Fitness	Cardiorespiratory fitness was assessed by measuring heart rate in response to a submaximal exercise test on a friction-braked cycle ergometer. Dependent on the extent to which heart rate increased during a 2-min 50 W warm-up, the workload was adjusted to elicit a steady heart-rate in the range 130–170 beats per minute. After a further 6-min constant power output stage, the maximum heart rate was recorded and used to calculate predicted maximum oxygen uptake adjusted for body weight in milliliters per minute per kilogram (VO_2max) according to standard protocols (24).
Anthropometry	Height was measured to the nearest millimeter using a portable stadiometer (Harpenden; Holtain, Ltd). Weight was measured to the nearest 0.1 kg using calibrated scales. Individuals were weighed in light clothing. Body mass index (BMI) was calculated. Waist girth was the perimeter at the level of the noticeable waist narrowing located between the costal border and the iliac crest. Hip girth was taken as the perimeter at the level of the greatest protuberance and at about the symphysion pubic level anteriorly. Measurements were repeated and the average used to calculate Waist:hip ratio.
Lung function	We calculated post-albuterol forced expiratory volume in one second (FEV_1) and the ratio of FEV_1 to forced vital capacity (FVC) using measurements from spirometry conducted with a Sensormedics body plethysmograph (Sensormedics Corporation, Yorba Linda, CA, USA).
Blood pressure	Systolic and diastolic blood pressure were assessed according to standard protocols with a Hawksley random-zero sphygmomanometer with a constant deflation valve. Mean arterial pressure (MAP) was calculated using the formula *Diastolic Pressure*+1/3(*Systolic Pressure - Diastolic Pressure*).
Leukocyte telomere length	Leukocyte DNA was extracted from blood using standard procedures (25, 26). DNA was stored at -80°C. All DNA samples were assayed for leukocyte telomere length at the same time. Leukocyte telomere length was measured using a validated quantitative PCR method (27), as previously described,(28) which determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative PCR reactions for each subject; one for a single-copy gene (S) and the other in the telomeric repeat region (T). All DNA samples were run in triplicate for

	telomere and single-copy reactions. Measurement artifacts (e.g., differences in plate conditions) may lead to spurious results when comparing leukocyte telomere length measured on the same individual at different ages. To eliminate such artifacts, we assayed DNA triplicates from the same individual from all time points, on the same plate. CV for triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S).
Creatinine clearance	Serum creatinine (mmol/L) was measured by kinetic colorimetric assay on a Hitachi 917 analyzer (age 32) and Modular P analyzer (age 38) (Roche Diagnostics, Mannheim, Germany). For Pace of Aging analysis, creatinine was measured as creatinine clearance, calculated using a modified Cockcroft-Gault equation. The original equation [(140-*age*) x (*Wt in kg*) x (0.85 if *female*) / (72 x *Creatinine*)] (29) was modified as follows: (30, 31) For individuals with BMI of 18-23, we substituted "ideal body weight" in place of weight [for men, *Ideal Body Weight* = 50+(2.3x *Ht in inches* - 60); for women *Ideal Body Weight* = 50+(2.3x *Ht in inches* - 60)]. For individuals with BMI≥23, we substituted "adjusted body weight" for weight (*Adjusted Body Weight = Ideal Body Weight* + 0.4 x *Wt in kg - Ideal Body Weight*).
Urea nitrogen	Serum urea nitrogen (mmol/L) was measured by kinetic UV assay at age 26 (Hitachi 917 analyser) and by kinetic colorimetric assay at ages 32 and 38 (Hitachi 917 analyzer at age 32, Modular P analyzer at age 38).
Lipoprotein *(a)*	Serum lipoprotein *(a)* (mg/L) was measured by immunoturbidimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Non-fasting Triglycerides, Total cholesterol, and High-density lipoprotein (HDL) cholesterol	Serum non-fasting triglyceride, total cholesterol, and high-density lipoprotein levels (mmol/L) were measured by colorimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Gum health (combined attachment loss)	Examinations were conducted using calibrated dental examiners; three sites (mesiobuccal, buccal, and distolingual) per tooth were examined, and gingival recession (the distance in millimeters from the cementoenamel junction to the gingival margin) and probing depth (the distance from the probe tip to the gingival margin) were recorded using a PCP-2 probe. The combined attachment loss for each site was computed by summing gingival recession and probing depth (third molars were not included).
White blood cell count	Whole blood white blood cell counts ($x10^9$/L) were measured by flow-cytometry with a Coulter STKS (Coulter Corporation, Miami, FL) (age 26), a Sysmex XE2100 (Sysmex Corporation, Japan) (age 32) and a Sysmex XE5000 (Sysmex Corporation, Japan) (age 38). Counts were log-transformed for analysis.
C-reactive protein (hsCRP)	Serum C-reactive protein (mg/L) was measured by high sensitivity immunoturbidimetric assay on a Hitachi 917 analyzer (age 32) and Modular

	P analyzer (age 38). Values were log transformed for analysis.
Apolipoprotein *A1*	Serum apolipoprotein *A1* (g/L) was measured by immunoturbidimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Apolipoprotein *B100*	Serum apolipoprotein *B100* (g/L) was measured by immunoturbidimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Albumin	Serum albumin (g/L) was measured by immunoturbidimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Alkaline phosphatase	Serum alkaline phosphatase, ALP (U/L) was measured by enzymatic colorimetric assay on a Hitachi 917 analyzer (ages 26-32) and Modular P analyzer (age 38).
Cytomegalovirus Optical Density	Plasma cytomegalovirus (CMV) IgG antibodies (IU/ml) were measured by EIA assay (Diamedix, FL, USA) on a Molecular Devices Spectramax384 plate reader (Molecular Devices, CA, USA).

Supplemental Table 2. Pairwise correlations among Study-member specific intercepts (Panel A) and slopes (Panel B) for 18 biomarkers. Correlations with r>0.05 are highlighted in yellow. Correlations with r>0.1 are highlighted in pink.

Panel A.

Biomarker Intercept Correlations	Glycated Hemoglobin	Waist-Hip Ratio	Cardiorespiratory Fitness	FEV1/FVC ratio	FEV1	Mean Arterial Pressure	BMI	Telomere Length	Urea Nitrogen	Gums	Lipoprotein (a)	Triglycerides	Cholesterol	Creatinine Clearance	White Blood Cell Count	CRP	HDL Cholesterol	ApoB100/A1 Ratio
Glycated Hemoglobin																		
Waist-Hip Ratio	0.1																	
Cardiorespiratory Fitness	0.1	0.4																
FEV1/FVC ratio	0.0	0.1	0.1															
FEV1	0.1	0.1	0.0	0.3														
Mean Arterial Pressure	0.1	0.2	0.3	0.0	0.0													
BMI	0.1	0.5	0.8	0.1	0.1	0.2												
Telomere Length	0.1	0.0	0.1	0.0	0.1	0.1	0.0											
Urea Nitrogen	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0										
Gums	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0									
Lipoprotein (a)	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0								
Triglycerides	0.1	0.4	0.3	0.0	0.1	0.2	0.3	0.1	0.1	0.0	0.1							
Cholesterol	0.1	0.2	0.2	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.2	0.4						
Creatinine Clearance	0.0	-0.1	-0.3	0.0	0.1	0.0	-0.2	0.1	0.1	0.0	0.0	0.0	0.0					
White Blood Cell Count	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.3	0.0	0.2	0.1	0.0				
CRP	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	-0.1	0.0			
HDL Cholesterol	0.0	0.3	0.3	0.0	0.1	0.1	0.3	0.1	0.0	0.1	0.0	0.4	-0.1	-0.1	0.1	0.0		
ApoB100/A1 Ratio	0.1	0.3	0.4	0.1	0.1	0.1	0.4	0.1	0.0	0.1	0.1	0.5	0.7	0.0	0.2	0.0	0.6	

Panel B.

Biomarker Slope Correlations	Glycated Hemoglobin	Waist-Hip Ratio	Cardiorespiratory Fitness	FEV1/FVC ratio	FEV1	Mean Arterial Pressure	BMI	Telomere Length	Urea Nitrogen	Gums	Lipoprotein (a)	Triglycerides	Cholesterol	Creatinine Clearance	White Blood Cell Count	CRP	HDL Cholesterol	ApoB100/A1 Ratio
Slope	0.13	0.10	0.10	0.07	0.06	0.06	0.04	0.03	0.03	0.04	0.03	0.02	0.02	0.03	0.00	-0.01	-0.02	-0.03
Glycated Hemoglobin																		
Waist-Hip Ratio	0.2																	
Cardiorespiratory Fitness	0.0	0.2																
FEV1/FVC ratio	0.0	-0.1	0.1															
FEV1	0.1	0.0	0.1	0.4														
Mean Arterial Pressure	0.1	0.2	0.2	-0.1	0.0													
BMI	0.1	0.4	0.6	-0.1	0.1	0.3												
Telomere Length	0.0	0.0	0.0	0.0	0.0	0.0	0.0											
Urea Nitrogen	0.0	0.0	0.0	0.0	-0.1	0.0	0.0	-0.1										
Gums	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0									
Lipoprotein (a)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0								
Triglycerides	0.2	0.3	0.2	0.0	0.1	0.2	0.4	0.0	0.1	0.0	0.0							
Cholesterol	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.3						
Creatinine Clearance	0.0	0.0	-0.1	-0.1	-0.1	0.0	-0.2	0.0	0.1	0.0	0.0	-0.2	-0.1					
White Blood Cell Count	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.2	0.2	0.0				
CRP	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	-0.1	0.0	0.0	0.0	0.0	-0.1	0.0			
HDL Cholesterol	0.1	0.2	0.2	0.0	0.1	0.0	0.4	0.0	0.0	0.0	-0.1	0.4	-0.1	-0.1	0.1	0.1		
ApoB100/A1 Ratio	0.1	0.2	0.3	0.1	0.1	0.1	0.4	0.0	-0.1	0.0	0.0	0.3	0.4	-0.1	0.1	0.1	0.5	