OXFORD

Precision Clinical Medicine, 2024, 7: pbae019

DOI: 10.1093/pcmedi/pbae019

Research Article

Phenotypic age mediates effects of Life's Essential 8 on reduced mortality risk in US adults

Yuxuan Zhao [1,†] Haiming Yang,[1,†] Rong Jiao,[1] Yueqing Wang,[1] Meng Xiao,[1] Mingyu Song,[1] Huan Yu,[1] Chunxiao Liao,[1] Yuanjie Pang [1] Wenjing Gao,[1] Tao Huang,[1] Canqing Yu [1,2,3] Jun Lv [1,2,3] Shengxu Li,[4] Lu Qi [5,6,7] Liming Li,[1,2,3] Dianjianyi Sun[1,2,3,*]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, Beijing 100191, China
[2]Peking University Center for Public Health and Epidemic Preparedness & Response, Beijing 100191, China
[3]Key Laboratory of Epidemiology of Major Diseases (Peking University), Ministry of Education, Beijing 100191, China
[4]Children's Minnesota Research Institute, Children's Minnesota, Minneapolis, MN 55404, USA
[5]Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70118, USA
[6]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
[7]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
*Corresponding author: Dianjianyi Sun, E-mail: dsun1@bjmu.edu.cn
[†]These authors contributed equally to this work.

Abstract

Objective: This study aimed to find out whether phenotypic age could mediate the protective effects of a healthy lifestyle on mortality.

Methods: We included adult participants with available data for individual phenotypic age (PhenoAge) and Life's Essential 8 (LE8) scores from the National Health and Nutrition Examination Survey 2005–2010 (three cycles) and linked mortality records until 31 December 2019. Adjusted hazard ratios (HR) were estimated to evaluate the associations of PhenoAge and LE8 scores with all-cause and cardiovascular mortality risk. Mediation analyses were performed to estimate the proportional contribution of PhenoAge to the effect of LE8 on mortality risks.

Results: A 1-year increment in PhenoAge was associated with a higher risk of all-cause (HR = 1.04 [95% confidence interval, 1.04–1.05]) and cardiovascular (HR = 1.04 [95% confidence interval, 1.04–1.05]) mortality, independent of chronological age, demographic characteristics, and disease history. High level of LE8 (score: 80–100) was associated with a 3.30-year younger PhenoAge. PhenoAge was estimated to mediate 36 and 22% of the effect of LE8 on all-cause and cardiovascular mortality, respectively (all $P < 0.001$). As for single-metric scores of LE8, PhenoAge mediated 30%, 11%, 9%, and 7% of the effects of the healthy diet, smoking status, blood pressure, and physical activity on all-cause mortality risk, respectively (all $P < 0.05$).

Conclusion: Adherence to LE8 recommendations slows phenotypic aging. PhenoAge could mediate the effect of LE8 on mortality risk.

Keywords: phenotypic age; Life's Essential 8; all-cause mortality; cardiovascular mortality

Introduction

Aging is one of the major risk factors for a variety of chronic diseases and death [1]. However, chronological age does not account for the individual differences in the underlying process of biological aging [2]. Phenotypic age (PhenoAge), a composite score of routine clinical markers, has been newly developed to differentiate individuals with increased or decreased risk for morbidity and mortality among people within the same chronological age group [3].

Emerging evidence suggests that various modifiable lifestyle factors, such as diet [4–6], physical activity [7, 8], smoking [9], drinking [10, 11], obesity [12, 13], and sleep [14, 15] are associated with accelerated or decelerated biological aging measured by PhenoAge or other indexes. Life's Essential 8 (LE8), recommended by the American Heart Association (AHA), is a set of eight health behaviors that people can improve through lifestyle changes to achieve ideal cardiovascular health (CVH) [16, 17]. Meeting more LE8 metrics has been associated with lower risks of all-cause and cause-specific morbidity and mortality [18–22]. Adherence to LE8 among asymptomatic adults is vital for achieving "longer and healthier lives for all" outlined by AHA 2030 impact goals [23]. However, whether PhenoAge can serve as an effective indicator of biological aging mediating the effect of LE8 on reduced mortality risk is largely unknown.

In the current study, we used data from three cycles (2005–2010) of the National Health and Nutrition Examination Survey (NHANES) with linked death records till 31 December 2019, to investigate the relations between PhenoAge and LE8, the prospective associations between PhenoAge, LE8, and mortality risks, and whether PhenoAge mediated effects of LE8 and its single components on all-cause and cause-specific mortality.

Methods

Study population

The NHANES is a nationally representative cross-sectional survey of the civilian non-institutionalized US population. All participants provided written informed consent and the protocols were

Received 15 July 2024; accepted 28 August 2024. published 16 September 2024

approved by the research ethics boards of the National Center for Health Statistics. Overall, the continuous NHANES surveys since 1999 used complex multi-stage probability sampling methods to enroll ~5000 people for each 2-year cycle. Data was acquired by at-home interviews and health examinations conducted in mobile examination centers (MECs) and was made publicly available for analysis (https://www.cdc.gov/nchs/nhanes/index.htm) [24].

There were 17 132 participants aged ≥20 years from three cycles of NHANES IV from 2005 to 2010. Participants with ineligible follow-up data, invalid MEC follow-up time, and insufficient data for calculating PhenoAge or LE8 were excluded. Detailed inclusion criteria and baseline characteristics of two sub-populations are listed in supplementary Fig. 1 and supplementary Tables 2–3, see supplementary online material.

PhenoAge calculation

PhenoAge was calculated using the method described elsewhere [25]. In general, PhenoAge is a composite measure (in units of years) calculated with a linear combination of 10 variables, including chronological age and nine routine clinical biomarkers [serum albumin, creatinine, glucose, C-reactive protein (CRP), lymphocyte %, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count]. It was developed to reflect one's mortality risk compared with others of the same chronological age. Phenotypic age acceleration (PhenoAgeAccel) was also computed accordingly and defined as the residual when regressing PhenoAge on chronological age in a linear model. It signifies the difference between a person's chronological age and PhenoAge, which reflects relative health status and disease susceptibility compared with others of the same chronological age. A positive value of PhenoAgeAccel suggests older biological age than chronological age and vice versa.

LE8

The AHA LE8 was adapted to ascertain CVH. The LE8 includes four modifiable CVH behaviors (not smoking, eating healthily, being physically active, and healthy sleeping) and four CVH factors [body mass index (BMI), blood pressure, blood non-high-density lipoprotein (non-HDL) cholesterol, and blood glucose] [17]. An ordinal point-scoring system (ranging from 0 to 100) was applied to each metric (supplementary Table 1, see supplementary online material). The unweighted average of the eight metric scores was calculated to scale the overall CVH, yielding a summation LE8 score ranging from 0 to 100 for each individual. CVH level was categorized based on the LE8 score as high (80–100), moderate (50–79), and low (0–49). Information on smoking status, physical activity levels, diet and sleep duration were acquired through interviewer-administered questionnaires. Smoking status was scored according to current cigarette use and the time since quitting. One's physical activity level was assessed by the intensity and frequency of leisure-time or recreational physical activity during the past 7 or 30 days. The original definition of a healthy diet is meeting no less than four out of five components of the healthy dietary pattern defined by the AHA [16]. The Healthy Eating Index (HEI) [26] was used to define the dietary metric of LE8. The HEI is a more comprehensive measurement designed to evaluate how well a person's diet aligns with the dietary recommendations from the Dietary Guidelines for Americans [26]. Sleep health was scored by the average hours of sleep per night. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Blood pressure data was acquired during physical examination in the MECs. The blood pressure used in the current analysis was the mean of all readings available. Blood glucose and non-HDL cholesterol levels were measured using participants' blood specimens collected in the MECs.

Asertainment of mortality

All-cause mortality and cause-specific mortality data were acquired from the open-source linkage data from the National Death Index; data can be accessed at https://www.cdc.gov/nchs/data-linkage/mortality-public.htm. Participants aged ≥18 years with sufficient identifying data were eligible for follow-up, from the date of the interview to 31 December 2019. The leading cause of death was provided based on the International Statistical Classification of Diseases 10th revision code included in the restricted-use linkage data. Cardiovascular mortality was defined as any death caused by heart diseases or cerebrovascular diseases.

Covariates

The demographic characteristics included sex, ethnicity, marital status, annual family income, and educational attainment. Participants who reported physician-diagnosed congestive heart failure, coronary heart disease, angina/angina pectoris, heart attack, or stroke were classified as having cardiovascular disease (CVD). Chronic obstructive pulmonary disease (COPD) was defined as self-reported diagnosed emphysema or chronic bronchitis. Cancer was defined as self-reported diagnosed cancer or malignancy of any kind. Diabetes was defined both by self-reporting or Hemoglobin A1c (HbA1c) ≥ 6.5%. Hypertension [systolic pressure (SBP) ≥ 140 mmHg or diastolic pressure (DBP) ≥ 90 mmHg] and hyperlipidemia (total cholesterol ≥ 200mg/dl) were defined by self-report or examination and laboratory results. Missing covariates were coded as "not defined" to preserve the sample size.

Statistical methods

Baseline characteristics were compared across three different groups of PhenoAge (sex-specific bottom quintile vs. 2nd to 4th quintile vs. top quintile) and LE8 (high vs. moderate vs. low) statistical comparisons were performed with the χ^2 test for categorical variables and analysis of variance (ANOVA) for continuous variables (supplementary Tables 2–3).

We first estimated the effects of PhenoAge on mortality risk. Cox proportional hazard models were used to examine the associations of PhenoAge [both continuous and categorical variables, PhenoAgeAccel, and its ten biomarkers (values were standardized and centered)] and LE8-score [both continuous and categorical variables, and single-metric scores (continuous values were standardized and centered)] with all-cause and cardiovascular mortality risks. Crude mortality rates were calculated as events per 1000 person-years of follow-up time until 31 December 2019, or death, whichever occurred first. Adjusted hazard ratios (HRs) were estimated with adjustment for chronological age, sex, ethnicity, marital status, educational attainment, annual family income, research cycle, and disease histories of CVD, COPD, cancer, diabetes, hypertension, and hyperlipidemia.

Then, we evaluated the relationships between PhenoAge and LE8. The least-squared means of PhenoAgeAccel were estimated in three different LE8-score groups and single-metric-score groups (high vs. moderate vs. low), adjusted for demographic characteristics and histories of diseases. When estimating the least-squared means of each component of LE8, the other seven metrics were additionally adjusted. The adjusted means of PhenoAgeAccel were calculated using the R package 'lsmeans' [27].

Table 1. Adjusted HRs for all-cause and cardiovascular mortality by levels of PhenoAge.

	No. of cases (incidence rate per 1000 person years)	All-cause mortality		Cardiovascular mortality	
		aHR[c] (95% CI)	*P*	aHR for (95% CI)	*P*
PhenoAge					
Bottom quintile	19 (0.53)	0.33 (0.20–0.53)	0.990	0.66 (0.25–1.72)	0.393
2nd to 4th quintiles	767 (7.33)	1.00		1.00	
Top quintile	1736 (66.91)	1.95 (1.72–2.22)	<0.001	2.19 (1.71–2.79)	<0.001
1 point increment (continuous)[a]		1.04 (1.04–1.05)	<0.001	1.04 (1.04–1.05)	<0.001
PhenoAgeAccel:					
Bottom quintile	396 (11.75)	0.81 (0.72–0.91)	<0.001	0.77 (0.62–0.95)	0.014
2nd to 4th quintile	1205 (11.78)	1.00		1.00	
Top quintile	921 (29.88)	2.04 (1.86–2.24)	<0.001	1.92 (1.62–2.27)	<0.001
1 point increment (continuous)		1.04 (1.04–1.05)	<0.001	1.04 (1.04–1.05)	<0.001
Z-Score of biomarkers[b]					
Albumin (g/l)		0.74 (0.71–0.78)	<0.001	0.79 (0.72–0.85)	<0.001
Creatinine (μmol/l)		1.13 (1.10–1.16)	<0.001	1.13 (1.09–1.18)	<0.001
Glucose, serum (mmol/l)		1.10 (1.06–1.14)	<0.001	1.12 (1.05–1.20)	<0.001
Log C-reactive protein (mg/dl)		1.19 (1.14–1.24)	<0.001	1.16 (1.07–1.26)	<0.001
Lymphocyte percent (%)		0.83 (0.80–0.87)	<0.001	0.79 (0.73–0.85)	<0.001
Mean cell volume (fl)		1.13 (1.09–1.18)	<0.001	1.10 (1.02–1.19)	0.015
Red cell distribution width (%)		1.23 (1.20–1.27)	<0.001	1.22 (1.16–1.29)	<0.001
Alkaline phosphatase (U/l)		1.19 (1.16–1.22)	<0.001	1.17 (1.11–1.23)	<0.001
White blood cell count (1000/μl)		1.03 (1.01–1.06)	0.001	1.03 (0.98–1.08)	0.226
Chronological age (years)		1.08 (1.08–1.08)	<0.001	1.10 (1.09–1.11)	<0.001

All models were adjusted for chronological age, sex, ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, other Hispanic, and others), marital status (married/living with a partner, divorced/separated/widowed, never married), annual family income (<25 000 dollars/year, 25 000–75 000 dollars/year, >75 000 dollars/year), and educational attainment (less than high-school, high-school, Bachelor's degree or above), as well as disease histories including history of COPD, CVD, cancer, diabetes, hypertension, and hyperlipidemia.

[a]A 1 point increment equals 1 SD increment in the original value. [b]The biomarkers listed are those incorporated in the construction of PhenoAge and the values were standardized and centred before inclusion in Cox models. [c]aHR, adjusted hazard ratio.

Third, we assessed the associations of LE8 with mortality risk. Cox proportional hazard models were applied, and model 1 was adjusted for chronological age, sex, ethnicity, marital status, educational attainment, annual family income, research cycle, and disease histories of COPD and cancer. Besides, we introduced PhenoAge as an additional covariate in model 2. We did not include diabetes, hypertension, and hyperlipidemia as covariates since blood pressure, fasting glucose, and total cholesterol were included in the calculation of LE8. To test the potential ability of PhenoAge to discriminate high-risk individuals independent of established risk factors and LE8, we computed reclassification and discrimination statistics for different survival analyses models. C-Indexes, net reclassification improvement, and integrated discrimination improvement were calculated and compared with R packages 'survcomp' [28] and 'survIDINRI' [29].

Mediation analyses were performed to test whether the LE8 score and its components affect all-cause and cardiovascular mortality risks via PhenoAge and to quantify the mediated proportions [30]. Multivariate generalized linear regressions were fitted for the exposure–mediator models, while parametric survival regressions were fitted for the exposure–outcome and mediator–outcome models. The covariates included in both the linear regression models and parametric survival regression models were consistent with that included in the Cox proportional hazard models assessing the associations of LE8 with mortality. The proportions of the mediated effect were estimated using the R package 'mediation' [31].

We conducted several sensitivity analyses. Firstly, since blood glucose was a component of both PhenoAge and LE8, we excluded blood glucose from the calculation of LE8 in evaluating the associations of PhenoAge and LE8 and in the mediation analyses. Secondly, we replaced PhenoAge with PhenoAgeAccel in the mediation analyses.

All statistical analyses were performed with R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided *P* value < 0.05 was considered statistically significant.

Results

The baseline descriptive characteristics of study participants across PhenoAge quintiles are shown in supplementary Table 2 (mean age 49.5 years, 48.6% females, 49.1% White). During a median follow-up time of 11.17 years, 2522 death events were recorded among 15 320 adults. In Table 1, participants in the top quintile of PhenoAge had a 1.95- and 2.19-fold all-cause and cardiovascular mortality risk (all $P < 0.001$), respectively, compared with ones in the 2nd to 4th quintiles of PhenoAge after adjusting for chronological age, demographics, and disease histories. A 1-year increment in PhenoAge yielded a HR of 1.04 (95% confidence interval (CI), 1.04–1.05) for all-cause mortality and 1.04 (1.04–1.05) for cardiovascular mortality.

The baseline characteristics of sub-population 2 are shown in supplementary Table 3. Overall, 17.5%, 67.7%, and 14.8% of the 11 060 participants met the high, moderate, and low level of CVH at the time of examination, respectively. The increment in LE8 was associated with a significant reduction in PhenoAge after adjustment of chronological age, demographic characteristics, and histories of COPD and cancer (Fig. 1A). The adjusted means of PhenoAgeAccel for the high, moderate, and low levels of LE8-score were −3.30 years, −1.07 years, and 3.02 years, respectively (Fig. 1B, supplementary Table 4, see supplementary online material). High levels of all single-metric scores were associated with a reduced PhenoAge compared with low levels, except for the score of non-HDL cholesterol (Fig. 1B, supplementary Table 4).

During a median follow-up of 11.17 years, 1659 deaths were recorded. Table 2 shows the associations of LE8 with all-casue

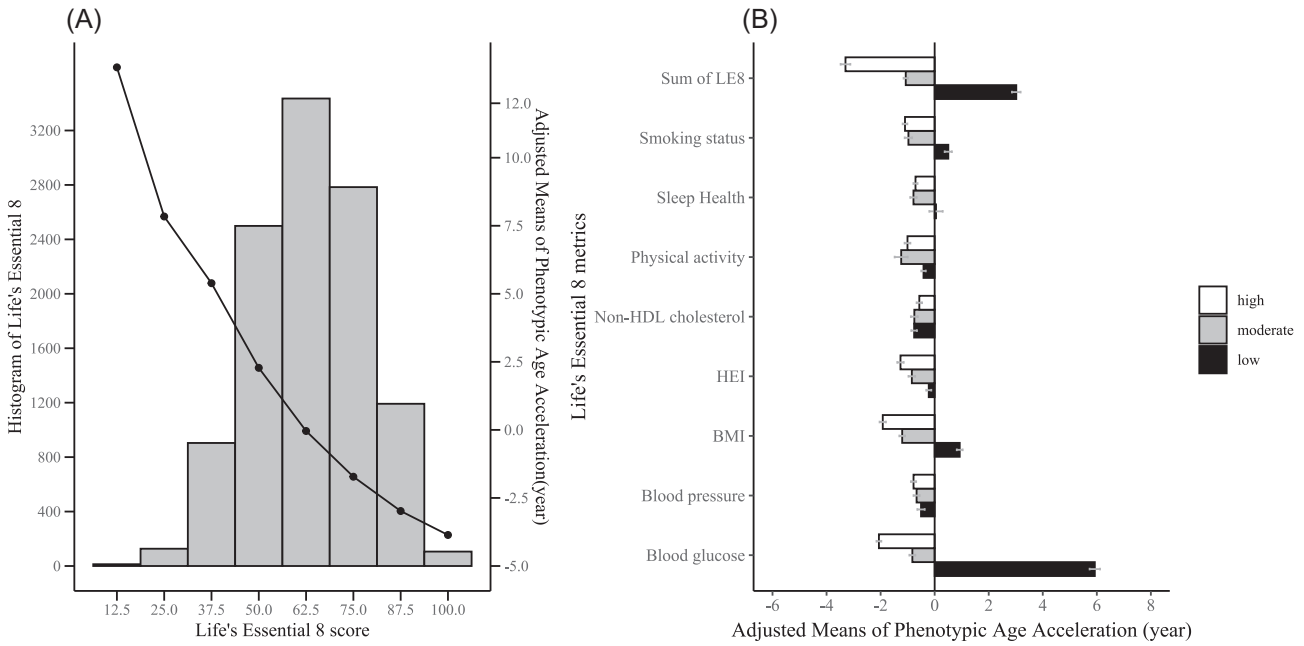

Figure 1. Adjusted means of PhenoAgeAceel by levels of LE8 and single components. Least-squared means of PhenoAgeAccel are presented for each possible score of LE8 (**A**) and levels of LE8 and its single components (**B**). In (A), the bars represent the frequency distribution of LE8 scores and the broken line represents the least-squared means of PhenoAgeAcc in eight LE8 groups (from 0 to 100 divided by 12.5). Means were adjusted for chronological age, sex, ethnicity, marital status, education attainment, annual family income, and histories of COPD, CVD, and cancer. In (B), white, gray, and black represent the least-squared means of PhenoAgeAcc in high, moderate, and low levels of LE8, respectively. Means were adjusted for sex, ethnicity, marital status, education attainment, annual family income, and histories of COPD, CVD, and cancer. For LE8 scores, 0–49 was categorized as low, 50–79 as moderate, and 80–100 as high. Error bars represent standard error. When estimating the adjusted means of each component of LE8, the other seven metrics were additionally adjusted. A positive value of PhenoAgeAccel indicates older PhenoAge compared with chronological age, and vice versa. Overall, participants with higher LE8 scores had lower PhenoAgeAccel.

Table 2. Adjusted HRs for all-cause and cardiovascular mortality by level of LE8.

	No. of cases (incidence rate per 1000 person years)	All-cause mortality		Cardiovascular mortality	
		Model 1 aHRs[a] (95% CI)	Model 2[b] aHRs (95% CI)	Model 1 aHRs (95% CI)	Model 2[b] aHRs (95% CI)
Sum of LE8					
High	105 (5.64)	0.77 (0.63–0.94)	0.77 (0.63–0.95)	0.64 (0.42–0.96)	0.66 (0.43–0.99)
Moderate	1094 (13.25)	1.00	1.00	1.00	1.00
Low	460 (22.81)	1.37 (1.22–1.53)	1.19 (1.06–1.33)	1.55 (1.27–1.90)	1.36 (1.11–1.67)
1-SD increment of LE8		0.79 (0.75–0.84)	0.92 (0.87–0.97)	0.71 (0.64–0.79)	0.76 (0.69–0.85)
Single-metric score (1-SD increment)[c]					
HEI		0.95 (0.90–1.00)	0.96 (0.91–1.01)	0.91 (0.83–0.99)	0.92 (0.83–1.00)
Blood glucose		0.88 (0.83–0.92)	0.98 (0.93–1.03)	0.88 (0.80–0.97)	0.98 (0.89–1.08)
BMI		1.08 (1.02–1.14)	1.13 (1.07–1.19)	0.98 (0.89–1.08)	1.02 (0.92–1.12)
Physical activity		0.82 (0.78–0.87)	0.83 (0.78–0.87)	0.85 (0.77–0.94)	0.86 (0.77–0.95)
Blood pressure		0.92 (0.87–0.97)	0.92 (0.87–0.97)	0.85 (0.78–0.94)	0.86 (0.78–0.94)
Smoking status		0.79 (0.75–0.84)	0.81 (0.77–0.86)	0.81 (0.72–0.91)	0.83 (0.74–0.93)
Total non-HDL cholesterol		1.11 (1.05–1.17)	1.09 (1.04–1.15)	1.00 (0.91–1.10)	0.99 (0.90–1.18)
Sleep health		0.98 (0.94–1.03)	0.98 (0.94–1.03)	1.00 (0.92–1.08)	0.99 (0.91–1.08)

All models were adjusted for chronological age, sex, ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, other Hispanic, and others), marital status (married/living with a partner, divorced/separated/widowed, never married), annual family income (<25 000/year, 25 000–75 000/year, >75 000/year), educational attainment (less than high-school, high-school, Bachelor's degree or above) and histories of COPD, CVD, and cancer. All metrics of LE8 were categorized into different levels and assigned a metric score respectively, yielding an unweighted average of summation LE8-score ranging from 0–100, which was also categorized into three levels (high: 80–100, intermediate: 50–79, poor: 0–49). When treated as a continuous variable, the sum of the LE8 score and scores of each metric were standardized and centered.
[a]aHR indicates adjusted hazard ratio. [b]In model 2, PhenoAge was additionally adjusted.
[c]For analyses on single metrics, scores of the other seven components were additionally adjusted.

mortality and cardiovascular mortality. A 1-SD increment of LE8-score was inversely associated with all-cause and cardiovascular mortality risks [HR (95%CI) = 0.79 (0.75–0.84) and 0.71 (0.64–0.79), respectively. Table 2, results for model 1]. Compared with participants who met the moderate level of LE8, those with low level had increased all-cause and cardiovascular mortality risks [HR (95%CI) = 1.37 (1.22–1.53) and 1.55 (1.27–1.90), respectively. Table 2, results for model 1]. As shown in suppelementary Fig. 2 (see supplementary material), the 5- and 10-year cumulative mortality rates from all-causes and CVD were both lower in

Table 3. Proportion of effect of LE8 on risk of all-cause and cardiovascular mortality mediated by PhenoAge.

Lifestyle metric	All-cause mortality		Cardiovascular mortality	
	Mediated % (95% CI)	*P*	Mediated % (95% CI)	*P*
Sum of LE8	36 (28–48)	<0.001	22 (15–34)	<0.001
Single-metric[a]				
HEI	30 (12–207)	0.028	14 (5–78)	0.038
Physical activity	7 (4–11)	<0.001	8 (4–19)	<0.001
Blood pressure	9 (2–24)	0.004	4 (1–12)	0.018
Smoking status	11 (8–16)	<0.001	11 (6–28)	<0.001

In mediation analyses, multivariate generalized linear regressions were fitted for the exposure–mediator models, while parametric survival regressions were fitted for the exposure–outcome and mediator–outcome models. Both models were adjusted for chronological age, demographic features including sex, ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, other Hispanic, and others), marital status (married/living with a partner, divorced/separated/widowed, never married), annual family income (<25 000/year, 25 000–75 000/year, >75 000/year), educational attainment (less than high-school, high-school, Bachelor's degree or above) and histories of COPD, CVD, and cancer. Only significant mediation results are shown in this table.
[a]For analyses on single metrics, the other seven metrics were additionally adjusted.

respondents who met a high level of LE8 than those who met moderate or low levels of LE8, indicating better survival for respondents who met a high level of LE8. A 1-SD increment of single-metric scores in smoking status, physical activity, diet, blood pressure, and blood glucose was also associated with lower all-cause mortality risk (Table 2, all $P < 0.05$). A 1-SD increment of single-metric scores in BMI and non-HDL cholesterol was positively associated with all-cause mortality risk (Table 2, all $P < 0.05$). Introducing PhenoAge as an additional variable in this model yielded a significant increase in C-index, indicating an added predictive power of PhenoAge independent of demographics and LE8 (supplementary Table 5, see supplementary online material).

After introducing PhenoAge as an additional covariate in assessing the associations of LE8 with mortality risks, the effects of sum-LE8 on mortality were attenuated and were closer to the null hypothesis (Table 2, results for model 2). Besides, the effects of PhenoAge on mortality remained significant. Therefore, we introduced mediation analyses to test whether PhanoAge could mediate the effects of LE8 on mortality.

In mediation analysis on the total LE8, PhenoAge was calculated to mediate 36% and 22% of the effects of LE8 on all-cause and cardiovascular mortality, respectively (both $P < 0.001$ in Table 3). In mediation analyses on single metrics of LE8, non-HDL cholesterol and BMI were excluded from mediation analyses due to their positive associations with mortality risks (Table 3). Blood glucose was not analyzed either, as it was one of the biomarkers used to construct PhenoAge. Among the remaining single-metric scores, PhenoAge mediated a considerable proportion of the effect of HEI score (30%, $P = 0.028$) on all-cause mortality risk, followed by smoking status score (11%, $P < 0.001$), blood pressure score (9%, $P = 0.004$) and physical activity score (7%, $P < 0.001$).

In sensitivity analysis 1, we first excluded blood glucose from the calculation of LE8 in analyses evaluating the associations of PhenoAge and LE8 and in the mediation analyses. The low level of LE8 (without blood glucose) was associated with slower PhenoAgeAccel compared with that before excluding blood glucose (1.72 for LE8 without blood glucose vs. 3.02 for LE8; see supplementary Table 6 in the supplementary online material). But the adjusted means of PhenoAgeAccel in high and moderate level of LE8 were consistent before and after excluding blood glucose. Besides, the increment of LE8 was still associated with a significant reduction of PhenoAge. In mediation analyses, the proportion of effects of LE8 (without blood glucose) on mortality mediated by PhenoAge was attenuated slightly (from 36% to 30% for all-cause mortality, and from 22% to 19% for CVD mortality; see supplementary Table 7 in the supplementary online material). In sensitivity analysis 2, we replaced PhenoAge with PhenoAgeAccel in the mediation analyses and the results were almost consistent with the main results (see supplementary Table 8 in the supplementary online material).

Discussion

In a nationally representative sample of US adults, we found that better adherence to a healthy lifestyle measured by LE8 is associated with a reduced PhenoAge, independent of chronological age and demographics. Our prospective analyses further validated the associations between PhenoAge, LE8-score, and their individual components, with all-cause mortality. Importantly, our study provides strong evidence that PhenoAge can mediate half the effect of LE8 on all-cause mortality and cardiovascular mortality.

Our study quantified the direct benefits of adherence to a healthy lifestyle and a high level of CVH, determined by eight metrics, on phenotypic aging. Our reuslts aligned with the inverse associations of LE8 with PhenoAge from a previous study [32]. But differently, through estimating the adjusted means of PhenoAgeAccel, our study provides straightforward information that a high level of CVH may slow PhenoAge by 3.30 years, independently of other factors. More importantly, our study demonstrated that adherence to each component of LE8 (except total cholesterol) could all exert reduction in PhenoAgeAccel to varying degrees independent of each other. The results also coincide with previous research demonstrating the contribution of behavioral factors to the variation of PhenoAge [33].

We confirmed that PhenoAge could predict all-cause and cardiovascular mortality, independent of chronological age, demographic characteristics, and medical history [3]. The significant increase in reclassification statistics further supported that PhenoAge is a functional and beneficial index for improving accuracy in risk stratification among mass population. Our findings are in line with previous studies observing similar associations of each biomarker included in PhenoAge with mortality risk [34–36].

Our analyses yielded similar results to previous studies showing that overall adherence to LE8, as well as smoking cessation, staying physically active, keeping a healthy diet, and maintaining an optimal blood pressure and glucose level were associated with a lowered risk for all-cause and cardiovascular

mortality [37–44]. Unexpectedly, our study found that increments in BMI and non-HDL cholesterol scores were positively associated with mortality from all causes. Several studies have reported inconsistent associations of BMI and non-HDL cholesterol with mortality from all causes, but none of them reported protective effects of high scores of BMI or non-HDL cholesterol [44–46]. Some of the studies have attributed the results partly to the U-shape associations of non-HDL cholesterol with all-cause mortality and CVD mortality among US adults [44, 47]. To further elucidate the results we also applied restricted cubic splines methods to our population and found significant nonlinear relationships of both BMI and non-HDL cholesterol with all-cause mortality (supplementary Fig. 2). The mechanisms underlying the associations have not been fully discussed, although the results should bring attention to re-considering the scoring system of LE8 for the two metrics because of the nonlinear associations. In addition, the associations of sleep duration with all-cause and CVD mortality were insignificant in our study. However, previous studies based on more recent NHANES cycles identified small but significant effects of sleep duration on all-cause mortality [44, 46], suggesting that it is still important to add sleep duration into LE8.

Noteably, our study introduced mediation analyses to examine the proportional contribution of PhenoAge to the effect of lifestyle on mortality risk. We found that PhenoAge mediated almost 51% of the effect of LE8 on all-cause mortality and half of the effect on cardiovascular mortality. Further, we estimated PhenoAge's mediation effect on mortality risk through the individual components of LE8. PhenoAge was originally trained as a predictor for mortality risk [25], without considering biological mechanisms. The results of our mediation analyses support that, although aging is a complex process with numerous potential mechanisms involved, PhenoAge is an efficacious composite measure that captures and quantifies the long-term effect of lifestyle on the aging process. Besides, previous studies have indicated the relationship among lifestyle factors, individual biomarkers included in PhenoAge, and the aging process. For instance, cigarette smoking has been shown to be strongly and inversely associated with low albumin levels [48], indicating malnutrition and accelerated aging [49, 50]. High C-reactive protein levels, a reflection of chronic inflammation and aging, can be lowered by lifestyle interventions including weight control and physical activities [51, 52]. Higher levels of mean cell volume and red blood cell distribution width were associated with older age, probably due to slowed hemolysis rate and age-related anemia [53, 54].

Given that PhenoAge may be an independent predictor of mortality, and a mediator between adherence to a healthy lifestyle and long-term health outcomes, our study might have important clinical relevance for public health. First, PhenoAge can be used as an indicator to help promote the willingness to adhere to a better lifestyle at the individual level. Previous lifestyle-intervention RCTs using single-metric outcome measures have demonstrated declines in compliance towards the end of the trials [55, 56], indicating a possible inadequacy of motivation among participants. PhenoAge quantifies the long-term effects of a healthy lifestyle and links the benefits directly to mortality risks. A decreased PhenoAge may serve as a better incentive than single-metric measures for promotion of a better lifestyle. Second, PhenoAge can be used for risk stratification and implementation of targeted lifestyle interventions at the population level, as it remained predictive of mortality risks independent of chronological age, demographic characteristics, and prevalent disease histories, and yielded additional predictive power on top of established risk factors.

The results of our study should also be interpreted in the context of the following limitations. Consistent with previous study, as the development of PhenoAge was based on the NHANES sample [3], whether PhenoAge can be applied to populations from other countries is still unknown. The current analysis cannot determine causal relationships between lifestyle and PhenoAge, nor can it determine the relationship between PhenoAge and mortality risks. However, the habitual lifestyle behaviors reported by participants pre-dated the interviews and MEC examinations, coinciding with the time sequence of temporal relationships and providing our interpretation with more credibility. In addition, the covariates included in our study were only demographic characteristics and histories of diseases; however, some unmeasured factors including environmental exposures could influence PhenoAge and mortality risk and thus influence the mediation results. Nevertheless, whether PhenoAge and PhenoAgeAccel can be modified by changes in lifestyle warrants further prospective studies or clinical trials.

Conclusions

In conclusion, adherence to LE8 recommendations was significantly associated with slowed PhenoAge, leading to reduced risks of all-cause and cardiovascular mortality. Our results proved that PhenoAge could serve as a mediator of the effects of LE8 on mortality, which supports the potential utilization of PhenoAge in promoting long-term CVH and well-being through realizing the promise of LE8.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Grants No. 82103920 and 81941018) and the National Key Research and Development Program (Grant No. 2023YFC2509400). The funding organizations were not involved in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Author contributions

Study conceptualization and design: D.S. Statistical analysis and interpretation of data: Y.Z. and H.Y. Writing-original drafting of manuscript: Y.Z. and H.Y. Critical review and editing of the manuscript for important intellectual content: Y.Z., Y.W., M.X., M.S., H.Y., C.L., Y.P., W.G., T.H., C.Y., J.L., S.L., L.Q., L.L. and D.S. Study supervision: D.S.

Supplementary materials

Supplementary data is available at *PCMEDI* Journal online.

Conflicts of interest

The authors declare that they have no conflicts of interests.

Ethics approval and consent to participate

All participants provided written informed consent, and the protocols were approved by research ethics boards of the National Center for Health Statistics webpage: https://www.cdc.gov/nchs/nhanes/irba98.htm.

Availability of data and materials

The datasets that support the findings of this study are publicly available on the National Health and Nutrition Examination Survey (NHANES) website: https://www.cdc.gov/nchs/nhanes/index.htm.

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Received 15 July 2024; accepted 28 August 2024. published 16 September 2024