

Original Article

Lower Epigenetic Age Acceleration in Older Adults with Preserved Global Cognitive Performance

Felipe Salech^{1,2,3,4,5,8,10}, Carlos Marquez^{3,6,10}, Paulina Correa-Burrows^{3,5,11}, Rodrigo Saguez³, Daniela P Ponce⁴, Carol San Martin³, Christian Gonzalez-Billault^{5,8,9,10,12}, María Isabel Behrens^{2,4,7,8}, Cecilia Albala^{3,5,10*}

¹Clínica de Caídas y Fracturas, Sección de Geriátrica, Hospital Clínico Universidad de Chile, Santiago, Chile; ²Centro de Investigación Clínica Avanzada (CICA), Hospital Clínico Universidad de Chile, Santiago, Chile; ³Ageing and Quality of life Nucleus, INTA, Universidad de Chile, Santiago, Chile; ⁴Clínica Alemana de Santiago - Universidad del Desarrollo, Santiago, Chile; ⁵Centro de Gerociencia, Salud Mental y Metabolismo Procesos de Envejecimiento y Factores Asociados a la Salud y Bienestar de los Adultos GERO, Santiago, Chile; ⁶Departamento de Medicina Interna, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile; ⁷Departamento de Neurología y Neurocirugía, Facultad de Medicina, Universidad de Chile, Santiago, Chile; ⁸Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile, Santiago, Chile; ⁹Departamento de Biología, Facultad de Ciencias, Universidad de Chile; ¹⁰Unidad de Nutrición Pública, Instituto de Nutrición y Tecnologías de los alimentos (INTA), Universidad de Chile; ¹¹Unidad de Nutrición Humana, Instituto de Nutrición y Tecnologías de los alimentos (INTA), Universidad de Chile; ¹²The Buck Institute for Research on Aging, Novato, CA, USA.

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ABSTRACT: Epigenetic clocks estimate biological age, yet evidence linking decelerated epigenetic aging to healthy cognitive performance in community-dwelling older adults is limited. Cross-sectional study within the population-based ALEXANDROS cohort (Santiago, Chile). Healthy Cognitive Agers (HCA) were participants aged ≥ 80 years who consistently maintained a MMSE score ≥ 30 across all cohort assessments. Controls were cognitively normal peers from the same cohort (MMSE 28.5 ± 2.9). Epigenetic age was estimated using the Horvath, GrimAge, and PhenoAge clocks. Epigenetic age acceleration (EAA) was calculated as residuals from regressing control-calibrated epigenetic age on chronological age. DNA methylation-derived telomere length (DNAmTL, kb) was also assessed. Eighty community-dwelling older adults were included (40 HCA and 40 controls). HCA exhibited substantially lower control-calibrated EAA than controls across all clocks, including Horvath (-13.55 ± 5.28 vs 0.00 ± 4.34 years; $p < 0.001$; Cohen's $d = -2.80$), PhenoAge (-9.72 ± 8.37 vs 0.00 ± 4.56 years; $p < 0.001$; Cohen's $d = -1.44$), and GrimAge (-3.16 ± 3.08 vs 0.00 ± 4.14 years; $p < 0.001$; Cohen's $d = -0.87$). HCA also exhibited substantially longer DNAm-derived telomere length (6.39 ± 0.27 vs 5.39 ± 0.21 kb; $p < 0.001$; Cohen's $d = 4.15$). Preserved cognitive performance in late life, based primarily on persistent MMSE performance, is associated with a decelerated biological aging profile in community-dwelling older adults. Although longitudinal studies are required to clarify temporality, these findings are consistent with the hypothesis that DNA methylation-derived epigenetic markers may help characterize healthy cognitive aging trajectories.

Keywords: Cognitive Aging, Epigenetic Clocks, DNA Methylation, Biological Aging, Intrinsic capacity

Introduction

The widening gap between lifespan and healthspan represents one of the most pressing challenges in public

health and aging research [1]. While lifespan refers to the total number of years an individual lives, healthspan encompasses the period during which life is spent in good

*Correspondence should be addressed to: Dr. Cecilia Albala, INTA, Universidad de Chile, Santiago, Chile. Email: calbala@uchile.cl

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health and functional independence [2, 3]. Epidemiological data indicate that healthspan is not increasing at the same rate as lifespan, resulting in a growing population affected by chronic diseases, frailty, and functional decline [2, 4]. This imbalance has made understanding the mechanisms and determinants of healthy longevity a core goal of modern medical research.

Healthy cognitive aging is a critical component of healthy longevity, referring to the maintenance of cognitive performance and adaptability that mitigates the effects of age-related neural, behavioral, and social decline [5, 6]. Preserving cognitive health supports essential daily functions—such as memory, problem-solving, and reasoning—and contributes to independence and overall well-being [7, 8]. The preservation of cognitive and functional capacity is, therefore, a key indicator of successful aging. Considering the multiple cognitive domains, operational definitions of healthy cognitive aging vary widely across studies [5, 9]. Given the lack of a standardized definition, studies often rely on operational criteria to identify individuals with exceptional cognitive preservation, although such definitions may not fully capture all domains of cognitive function [5].

The biological mechanisms underlying healthy cognitive aging remain poorly understood. The conceptual framework of geroscience and the hallmarks of aging has provided a foundation for identifying the cellular and molecular processes that contribute to age-related decline, including genomic instability, mitochondrial dysfunction, altered proteostasis, and epigenetic alterations [10, 11]. Among these, epigenetic changes have emerged as a central feature of the aging process, reflecting cumulative biological adaptations to intrinsic and environmental influences. Advances in epigenomic profiling now offer new avenues for understanding how these molecular signatures relate to health status and functional capacity, enabling more accurate and individualized assessments of biological aging than chronological age alone [12–15]. Within this context, biological age—a composite measure reflecting an individual's physiological and molecular state—has become a promising indicator of both healthy aging and disease risk [15].

Several approaches have been developed to estimate biological age, including telomere length, transcriptomic, proteomic, and metabolomic signatures. However, epigenetic clocks based on DNA methylation patterns have demonstrated superior accuracy in predicting morbidity, mortality, and cognitive decline compared to traditional markers [12, 13, 15]. Measures such as DNAmGrimAge, PhenoAge, and Horvath's clock have shown strong correlations with both lifespan and healthspan, suggesting that accelerated epigenetic aging

reflects underlying biological processes contributing to functional deterioration [15, 16]. Conversely, decelerated epigenetic aging has been associated with resilience and cognitive preservation in older adults, indicating that epigenetic age may serve as a molecular indicator of healthy brain aging [17, 18]. However, much of the existing evidence linking epigenetic clocks to aging outcomes derives from selected populations, clinical samples, or cohorts enriched for disease, which may limit generalizability to older adults aging successfully in the community [19–21]. As a result, it remains unclear whether exceptional cognitive aging in late life is associated with a distinct and consistently decelerated biological aging profile at the molecular level.

Considering these elements, the aim of the present study was to examine whether older adults with preserved cognitive performance into advanced age—defined as Healthy Cognitive Agers (HCA), framed in this study as an operational phenotype based primarily on persistent MMSE performance—exhibit a distinct biological aging profile compared with cognitively typical peers. Specifically, we evaluated multiple epigenetic clocks and DNA methylation-derived telomere length to determine whether exceptional cognitive aging is associated with decelerated biological aging. By leveraging longitudinal cognitive data from a well-characterized population-based cohort, this study seeks to provide molecular-level evidence linking cognitive resilience to systemic markers of biological aging in late life.

METHODS

Study Setting and Participants

Healthy Cognitive Agers were defined as individuals aged ≥ 80 years, belonging to the ALEXANDROS cohort, who had undergone at least three prior cohort assessments and maintained a perfect MMSE score (30 points) at all prior assessments, in addition to a confirmatory evaluation performed for this study. The ALEXANDROS cohort, initiated in 2000, was created to investigate disability associated with obesity in individuals aged 60 and above who reside in Santiago, Chile [22]. The subjects were identified from the cohort database and invited for a new in-person assessment to confirm their MMSE score. Information regarding cognitive and functional performance was available for 2,372 subjects at baseline. A control group was conceived to allow comparison between HCA and community-dwelling older adults without evidence of cognitive impairment, while also enabling comparisons with chronologically younger individuals. Importantly, the control group was designed to reflect the natural heterogeneity of cognitive performance in the general older population, rather than a

highly selected or cognitively optimal subgroup. This approach provides a real-world reference against which exceptional cognitive preservation can be contrasted. Control participants were community-dwelling adults aged 70 years or older, with no history of cognitive impairment, defined by a Mini-Mental State Examination (MMSE) score ≥ 24 (but an MMSE score < 30 at their most recent assessment, thereby not meeting criteria for Healthy Cognitive Agers). This MMSE range was selected to reflect normative cognitive data from Chile indicate that MMSE scores in the range of 24–29 are commonly observed among cognitively normal older individuals [23]. Additionally, none of the participants in the control group had a documented diagnosis of cognitive impairment in their clinical records, nor were they using memantine, donepezil, or rivastigmine among their medications. To account for chronological age differences between groups, age-equivalence analyses were conducted, and sensitivity analyses were performed restricting the control group to participants aged ≥ 80 years. These analytical strategies were specifically implemented to address potential confounding due to chronological age differences and group heterogeneity, and to ensure that observed associations were not driven solely by these factors. These approaches were used to assess the robustness of between-group differences and to ensure that observed associations were not driven by chronological age alone. All these individuals (healthy cognitive agers and controls) were invited to undergo an in-person interview to assess cognitive performance before being invited to participate in the study.

Phenotypical Characterization

After signing an informed consent, all subjects underwent face-to-face interviews including anthropometric measurements, self-reported chronic diseases (hypertension, diabetes, cancer, COPD, stroke, myocardial infarction and heart failure), and self-perceived symptoms of depression measured by the Short Form of the Geriatric Depression Scale (GDS-15). Cognitive evaluation was assessed with the MMSE and the MoCA test. Multi-morbidity was defined as having 3 or more chronic diseases. A dual-energy X-ray absorptiometry (DXA) scan was performed in the whole sample in order to assess body composition. Handgrip strength was measured by means of handgrip dynamometry (Hand Dynamometer T-18, Country Technology, Inc.), registering the best of two measurements with the dominant hand. Three meters walking speed was registered. Physical performance was evaluated using the Short Physical Performance Battery (SPPB). Anthropometric measurements of weight, height, and knee height as well as waist, hip, calf, and arm

circumferences were taken according to methods described previously [22, 24].

Their appendicular skeletal muscle mass (ASM) index (SMI) was calculated as the ratio of ASM and height² (kg/m²) with cutoff validated for Chilean population [24]. Low SMI was defined with cut-off points obtained for the Chilean population (men: < 7.19 kg/m²; women: < 5.77 kg/m²) [24]. Low muscle strength was defined with cut-off points previously determined in a large sample of the Chilean older population (≤ 25 th percentile: men 27kg; women 15kg) [22, 24]. Sarcopenia was defined using the consensus criteria and the algorithm of the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) [25].

Nutritional status and obesity were defined according to World Health Organization (WHO) standards. For three meters gait speed, we used the same cut-off point defined by the EWGSOP (0.8m/sec). Osteoporosis was diagnosed with a DXA, using WHO criteria: T-scores of bone mineral density (BMD) below -1 and -2.5 categorized the patient as osteopenic and osteoporotic, respectively.

Determination of Biological Age

Peripheral blood samples (25 mL) were collected in EDTA tubes during morning assessments following an overnight fast. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque density gradient centrifugation (GE Healthcare). Genomic DNA was extracted from PBMCs using DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany), quantified, and stored at -80°C until analysis. One microgram of purified DNA per participant was shipped on dry ice to the Clock Foundation (Torrance, CA, USA) for DNA methylation profiling. Genome-wide DNA methylation was measured using the Infinium MethylationEPIC BeadChip array (Illumina, San Diego, CA), which interrogates over 850,000 CpG sites. Genome-wide DNA methylation data underwent standard preprocessing and quality control procedures. Data quality was assessed through interarray correlation analysis, principal component analysis, and visual inspection of density plots and dendrograms to identify potential outliers or technical artifacts. Samples were randomly distributed across arrays to minimize batch effects, and beta values were normalized using standard procedures to reduce technical variability and ensure comparability across samples. These analytical steps are consistent with established workflows for EPIC array data and with previously published studies using similar methodologies [26]. DNA methylation was assessed in peripheral blood mononuclear cells (PBMCs). To account for immune-cell heterogeneity, leukocyte subpopulation proportions (including CD4+ T cells, CD8+ T

cells, B cells, NK cells, and monocytes) were bioinformatically estimated from the DNA methylation data using the advanced deconvolution algorithms embedded within the Clock Foundation pipeline. These software-estimated cell counts ensure that the calculated Epigenetic Age Acceleration (EAA) values for the Horvath and DNAmGrimAge clocks are robust to variations in blood cell composition, which is particularly relevant when evaluating cohorts of exceptionally long-lived individuals exhibiting immunosenescence.

Epigenetic age was estimated using the Horvath, PhenoAge and DNAmGrimAge clocks, which predict biological age based on methylation signatures at defined CpG sites. For each participant, epigenetic age acceleration (EAA) was computed as the residual from a linear regression of epigenetic age on chronological age, providing a measure independent of chronological age. Positive EAA values indicated accelerated biological aging, whereas negative values indicated decelerated aging. Because residual-based EAA was derived from regression models fitted in the pooled analytic sample, mean EAA values are centered around zero across the overall population. We estimated control-calibrated epigenetic age acceleration (EAA). Linear regression models relating epigenetic age to chronological age were fitted exclusively within the control group for each epigenetic clock (Horvath, PhenoAge, and GrimAge). The resulting regression coefficients were then applied to the entire study population to estimate expected epigenetic age, and control-calibrated EAA was calculated as the residual difference between observed and expected epigenetic age. This approach provides an estimate of biological age deviation relative to the normative aging trajectory observed in cognitively typical controls. The gap between biological age and chronological age was also evaluated. Aside from computing well-established first- and second-generation epigenetic clocks, the EPIC array also provides a methylation-based estimation of leukocyte telomere length (TL).

Statistical Analysis of Biological Age

Normality of continuous variables was assessed using the Shapiro–Wilk test. When variables deviated from normality, non-parametric sensitivity analyses were performed using the Mann–Whitney U test. Because sensitivity analyses yielded results consistent with the primary analyses, Welch’s t-test was retained as the main between-group comparison. Effect sizes for between-group comparisons were calculated using Cohen’s d. Effect sizes were interpreted according to conventional thresholds (small: 0.2, moderate: 0.5, large: 0.8). The homogeneity of the epigenetic age distribution across the

sample was assessed with the Pettitt test to detect potential change points related to group status. A two-sided $P < 0.05$ was considered statistically significant. Because the primary analyses were restricted to a small number of prespecified biological aging measures selected based on prior evidence, no formal adjustment for multiple comparisons was applied and p-values are reported as unadjusted. All analyses were conducted using Stata 16.0 (StataCorp LLC, College Station, TX) and XLSTAT-R 2024.3 (Addinsoft, Paris, France).

In addition to univariate comparisons, multivariable linear regression models were performed to assess the association between group status (Healthy Cognitive Agers vs Controls) and biological aging outcomes, including epigenetic age acceleration measures and DNAm-derived telomere length. Models were adjusted for sex, years of education, depressive symptoms (GDS score), SPPB, and comorbidity burden.

Ethics

The study protocol was reviewed and approved by the Institutional Review Board of the Institute of Nutrition and Food Technology, Universidad de Chile. All participants provided written informed consent prior to enrollment.

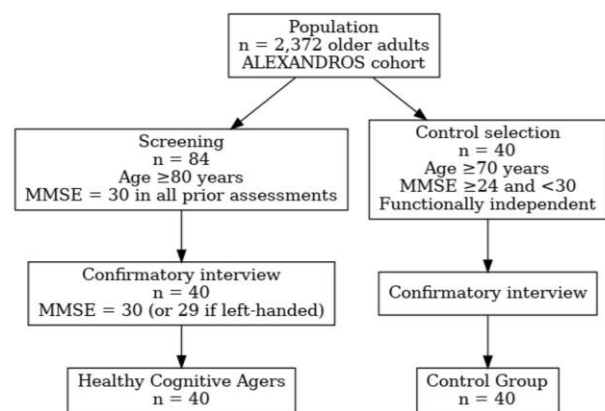


Figure 1. Flowchart of participant selection for the study.

This figure illustrates the selection process for participants in the study from the Alexandros Cohort. Out of 2,372 older adults, 84 individuals aged 80 years or older with a perfect MMSE score of 30 in all assessments were initially screened. Of these, 40 participants who maintained an MMSE score of 30 during the interview were included in the Healthy Cognitive Ager Group. A control group of 40 participants was also selected, characterized by an MMSE of ≥ 24 and < 30 .

RESULTS

Out of $n = 2,372$ subjects in the ALEXANDROS cohort, 84 individuals aged over 80 years who met the inclusion criterion of maintaining an MMSE score of 30 in all

previous assessments were identified and invited to participate. Among these, 40 maintained an MMSE score of 30 at the confirmatory interview and agreed to participate in the study. A 40 subjects control group was conceived to allow comparison between HCA and community-dwelling older adults without evidence of cognitive impairment, while also enabling comparisons with chronologically younger individuals (Fig. 1). The average age and MMSE score in the control group was 78.44 ± 4.7 and 28.5 ± 2.9 respectively, which is consistent with expected values in population-based samples of older adults. MoCA scores were also higher in Healthy Cognitive Agers compared with controls (mean $\pm$ SD: 24 ± 3.5 vs 15.6 ± 5.4 ; $p < 0.01$) (Table 1), supporting

the presence of consistently better global cognitive performance in the HCA group.

The study sample was predominantly female (57.5%), with a mean age of 80.44 ± 4.42 years and a mean educational attainment of 9.68 ± 3.83 years. There were no significant differences between HCA and controls with respect to sex distribution, education, depression, comorbidities or nutritional status. The prevalence of depressive symptoms (14%) and multimorbidity, defined as the presence of three or more chronic conditions (30.1%), did not differ between groups. The epidemiological characteristics of the study participants are summarized in (Table 1).

Table 1. Demographic, Clinical, Cognitive, Physical performance and frailty characteristics of the study population.

	Healthy Cognitive Agers (n=40)	Control (n=40)	Total (n=80)	p
Gender				
Female	24 (60)	22 (55)	46 (57.5)	0.65
Male	16 (40)	18 (45)	34 (42.5)	
Age				
Average $\pm$ SD	82.4 ± 3.05	78.44 ± 4.7	80.44 ± 4.42	0.0001
CI 95%	81.4 – 83.42	76.93 – 79.94	79.46 – 81.43	
BMI (kg/m²)				
Average $\pm$ SD	28.54 ± 4.39	28.83 ± 5.0	28.68 ± 4.68	0.78
Nutritional State (BMI)				0.9
20-24.9 kg/m ²	8 (20)	9 (22.5)	17 (21.25)	
25 – 29.9 kg/m ²	18 (45)	16 (40)	34 (42.5)	
≥ 30 kg/m ²	14 (35)	15 (37.5)	29 (36.25)	
Education (y)				
Mean $\pm$ DS	9.62 ± 4.01	9.76 ± 3.64	9.68 ± 3.83	0.88
CI95%	8.28 – 10.96	8.29 – 11.24	8.71 – 10.64	
Depression (GDS 15) n (%)	6 (15)	8 (20.51)	14 (17.72)	0.52
MMSE	30	28.5 ± 2.4	29.2 ± 1.8	0.0003
Mean $\pm$ SD				
MoCA	24 ± 3.5	15.6 ± 5.4	19.8 ± 6.2	0.01
Mean $\pm$ SD				
Comorbidities (number)	2.2 ± 1.04	2.3 ± 1.55	2.25 ± 1.31	0.73
Mean $\pm$ SD	2 (1 - 3)	2 (1-4)	2 (0.5 – 4)	
Multimorbidity				
≥ 3 Diseases	13 (32.5)	17 (42.5)	30 (37.5)	0.35
Cancer	9 (22.5)	10 (25)	19 (23.75)	0.79
SPPB mean $\pm$ SD	11.12 ± 1.66	9.55 ± 2.91	10.35 ± 2.47	0.004
Sarcopenia % (n)	5 (2)	22.5 (9)	13.92 (11)	0.02
Handgrip (kg) mean $\pm$ SD	26.25 ± 6.9	24.2 ± 11.14	25.2 ± 9.2	0.35
Male	32.3 ± 4.2	34.1 ± 7.5	33.4 ± 6.1	0.27
Female	21.3 ± 4	17.2 ± 6.8	19.1 ± 5.9	0.02
Walk speed mean $\pm$ SD	0.8 ± 0.15	0.81 ± 0.25	0.8 ± 0.2	0.83
5 CST mean $\pm$ SD	10.1 ± 3.3	12.41 ± 5.4	11.19 ± 4.5	0.03
Frailty (phenotype) % (n)	22.5 (9)	25 (10)	23.75 (19)	0.89

Comparison between healthy cognitive agers and control subjects across various parameters, including gender distribution, age, nutritional status, education level, depression (GDS 25), cognitive performance (Minimental and MoCA test), and multimorbidity. Data are presented as mean $\pm$ standard deviation (SD) or percentage (%), with 95% confidence intervals (CI) where applicable. Statistically significant differences between groups are indicated (* $p < 0.05$, ** $p < 0.01$). Between-group comparisons were conducted using Student's t-test for independent samples for continuous variables and Fisher's exact test for categorical variables.

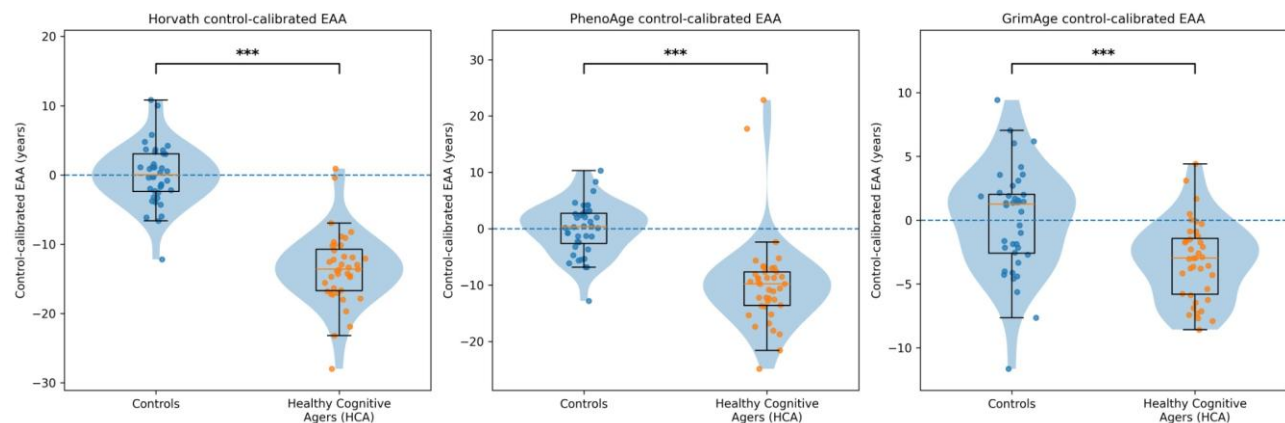


Figure 2. Distribution of control-calibrated epigenetic age acceleration (EAA) in Healthy Cognitive Agers and controls. Violin plots and individual participant values are shown for the Horvath, PhenoAge, and GrimAge epigenetic clocks. Control-calibrated EAA was calculated by fitting linear regression models of epigenetic age on chronological age exclusively in the control group and applying the resulting coefficients to the entire study population. Healthy Cognitive Agers ($n = 40$) exhibited significantly lower control-calibrated EAA than controls ($n = 40$) across all three epigenetic clocks, including Horvath ($t = -12.54$, $p < 0.001$), PhenoAge ($t = -6.45$, $p < 0.001$), and GrimAge ($t = -3.88$, $p < 0.001$). Between-group comparisons were performed using Welch's t -test. P-values are two-sided and unadjusted. Dashed horizontal lines indicate zero control-calibrated EAA, corresponding to the expected epigenetic age for a given chronological age based on the control-derived reference model. Asterisks indicate statistical significance (***) $p < 0.001$.

Epigenetic Age Acceleration

HCA showed lower epigenetic age across all three clocks compared with controls (Fig. 2). For the Horvath DNAmAge clock, HCA exhibited a mean age acceleration of -13.55 ± 5.28 years, whereas controls showed a positive acceleration of 0.00 ± 4.34 years. The between-group difference was significant ($t = -12.54$, $p < 0.001$), with a large effect size (Cohen's $d = -2.80$) (Table 2).

A similar pattern was observed with the GrimAge clock (Fig. 2, Table 2). HCA demonstrated a mean acceleration of -3.16 ± 3.08 years, whereas controls

showed substantial age acceleration (0.00 ± 4.14 years; $t = -3.88$, $p = 0.001$; Cohen's $d = -0.87$). Although the magnitude of the group difference was smaller than that observed for Horvath DNAmAge, the direction was consistent and the effect size remained large.

Using the PhenoAge clock, HCA again presented substantially lower biological age than expected for their chronological age (Fig. 2, Table 2). Their mean acceleration was -9.72 ± 8.37 years, compared with 0.00 ± 4.56 years in controls ($t = -6.45$, $p < 0.001$; Cohen's $d = -1.44$).

Table 2. Comparison of control-calibrated epigenetic age acceleration (EAA) and DNAm-derived telomere length between Healthy Cognitive Agers and controls.

Clock	Healthy Cognitive Agers (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	t (Welch)	p -value	Cohen's d
Horvath control-calibrated EAA	-13.55 ± 5.28	0.00 ± 4.34	-12.54	<0.001	-2.80
PhenoAge control-calibrated EAA	-9.72 ± 8.37	0.00 ± 4.56	-6.45	<0.001	-1.44
GrimAge control-calibrated EAA	-3.16 ± 3.08	0.00 ± 4.14	-3.88	<0.001	-0.87
DNAmTL (kb)	6.39 ± 0.27	5.39 ± 0.21	18.56	<0.001	4.15

Values are presented as mean $\pm$ standard deviation. Control-calibrated EAA was calculated by fitting linear regression models of epigenetic age on chronological age in the control group and applying the resulting coefficients to the entire study population. DNAm-derived telomere length (DNAmTL) is expressed in kilobases, with higher values indicating longer telomeres. Between-group comparisons were performed using Welch's t -test. P-values are two-sided and unadjusted. Effect sizes are reported as Cohen's d . Negative Cohen's d values indicate lower control-calibrated EAA in Healthy Cognitive Agers, whereas positive Cohen's d values indicate longer DNAmTL.

We also evaluated biological aging using the epigenetic age gap (epigenetic age minus chronological age). Consistent with the residual-based EAA analyses, significant differences between Healthy Cognitive Agers and controls were observed across the three epigenetic

clocks. These results are presented in Supplementary Table 1 and Supplementary Figure 1.

In multivariable linear regression models adjusting for sex, education, depressive symptoms, SPPB, and multimorbidity, HCA status remained significantly

associated with lower epigenetic age acceleration for the Horvath and PhenoAge clocks. No significant association

was observed for GrimAge after multivariable adjustment (Table 3).

Table 3. Multivariable linear regression models of epigenetic age acceleration with HCA status (HCA vs control) as the main predictor.

Adjusted Variable	Beta (95% CI)	p-value
Horvath EAA		
HCA vs control	-6.89 (-13.20 to -0.58)	0.034
Female sex	0.59 (-5.45 to 6.63)	0.844
Education ≤ 8 years	-2.26 (-8.46 to 3.94)	0.462
Multimorbidity ≥ 3	-1.46 (-7.64 to 4.71)	0.631
Depressive symptoms	-0.18 (-7.80 to 7.45)	0.963
SPPB total score	-1.30 (-3.34 to 0.74)	0.204
PhenoAge EAA		
HCA vs control	-10.38 (-19.72 to -1.04)	0.031
Female sex	-1.92 (-10.85 to 7.01)	0.664
Education ≤ 8 years	-6.37 (-15.54 to 2.80)	0.166
Multimorbidity ≥ 3	-3.16 (-12.29 to 5.98)	0.485
Depressive symptoms	0.61 (-10.66 to 11.88)	0.912
SPPB total score	-2.98 (-6.00 to 0.04)	0.053
GrimAge EAA		
HCA vs control	3.36 (-9.67 to 16.39)	0.602
Female sex	6.50 (-5.96 to 18.97)	0.295
Education ≤ 8 years	6.08 (-6.72 to 18.88)	0.339
Multimorbidity ≥ 3	5.94 (-6.81 to 18.69)	0.349
Depressive symptoms	-6.73 (-22.46 to 9.01)	0.389
SPPB total score	0.21 (-4.00 to 4.42)	0.920

Values are β coefficients (95% confidence intervals) derived from multivariable linear regression models. All models included HCA status (HCA vs control) as the primary independent variable and were adjusted for sex, education (≤ 8 years), depressive symptoms, multimorbidity (≥ 3 chronic conditions), and physical performance (Short Physical Performance Battery [SPPB] total score). Negative β coefficients indicate lower epigenetic age acceleration. Abbreviations: HCA, Healthy Cognitive Agers; EAA, Epigenetic Age Acceleration.

DNA Methylation-Derived Telomere Length (DNAmTL)

DNAmTL values also differed significantly between groups (Table 2). HCA exhibited longer telomeres (6.39 ± 0.27 kb) than controls (5.39 ± 0.21 kb), corresponding to the largest effect size observed among all biomarkers ($t = 18.56$, $p = 1.6 \times 10^{-29}$; Cohen's $d = 4.15$). This finding is consistent with a globally younger biological profile in the HCA group.

Age-equivalence and sensitivity analyses

Chronological age differed between groups by design, with HCA being older than controls. To evaluate whether this difference accounted for the observed associations with biological aging, we performed complementary age-equivalence and sensitivity analyses (Supplementary Table 2). In age-equivalence analyses, HCA (mean chronological age 82.4 ± 3.05 years) exhibited substantially younger biological ages compared with chronologically younger controls (Fig. 3). For the Horvath clock, HCA showed a mean biological age of 68.9 ± 6.0 years, compared with 77.9 ± 5.4 years in

controls approximately 5 years younger and 79.7 ± 6.2 years in controls approximately 10 years younger. Similarly, PhenoAge estimates were lower in HCA (66.8 ± 8.6 years) than in younger control groups (73.5 ± 5.9 and 75.6 ± 6.4 years, respectively). For GrimAge, biological age in Healthy Cognitive Agers (81.4 ± 4.3 years) was comparable to that observed in controls approximately 10 years younger (81.2 ± 5.1 years) (Fig. 3).

As a sensitivity analysis, between-group comparisons were repeated restricting the control group to participants aged ≥ 80 years (Supplementary Table 2). Under this age-restricted comparison, differences remained statistically significant for Horvath age acceleration (-2.34 ± 5.73 vs 11.71 ± 4.91 years), PhenoAge acceleration (-1.56 ± 8.52 vs 7.79 ± 3.53 years), and DNAm-derived telomere length (6.39 ± 0.27 vs 5.41 ± 0.18 kb), with effect sizes comparable to the primary analysis. In contrast, GrimAge acceleration differences were attenuated and no longer statistically significant. Shapiro-Wilk tests indicated that most biological aging variables were normally distributed; however, PhenoAge showed evidence of non-normality in the HCA group. Non-parametric sensitivity

analyses using the Mann–Whitney U test yielded results consistent with the primary Welch's t-test analyses.

Together, these analyses indicate that the younger biological aging profile observed in HCA is not solely

explained by differences in chronological age but is consistent with a distinct pattern of biological aging associated with preserved cognitive function into advanced age.

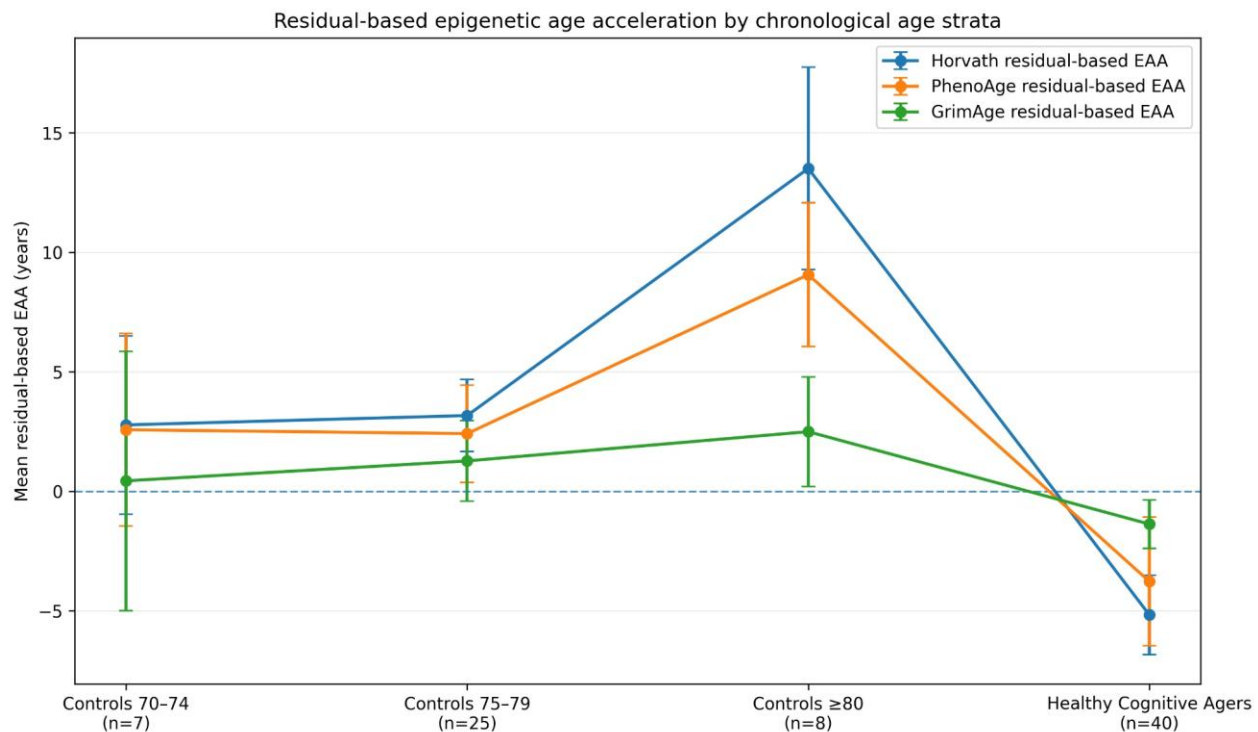


Figure 3. Age-equivalence analysis of biological aging in Healthy Cognitive Agers and chronologically younger controls. Mean biological age estimated using the Horvath, PhenoAge, and GrimAge epigenetic clocks is shown for Healthy Cognitive Agers and for control participants stratified by chronological age (70–74 years, $n=7$; 75–79 years, $n=25$; and ≥ 80 years, $n=8$). This complementary analysis was performed to contextualize the magnitude of biological aging differences observed between groups. Despite being chronologically older (82.4 ± 3.0 years), Healthy Cognitive Agers exhibited biological ages comparable to, or lower than, those observed in chronologically younger control strata, particularly for the Horvath and PhenoAge clocks. Data are presented as mean $\pm$ 95% confidence intervals. Between-group comparisons across strata were performed using Welch's t-test. P-values are two-sided and unadjusted.

Phenotypical Characterization of study participants

Considering that participants were selected based on their cognitive performance, we examined whether preserved cognition was associated with differences in physical performance and muscle status. As shown in Table 1, the HCA exhibited significantly higher scores on the Short Physical Performance Battery (SPPB) compared with the control group (11.12 ± 1.66 vs. 9.55 ± 2.91 , $p = 0.0044$). The prevalence of sarcopenia, defined according to EWGSOP2 criteria, was markedly lower among HCA (5.0%) than in controls (24.2%, $p = 0.02$). In contrast, there were no significant differences between groups in lower limb muscle mass assessed by DXA (12.01 ± 2.51 vs. 12.94 ± 3.60 kg, $p = 0.18$). Similarly, no significant differences were observed in the prevalence of frailty, as determined by the Fried phenotype. These findings suggest that the superior physical performance observed

in HCA may not be explained by greater muscle mass but may instead reflect more efficient motor control or neuromuscular function.

DISCUSSION

In this study, we characterized in a group of community-dwelling older adults who have maintained cognitive performance well into advanced age. These individuals, referred to as Healthy Cognitive Agers, represent a highly selected subgroup characterized by exceptional cognitive preservation based on longitudinal MMSE performance, also exhibited preserved physical function and a substantially younger biological profile. To our knowledge, this is the first study to evaluate epigenetic age in older adults with preserved healthy cognition in a Latin American population.

DNA methylation-based ‘epigenetic clocks’ provide molecular estimates of biological aging that complement clinical phenotyping, but clocks differ in training targets and may index partially distinct aging domains [14, 15, 27, 28]. In our study, biological age assessed by the Horvath clock was, on average, approximately 10 years lower than chronological age among HCA, whereas controls showed only a modest difference of about 2 years. The smaller and less robust differences observed with GrimAge may reflect limited power due to sample size and/or differences in what these clocks capture (e.g., chronological-age prediction vs morbidity/mortality-related signatures), rather than a definitive lack of biological aging differences. These findings align with prior research indicating that slower epigenetic aging correlates with preserved cognition and functional capacity in older adults [13, 14, 16–18, 27, 29]. Nevertheless, given the cross-sectional nature of our analysis and the limited sample size, these results should be interpreted with caution.

The magnitude of the observed effect sizes warrants careful consideration. While large standardized differences are less commonly reported in observational aging studies, several factors may contribute to this finding. First, the comparison of a highly selected phenotype of exceptional cognitive preservation with a community-based reference group increases biological contrast. Second, DNA methylation-based biomarkers represent integrative measures of systemic aging processes and may be particularly sensitive to cumulative biological differences. Consistent with this interpretation, previous studies examining strong exposures or extreme phenotypes have reported similarly large effect sizes in epigenetic aging measures. Importantly, the observed differences in biological aging remained robust after adjustment for key demographic and clinical variables, supporting the independence of the association between exceptional cognitive preservation and biological aging measures.

The association between high cognitive and physical performance observed in this study supports the concept of shared mechanisms underlying cognitive and motor resilience. Because sarcopenia and frailty did not differ between groups, superior physical performance in HCA could plausibly relate to central motor control, sensorimotor integration, or other neural factors; however, this remains speculative in the absence of neuroimaging or detailed gait/dual-task measures. Consistent with this cognitive–motor coupling, slower gait speed and related constructs has shown predict subsequent cognitive impairment in older adults [30]. This interpretation aligns with the growing recognition of motor function as an integrative marker of brain health in aging [31].

A noteworthy observation is that despite similar comorbidity burden across groups, individuals with HCA maintained superior cognitive and physical performance. This finding supports the World Health Organization’s definition of healthy aging [32] as a state determined not by the absence of disease but by the preservation of functional ability and resilience [33]. The lower biological age observed in healthy cognitive agers could reflect molecular mechanisms contributing to this resilience [34, 35]. However, given the observational design, we cannot infer causality between slower biological aging and preserved cognition. Longitudinal analyses will be necessary to determine whether decelerated epigenetic aging precedes or results from the maintenance of cognitive function.

The control group in this study was intentionally defined to reflect the heterogeneity of cognitive performance observed in community-dwelling older adults. While this may introduce variability and potentially attenuate or amplify group differences, it also enhances the ecological validity of the comparison. Importantly, the consistency of findings across age-equivalence and sensitivity analyses suggests that the observed differences in biological aging are not solely attributable to chronological age or group definition, but rather reflect a distinct biological profile associated with exceptional cognitive preservation.

Strengths and Limitations: This study has several strengths. Participants were selected from a well-characterized, population-based cohort (ALEXANDROS), reducing selection bias and increasing generalizability. The comprehensive phenotyping—including cognitive, physical, and molecular measures—provides an integrated perspective of healthy cognitive aging. Additionally, the use of high-sensitivity epigenetic assays ensures analytical robustness. However, some limitations must be acknowledged. A limitation of this study is the use of persistent MMSE = 30 as the defining criterion for the HCA group. While this approach provides a stringent and reproducible method to identify individuals with consistently optimal global cognitive performance, the MMSE is a screening tool with known ceiling effects and limited sensitivity to subtle deficits across cognitive domains. Therefore, this operational definition likely identifies a subgroup with cognitive preservation rather than fully capturing the broader construct of healthy cognitive aging. MoCA results provided additional support for the characterization of the HCA group, showing consistently higher global cognitive performance compared with controls; however, these findings should be interpreted with caution, as they may be influenced by the educational level of the cohort participants. However, both MMSE and MoCA are screening tools and do not capture domain-specific

cognitive functions in detail. Therefore, while these findings reinforce the identification of individuals with exceptional cognitive preservation, they should not be interpreted as a comprehensive assessment of cognitive health. The sample size was modest, limited by the rarity of individuals maintaining exceptional cognition beyond 80 years. Given the cross-sectional design, the directionality of the association between cognitive preservation and biological aging cannot be determined. Future work could incorporate pace-of-aging measures such as DunedinPACE, which was developed to quantify the rate of biological aging and has shown associations with morbidity/disability and cognitive impairment/dementia outcomes in some cohorts [36, 37]. Moreover, while we employed two validated epigenetic clocks, additional measures (e.g., DunedinPACE) might provide complementary insights. An additional limitation is the bioinformatic adjustment for cell-type composition in DNA methylation analyses. Although the use of PBMCs reduces variability associated with major leukocyte subtypes, residual heterogeneity within mononuclear cell populations may influence methylation patterns. Therefore, we cannot exclude the possibility that part of the observed differences in epigenetic aging reflects variation in cellular composition. However, the consistency of results across multiple epigenetic clocks and DNAm-derived telomere length, as well as across sensitivity analyses, suggests that the main findings are unlikely to be fully explained by this factor. Finally, as the cohort consists predominantly of Chilean older adults, findings may not be directly generalizable to other populations with different genetic or environmental backgrounds.

Conclusion

In summary, our findings indicate that exceptional cognitive preservation in late life, based on persistent MMSE performance, is associated with a decelerated biological aging profile in community-dwelling older adults. These differences persisted across age-equivalence and sensitivity analyses, indicating that they are not solely explained by chronological age. Our findings provide molecular-level evidence that healthy cognitive aging is associated with a distinct biological aging trajectory in late life. Given the cross-sectional design, these findings should be interpreted as associative rather than causal. Further longitudinal studies are needed to determine whether biological aging trajectories influence cognitive outcomes over time.

Competing Interests

All the authors declare that they have no financial or non-financial interests, either directly or indirectly, related to the work submitted for publication.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Felipe Salech, Carlos Marquez, Daniela P Ponce, Carol San Martin, Paulina Correa, María Isabel Behrens, Cecilia Albala. The first draft of the manuscript was written by Felipe Salech and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data/code availability statement

De-identified participant data, including methylation-derived outputs, calculated epigenetic clock estimates (Horvath and DNAmGrimAge), and summary data, will be made available upon reasonable request from the corresponding author at the time of publication. Custom analysis scripts used in this study will also be provided upon request. To protect participant confidentiality and adhere to institutional review board guidelines, data and code will be shared exclusively with qualified researchers for scientific research purposes. Access requires the submission and approval of a formal research proposal, alongside a signed data access agreement.

Declaration of AI Use

The authors (FS) used ChatGPT (OpenAI) and Nature Research Review to support technical tasks during manuscript preparation, including statistical calculations from provided datasets, graphical output generation, English translation, and language editing. These tools did not contribute to the conception of the study, interpretation of findings, or drafting of any sections requiring expert judgment. All intellectual content and conclusions were developed and verified by the authors, in accordance with COPE recommendations regarding the use of AI tools.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2026.0311.

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