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ORIGINAL RESEARCH

Epigenetic Age Mediates the Association of Life's Essential 8 With Cardiovascular Disease and Mortality

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BACKGROUND: Life's Essential 8 (LE8) is an enhanced metric for cardiovascular health. The interrelations among LE8, biomarkers of aging, and disease risks are unclear.

METHODS AND RESULTS: LE8 score was calculated for 5682 Framingham Heart Study participants. We implemented 4 DNA methylation-based epigenetic age biomarkers, with older epigenetic age hypothesized to represent faster biological aging, and examined whether these biomarkers mediated the associations between the LE8 score and cardiovascular disease (CVD), CVD-specific mortality, and all-cause mortality. We found that a 1 SD increase in the LE8 score was associated with a 35% (95% CI, 27–41; *P*=1.8E-15) lower risk of incident CVD, a 36% (95% CI, 24–47; *P*=7E-7) lower risk of CVD-specific mortality, and a 29% (95% CI, 22–35; *P*=7E-15) lower risk of all-cause mortality. These associations were partly mediated by epigenetic age biomarkers, particularly the GrimAge and the DunedinPACE scores. The potential mediation effects by epigenetic age biomarkers tended to be more profound in participants with higher genetic risk for older epigenetic age, compared with those with lower genetic risk. For example, in participants with higher GrimAge polygenic scores (greater than median), the mean proportion of mediation was 39%, 39%, and 78% for the association of the LE8 score with incident CVD, CVD-specific mortality, and all-cause mortality, respectively. No significant mediation was observed in participants with lower GrimAge polygenic score.

CONCLUSIONS: DNA methylation-based epigenetic age scores mediate the associations between the LE8 score and incident CVD, CVD-specific mortality, and all-cause mortality, particularly in individuals with higher genetic predisposition for older epigenetic age.

Key Words: cardiovascular health ■ epigenetic age scores ■ Life's Essential 8 ■ methylation

Cardiovascular disease (CVD) is the leading cause of death in the United States.[1,2] In an effort to better assess and improve cardiovascular health (CVH), the American Heart Association (AHA) proposed a new CVH metric composed of 8 factors, including diet, physical activity, nicotine exposure, sleep health, body mass index (BMI), blood lipid levels, blood glucose levels, and blood pressure.[2] Previous studies show that optimal levels of these CVH factors are associated with reduced CVD risk, as well as lower risk for a broad range of other diseases[3–7] and mortality.[2,8] Recent prospective studies conducted in different populations have shown temporal associations between CVH and incident CVD, CVD mortality, and all-cause mortality,

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CLINICAL PERSPECTIVE

What Is New?

- A higher Life's Essential 8 score, representing better cardiovascular health, is associated with younger DNA methylation-based epigenetic age, independent of chronological age.
- Epigenetic age biomarkers partly mediate the associations between Life's Essential 8 score and incident cardiovascular disease, cardiovascular disease-specific mortality, and all-cause mortality.
- The potential mediation effects of epigenetic age biomarkers are noticeable in individuals with a high genetic predisposition for advanced epigenetic age.

What Are the Clinical Implications?

- Our study indicates that cardiovascular risk factors affect DNA methylation related to the biological aging process and increase the burden of new-onset cardiovascular disease and mortality.
- Our study highlights the importance of promoting cardiovascular health, particularly in individuals with a higher genetic predisposition to older epigenetic age.

Nonstandard Abbreviations and Acronyms

CARDIA	Coronary Artery Risk Development in Young Adults
CpG	5′-C-phosphate-G-3′
CVH	cardiovascular health
FHS	Framingham Heart Study
LE8	Life's Essential 8
LS7	Life's Simple 7
PGS	polygenetic score

suggesting a causal effect of CVH on these clinical outcomes.[9–13] The number of Americans with ideal CVH, however, is low, and this trend persists for people of all ages, including children as young as 12 years old.[2] A better understanding of the molecular mechanisms involved with CVH may identify subpopulations and help design better prevention and intervention strategies to meet their needs and improve CVH more efficiently.

DNA methylation is the most studied epigenetic modification. Studies show both genetics and lifestyle can affect DNA methylation levels.[14,15] Differential DNA methylation at >1000 5′-C-phosphate-G-3′ (CpGs) (ie, DNA methylation sites) has been shown to be associated with CVD and all-cause mortality.[14–20] For example, using Mendelian randomization analysis, Agha et al provided evidence for a putative causal effect of DNA methylation on incident coronary heart disease, and Huan et al identified 92 putatively causal CpGs for various CVD traits.[19,21] Because recognition of the relationship between DNA methylation and morbidity and mortality has grown, various DNA methylation-based epigenetic scores have been developed to predict age and age-associated phenotypes.[22–26] These epigenetic scores have often been characterized as biological aging biomarkers, with older epigenetic age hypothesized to represent faster biological aging, although the definition of biological aging has not been uniformly agreed upon. Multiple studies have linked higher epigenetic age scores with increased risk of CVD and mortality independent of chronological age.[26–28] These studies suggest that DNA methylation is a potential contributor to disease development.

Our previous study showed that DNA methylation-based age biomarkers could explain approximately 20% to 40% of the association between diet quality, an important component of CVH, and all-cause mortality.[29,30] Although the relationships between individual CVH components, epigenetic age scores, and disease outcomes have been studied separately, research designed to examine the holistic status of CVH and epigenetic age is limited. We therefore hypothesized that DNA methylation is one of the molecular mechanisms underlying the relationship between CVH, represented by the Life's Essential 8 (LE8) score, and CVD and mortality. In this analysis, we examined the mediating roles that different epigenetic age scores may play in the associations between LE8 and CVD, CVD-specific mortality, and all-cause mortality in the community-based Framingham Heart Study (FHS). In addition, we examined whether genetic background may affect the observed mediation effect of epigenetic age scores on the outcome events.

METHODS

Data Availability

The data sets analyzed in the present study are available at the dbGAP repository phs000007.v33.p14 (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v33.p14).

Study Population

We analyzed data from participants in the FHS Offspring and Third Generation cohorts.[31] Data for the Offspring cohort were collected at exam 8 (2005–2008) and data for the Third Generation cohort were collected at exam 2 (2008–2011). Among the 6432 participants who attended the baseline exam (Figure 1), 5682 participants had complete LE8 data, 4130 participants had epigenetic

age score data, and 3693 participants had both LE8 data and epigenetic age score data. The FHS protocols and procedures were approved by the Institutional Review Board for Human Research at Boston University Medical Center, and all participants provided written informed consent. The current study was approved by the Institutional Review Board at Tufts University.

LE8 Scores

The full AHA rubric for scoring LE8 has been described previously.[2] Using dietary intakes that were assessed by a semiquantitative Harvard 2007 grid food frequency questionnaire, we calculated the Dietary Approaches to Stop Hypertension diet score as described in our recent study.[29] We assigned participants a diet score based on quantiles of the Dietary Approaches to Stop Hypertension diet score using the same threshold suggested by the AHA. Similarly, we used the AHA suggested cutoff values for sleep hours, BMI (weight in kilograms divided by the square of height in meters), blood pressure (millimeters of mercury), non-high-density lipoprotein cholesterol (milligrams per deciliter), and fasting glucose. A modified strategy was used to evaluate physical activity. We calculated a physical activity index based on the intensity and time spent for each type of activity and then categorized study participants into 7 groups based on index percentiles.[32] For instance, individuals in the top percentile of the physical activity index were assigned a score of 100, whereas those in the lowest percentile were assigned a score of 0. For smoking, we applied the same scoring strategy suggested by the AHA, with the exception that we assigned a score of 25 to individuals who have quit smoking for <2 years. We also did not consider exposure to secondhand smoke because of a lack of robust data. Each component was scored from 0 to 100, where 100 indicates complete adherence to LE8 recommendations. The overall LE8 score is an unweighted average of the 8 individual components. In

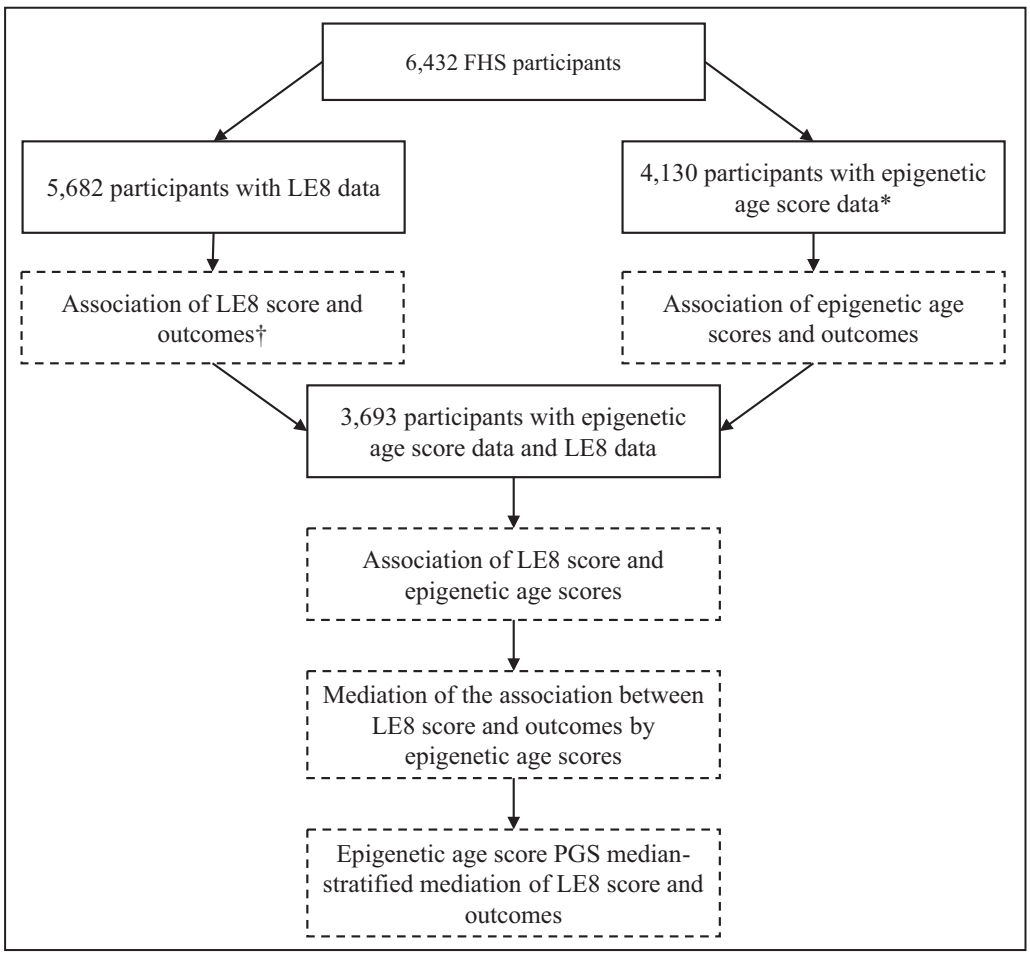

Figure 1. Study design flowchart.
Solid-lined boxes represent data sources, and dashed-lined boxes represent analyses. *Epigenetic age scores: DunedinPACE, PhenoAge, GrimAge, and DNAmTL scores. †outcomes: incident CVD, CVD-specific mortality, and all-cause mortality. CVD indicates cardiovascular disease; FHS, Framingham Heart Study; LE8, Life's Essential 8; and PGS, polygenic score.

the present analysis, we calculated the LE8 score only if participants had complete and valid measurements for all 8 components.

DNA Methylation Measurement and Score Building

DNA methylation profiles were measured using whole blood samples collected at the same time the LE8 components were assessed. Details of DNA collection and assessment of DNA methylation have been described previously.[21] Briefly, DNA methylation status for ≈450 000 CpGs was assayed using the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA), followed by standard quality control and normalization procedures. Data were normalized using the R package Watermelon. The methylation status at each CpG was quantified as a β value, calculated as the proportion of methylated signal intensity over the combined methylated and unmethylated probes.

We calculated 4 DNA methylation-based epigenetic age scores based on established algorithms for DunedinPACE Score,[26] PhenoAge,[23] DNAmTL,[33] and GrimAge.[24,34] Briefly, the DunedinPACE score was developed by tracking the longitudinal measurements in 19 biomarkers critical to aging and morbidity.[26] Then, an elastic net regression analysis determined the set of CpGs to predict these biomarkers. DunedinPACE scores were calculated as the sum of weighted DNA methylation β values of these selected CpGs. PhenoAge and GrimAge were also developed using similar CpG selection process, but based on different sets of biomarkers and clinical risk factors; PhenoAge was calculated based on 9 clinical biomarkers and chronological age,[23] and GrimAge was developed based on 10 DNA methylation biomarkers, sex, and chronological age.[34] The DNAmTL score was calculated using 140 CpGs that were chosen because of their tight relationship with leukocyte telomere length.[35] Before association analysis, we used linear regression models to derive methylation score residuals by regressing the methylation scores on chronological age, white cell composition estimated using the Houseman approach,[36] sample distribution across chips (row numbers and column numbers as categorical variables), and 15 DNA methylation-based principal components. The standardized residuals were then used for subsequent analyses. Higher DunedinPACE score, PhenoAge, and GrimAge residuals and lower DNAmTL residuals indicate older epigenetic age or faster biological aging.

Polygenetic Scores and Interaction Analyses

We calculated polygenetic scores (PGS) for PhenoAge and GrimAge scores using a Bayesian regression framework (named polygenic risk scores–continuous shrinkage) based on default settings.[37] Polygenic risk scores–continuous shrinkage infers posterior effect sizes under continuous shrinkage priors based on imputed genotype information in the FHS and summary statistics from genome-wide association studies. Because large-scale genome-wide association studies have been performed for PhenoAge and GrimAge,[38] we calculated PGS for PhenoAge and GrimAge. Genotyping was obtained with the Affymetrix 550K Array and imputed to the 1000 Genomes Project reference panel.[39,40] The PGSs were calculated using genotype dosage for ≈10 million biallelic single nucleotide polymorphisms (imputation quality R^2>0.5 and minor allele frequency >0.005). To perform the continuous shrinkage, we used the linkage disequilibrium matrix for European people from the 1000 Genomes Project.[40] The dosage of these single nucleotide polymorphisms was weighted by the posterior effect size and summed and scaled by the number of single nucleotide polymorphisms to calculate the PGS.

Clinical Outcome Ascertainment

The primary clinical outcomes were incident CVD, CVD-specific mortality, and all-cause mortality, and were derived from data obtained during a mean follow up of 14 years in Offspring participants and 11 years in Third Generation participants. A panel of 3 physicians reviewed and adjudicated outcome events after examining pertinent information such as medical and hospital records, death certificates, and next-of-kin interviews.[36] CVD comprised myocardial infarction, coronary heart disease, stroke, heart failure, and death from any cardiovascular conditions.[37] Individuals with prevalent CVD at baseline were excluded from analysis of incident CVD.

Statistical Analysis

As depicted in Figure 1, 3 main analyses were conducted to examine (1) cross-sectional association between LE8 score and 4 DNA methylation scores (DunedinPACE Score, PhenoAge, DNAmTL, and GrimAge); (2) prospective associations of LE8 and epigenetic age scores with incident CVD, CVD-specific mortality, and all-cause mortality; and (3) potential mediation effects of epigenetic age scores on the relationship between LE8 score and incident CVD, CVD-specific mortality, and all-cause mortality.

Linear mixed models were used to examine the cross-sectional association between LE8 score and DNA methylation scores with sex, age, alcohol use, laboratory site, and leukocyte composition as fixed-effect covariates and familial relationship as the random-effect factor. Standardized LE8 scores were used in all statistical analyses. To assess the risk of incident CVD, CVD-specific mortality, and all-cause

mortality relative to LE8 score and methylation scores, we used mixed proportional hazard models to account for familial relationship. Sex, age, and alcohol use were adjusted for in all prospective analyses. For survival analyses, person-years were calculated using the date that the participant attended the FHS exam to the date of the event (CVD event, CVD-specific mortality, or death of any cause), the end of follow-up, or the end of the study (2019), whichever occurred first. In the analysis for all-cause mortality, additional adjustments were made for prevalent cancer (excluding nonmelanoma skin cancer) and CVD. In addition, differential leukocyte proportions were added in the prospective analyses for methylation scores. *P* value trend analyses were conducted for each epigenetic age score by assigning participants to the median value for the quartile to which they belong and running the same survival analysis on these median values. We repeated the above analysis for each of the 8 individual LE8 components, as well as for scores of LE8 health factors (blood glucose, blood lipids, blood pressure, and BMI) and health behaviors (smoking, sleep, physical activity, and diet).

We conducted mediation analyses using a modified approached proposed by Huang and Yang to investigate whether the associations between LE8 score and incident CVD, CVD-specific mortality, and all-cause mortality were mediated by the methylation scores.[41] We calculated 95% CIs and the significance levels (*P* values) for natural indirect effects (ie, mediation) using a resampling method that takes random draws from a multivariate normal distribution of estimates. The proportion of mediation was calculated as the ratio of indirect effects to the sum of both direct and indirect effects. To test the stability of the mediation estimations, we also performed mediation analysis using the R mediation package, with *P* values and confidence intervals calculated via bootstrap with 4000 resamples using the bias-corrected and accelerated approach.[42–44]

We added an interaction term between LE8 score and PGS of PhenoAge and GrimAge in the cross-sectional analysis between LE8 score and methylation scores to examine the potential interaction between LE8 score and participants' genetic background. To further examine whether individuals' genetic background may affect the potential mediation effect of methylation scores on the associations between LE8 and incident CVD, CVD-specific mortality, and all-cause mortality, we conducted stratified mediation analyses in participants with PGS below and above the median of PGS. SAS 9.4 was used to calculate the LE8 score, a Python-based program was used to calculate PGS, and R 4.0 was used for association and mediation analyses. A 2-tailed $P<0.05$ was considered statistically significant.

RESULTS

Participant Characteristics

Table 1 displays participant characteristics based on quartile categories of the LE8 score for those with complete data on LE8 components and all-cause mortality (n=5669). FHS participants had a mean LE8 score of 68.7 (range, 20.6–100; median, 68.8). Women and younger participants tended to have higher LE8 scores. The BMI component had the highest correlation with the LE8 score (Pearson r=0.59), followed by blood pressure (r=0.55), blood glucose (r=0.52), diet (r=0.50), blood lipids (r=0.46), physical activity (r=0.40), smoking (r=0.38), and sleep (r=0.26; Figure S1). The 4 epigenetic age scores were modestly to moderately correlated; pairwise Pearson r ranged from 0.28 to 0.68 (Figure S1).

Association of LE8 Score and Epigenetic Age Scores

After adjusting for age, sex, alcohol consumption, laboratory site, leukocyte differential composition, and family relatedness, we found that a greater LE8 score was associated with lower DunedinPACE, GrimAge, and PhenoAge residuals and higher DNAmTL residuals (Figure S2). A 1 SD increase in the LE8 score (13 points) was associated with 0.39 SD (95% CI, −0.42 to −0.36; *P*=1.2E-133) lower DunedinPACE score, 0.42 SD (95% CI, −0.45 to −0.39; *P*=7.9E-150) lower GrimAge, and 0.15 SD (95% CI, −0.18 to −0.12; *P*=3.3E-20) lower PhenoAge, and a 0.10 SD (95% CI, 0.06–0.13; *P*=5.5E-9) higher DNAmTL (Figure S2 and Table S1). The strength of the association between the LE8 score and the epigenetic age scores is comparable in the FHS Offspring and in the Third Generation cohorts (Table S2). With correction for multiple testing (ie, 0.05/4 epigenetic scores times 8 LE8 components), all LE8 components were individually associated with the GrimAge residuals, whereas 7, 5, and 2 components were associated with the DunedinPACE, PhenoAge, and DNAmTL scores, respectively (Table S3). Most of these associations remained significant or nominally significant ($P<0.05$) in analyses with adjustment for other LE8 components.

Association of LE8 Score and Incident CVD, CVD-Specific Mortality, and All-Cause Mortality

After adjustment for potential confounders, a higher LE8 score was associated with a lower risk of incident CVD, CVD-specific mortality, and all-cause mortality: each 1 SD increase in the LE8 score was associated with a 35% (95% CI, 27–41; *P*=1.8E-15) lower risk of incident CVD, a 36% (95% CI, 24–47; *P*=7E-7) lower risk of CVD-related mortality, and a 29% (95%

Table 1. Participant Characteristics Based on LE8 Score Quartiles

Characteristic	LE8 score				
	All participants	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Total participants, (N)*	5669	1438	1454	1438	1339
Mean age, y	55.5±13.1	59.3±12.6	57.5±13	54.9±13.2	49.8±11.7
Sex, % women	54.7	42.4	45.1	56.6	76.1
Alcohol, servings/wk	5.2±6.75	0±0	1±1.25	5±2.5	13±8
LE8 score	68.7±13.3	51.6±6.5	64.6±2.7	73.9±2.9	85.9±4.8
Smoking status, n (%)					
Never	4082 (72)	696 (48.4)	1002 (68.9)	1164 (80.9)	1120 (83.6)
Former	1070 (18.9)	426 (29.6)	314 (21.6)	217 (15.1)	113 (8.4)
Current	517 (9.1)	316 (22)	138 (9.5)	57 (4)	6 (0.4)
DASH diet score	24±5.6	20.8±4.7	22.8±5.2	25±5.2	27.8±4.7
Physical activity score	35.9±6.1	33.2±5.2	35.8 (6.2)	36.7 (6.1)	38 (5.7)
Sleep, h	7.3±1.1	7.2 (1.5)	7.2±1.1	7.3 (1)	7.3±0.8
BMI, kg/m^2	28.1±5.6	32.2 (6.2)	29.1±5	26.9 (4)	23.8±3.1
Systolic blood pressure, mm Hg	122 (17)	130 (16)	125±16	119 (15)	110 (12)
Diastolic blood pressure, mm Hg	74 (10)	77 (11)	75 (9)	73 (9)	69 (8)
Hypertension medications, n (%)	1768 (34.3)	759 (53)	572 (39.4)	336 (23.5)	101 (7.6)
Total cholesterol, mg/dL	186.3 (35.9)	192.3 (40)	187.6 (38.3)	186.3 (33.6)	178.6±29
Triglyceride, mg/dL	107.4±76	141.5±102.7	116.9±79.6	93.9±51.1	76.1±33.3
HDL, mg/dL	58.8±18	50.9±14.9	55.5±16.2	60.7±17.8	68.8±18.1
Lipid lowering medications, n (%)	1600 (28.2)	625 (43.5)	508 (34.9)	338 (23.5)	129 (9.6)
Blood glucose, mg/dL	100.7±20.3	111.9±28.7	102.8±18.6	96.7±12.6	90.8±7.9
HbA1c, %	5.6±0.6	5.9±0.8	5.6±0.5	5.5±0.4	5.3±0.3
Diabetic medications, n (%)	326 (5.9)	204 (14.5)	90 (6.4)	27 (1.9)	5 (0.4)
Prevalent cancer, n (%)	438 (7.7)	136 (9.5)	120 (8.3)	118 (8.2)	64 (4.8)
Prevalent CVD, n (%)	140 (2.5)	58 (4.0)	40 (2.8)	28 (1.9)	14 (1.0)

Values are mean±SD for quantitative traits and n (%) for binary traits.

BMI indicates body mass index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IQR, interquartile range; and LE8, Life's Essential 8.

*Participants with LE8 and all-cause mortality data.

CI, 22–35; *P*=7E-15) lower risk of all-cause mortality. When we separated participants into quartiles by their LE8 score, we found a dose–response relationship between LE8 score and incident CVD, all-cause mortality, and CVD-related mortality (Table 2 and Figure S3). Associations of individual LE8 score components with the 3 clinical outcomes are presented in Table S4. Overall, in our study participants, the LE8

Table 2. Associations of LE8 Score With CVD and Mortality-Related Outcomes

LE8 quartile	Incident CVD		CVD-specific mortality		All-cause mortality	
	No. of CVD cases	HR	No. of CVD deaths	HR	No. of deaths	HR
Q1	159	3.1 (2.2–4.36)	64	4.68 (2.42–9.05)	297	2.38 (1.73–3.28)
Q2	115	2.02 (1.42–2.88)	42	2.79 (1.41–5.52)	203	1.68 (1.21–2.33)
Q3	85	1.73 (1.2–2.51)	36	2.98 (1.49–5.95)	131	1.29 (0.92–1.82)
Q4	46	Ref	11	Ref	45	Ref
P for trend		8.1E-12		4.6E-6		7.5E-13

HRs are adjusted for sex, age, alcohol use, and familial relatedness. All-cause mortality analyses were also adjusted for prevalent cancer and prevalent CVD cases.

CVD indicates cardiovascular disease; HR, hazard ratio; LE8, Life's Essential 8; and Ref, reference.

health factors had stronger associations with incident CVD and CVD mortality, whereas the LE8 health behaviors had stronger associations with all-cause mortality.

Epigenetic Age Scores Mediate the Association Between LE8 Score and Incident CVD, CVD-Specific Mortality, and All-Cause Mortality

Higher DunedinPACE, GrimAge, and PhenoAge scores and lower DNAmTL score were associated with increased risk of incident CVD, all-cause mortality, and CVD-related mortality. DunedinPACE, PhenoAge, and GrimAge score residuals that were 1 SD higher were associated with a 36% (95% CI, 23–51; *P*=2.5E-9), 11% (95% CI, 0–23; *P*=0.04), and 47% (95% CI, 33–63; *P*=2.3E-14) higher hazards of incident CVD risk, respectively. A 1 SD higher DNAmTL score residual was associated with a 21% (95% CI, 13–29; *P*=2.5E-6) lower CVD risk (Table S5). For CVD-specific mortality, 1 SD higher DunedinPACE and GrimAge score residuals resulted in a 43% (95% CI, 22–67; *P*=6.3E-6) and 68% (95% CI, 44–95; *P*=1.4E-11) greater risk of death from CVD, respectively. A 1 SD higher in DNAmTL residual was associated with a 21% (95% CI, 9–33; *P*=1.9E-3) lower CVD-specific mortality. No significant association was observed between PhenoAge and CVD-specific mortality (hazard ratio, 1.11 [95% CI, 0.95–1.28]; *P*=0.18). For all-cause mortality, 1 SD higher DunedinPACE, PhenoAge, and GrimAge score residuals resulted in a 45% (95% CI, 35–56; *P*=2.2E-16), 16% (95% CI, 8–24; *P*=5.1E-5), and 63% (95% CI, 52–74; *P*=2.2E-16) higher hazards for all-cause mortality, respectively. A 1 SD greater DNAmTL score residual was associated with a 21% (95% CI, 15–27; *P*=1.4E-10) lower hazard for all-cause mortality. We also found a dose–response relationship between epigenetic age scores and incident CVD, CVD-specific mortality, and all-cause mortality (Table S5).

We ran mediation analyses for each epigenetic age score (Figure S4). Although we found that nearly all scores were statistically significant in mediating the associations between LE8 score and incident CVD, CVD-related mortality, and all-cause mortality, the DunedinPACE and GrimAge scores had greater proportions of mediation (Table 3). For incident CVD, the mean proportion of mediation was 14% and 21% by the DunedinPACE and GrimAge scores, respectively. For CVD-related mortality, the mean proportion of mediation was 14% and 21% by DunedinPACE and GrimAge scores, respectively. For all-cause mortality, the mean proportion of mediation was 42% and 65% by the DunedinPACE and GrimAge scores, respectively. Analysis using the R mediation package yielded similar results (Table S6). Because all LE8 components were associated with the GrimAge, we also examined the potential mediation by GrimAge for LE8 components and clinical outcomes. For example, the proportion of mediation by GrimAge was 36% for blood glucose and incident CVD, 21% for BMI and CVD mortality, 26% for sleep and all-cause mortality (Table S7).

Stratified Analysis by PGS

The Pearson correlation coefficient was 0.24 (*P*=2.7E-53) for GrimAge PGS and GrimAge, and 0.24 (*P*=7.8E-53) for PhenoAge PGS and PhenoAge. We found that LE8 and GrimAge PGS interact to explain the variation observed in GrimAge score residuals (*P*=2.1E-24). We found a similar interaction between LE8 and PhenoAge PGS values to explain variation in PhenoAge score residuals (*P*=2.0E-3). We explored the LE8 score–PGS interaction in Figure S5 by dichotomizing GrimAge and PhenoAge PGS using median values. As expected, compared with participants with lower GrimAge and PhenoAge PGS values, epigenetic age scores were higher in those with higher PGS values, suggesting a higher epigenetic burden in this group. In participants with high versus low PGS, the mean of GrimAge residuals (age, white cell composition, and technical variables adjusted) was 1.2 years and –1.3 years and the mean of PhenoAge residuals was 1.6 years and –1.6 years, respectively. In participants with higher PGS values, indicating higher epigenetic ages, the association between LE8 and the epigenetic age score residuals was greater than in individuals with lower PGS values. A 1 SD higher standardized LE8 score was associated with 1.83 years (95% CI, 1.65–2.01; *P*=5.95E-76) decrease in GrimAge residuals for participants with higher GrimAge PGS values, whereas for participants in the bottom half of GrimAge PGS values, the same SD increase in LE8 score was associated with 1.09 years (95% CI, 0.94–1.24; *P*=5.77E-44) decrease in GrimAge residuals. Similarly, a 1 SD higher LE8 score was associated with 0.73 years (95% CI, 0.50–0.96; *P*=8.1E-10) and 0.51 years (95% CI, 0.28–0.73; *P*=8.5E-6) lower PhenoAge score residuals in individuals with high and low PhenoAge PGS values, respectively. We also dichotomized the LE8 score and presented the estimated epigenetic age residuals in participants with low PGS with low LE8 score, low PGS with high LE8 score, high PGS with low LE8 score, and high PGS with high LE8 score in Figure 2.

We stratified our study participants by the median of PGS and repeated the mediation analyses in each stratum (Table 3). In participants in the upper GrimAge PGS group (greater than or equal to the median), the mean proportion of mediation by GrimAge was 39% (*P*=8.8E-4) for incident CVD, 39% (*P*=1.1E-3) for CVD-specific mortality, and 78% (*P*=2.2E-16) for all-cause mortality. In stratified analysis for PhenoAge PGS, we

Table 3. Proportion of Mediation (Indirect Effect) by DunedinPACE, PhenoAge, GrimAge, and DNAmTL Scores for the Association Between LE8 and Incident CVD, CVD-Specific Mortality, and All-Cause Mortality

Epigenetic age score	Direct-effect HR (95% CI)	*P* value	Indirect-effect HR (95% CI)	*P* value	Mean proportion mediated, % (95% CI)
Incident CVD					
DunedinPACE	0.64 (0.56–0.73)	2.2E-16	0.93 (0.89–0.97)	2.7E-3	14% (5%–24%)
PhenoAge	0.61 (0.54–0.69)	2.2E-16	0.99 (0.97–1.01)	0.23	
Lower PGS	0.53 (0.44–0.64)	2.2E-16	0.99 (0.96–1.01)	0.23	
Upper PGS	0.69 (0.57–0.84)	1.9E-4	0.99 (0.97–1.01)	0.54	
GrimAge	0.67 (0.59–0.77)	2.2E-16	0.90 (0.86–0.94)	5.4E-5	21% (11%–33%)
Lower PGS	0.56 (0.46–0.69)	2.2E-16	0.94 (0.88–1.01)	0.08	
Upper PGS	0.80 (0.67–0.96)	0.02	0.89 (0.83–0.95)	8.8E-4	39% (14%–80%)
DNAmTL	0.61 (0.54–0.69)	2.2E-16	0.98 (0.97–0.99)	0.02	3% (0%–6%)
CVD-specific mortality					
DunedinPACE	0.59 (0.48–0.73)	2.2E-16	0.92 (0.86–0.98)	0.01	14% (3%–28%)
PhenoAge	0.56 (0.46–0.69)	2.2E-16	0.99 (0.97–1.01)	0.36	
Lower PGS	0.44 (0.32–0.60)	2.2E-16	0.98 (0.94–1.01)	0.29	
Upper PGS	0.74 (0.54–1.01)	0.06	0.99 (0.96–1.02)	0.54	
GrimAge	0.68 (0.59–0.77)	2.2E-16	0.91 (0.86–0.95)	6.4E-5	21% (10%–33%)
Lower PGS	0.57 (0.46–0.70)	2.2E-16	0.94 (0.88–1.00)	0.06	
Upper PGS	0.82 (0.68–0.97)	0.02	0.89 (0.83–0.95)	1.1E-3	39% (14%–84%)
DNAmTL	0.57 (0.46–0.69)	2.2E-16	0.98 (0.97–1.00)	0.06	
All-cause mortality					
DunedinPACE	0.81 (0.74–0.90)	6.8E-5	0.87 (0.84–0.90)	2.2E-16	42% (28%–60%)
PhenoAge	0.74 (0.67–0.81)	2.2E-16	0.98 (0.97–1.00)	0.02	6% (1%–11%)
Lower PGS	0.66 (0.57–0.76)	2.2E-16	0.99 (0.97–1.01)	0.21	
Upper PGS	0.83 (0.72–0.95)	8.1E-3	0.98 (0.96–1.00)	0.05	12% (2%–36%)
GrimAge	0.89 (0.80–0.99)	0.03	0.82 (0.78–0.85)	2.2E-16	65% (45%–95%)
Lower PGS	0.85 (0.71–1.01)	0.06	0.95 (0.89–1.01)	0.13	
Upper PGS	0.92 (0.80–1.06)	0.27	0.80 (0.75–0.85)	2.2E-16	78% (46%–100%)
DNAmTL	0.73 (0.66–0.80)	2.2E-16	0.98 (0.97–0.99)	1.3E-03	6% (3%–11%)

In addition to analyses in the entire study participants, mediation analyses for PhenoAge and GrimAge were also conducted separately in participants with lower and higher PGS values. Lower PGS values are associated with younger epigenetic ages, whereas higher PGS values are associated with older epigenetic ages. Direct-effect HR quantifies the effect that LE8 has on the outcome without epigenetic age effects. The indirect-effect HR quantifies the effect of LE8 on the outcome via the epigenetic age score (ie, mediation effect). Linear mixed models and mixed Cox models were used with epigenetic age score residuals as mediators, adjusting for sex, age, alcohol use, laboratory site, leukocyte composition, and familial relatedness. All-cause mortality analyses were also adjusted for prevalent cancer and CVD.

CVD indicates cardiovascular disease; HR, hazard ratio; LE8, Life's Essential 8; and PGS, polygenic score.

observed a significant mediation effect by PhenoAge in relation to LE8 score and all-cause mortality in the upper PhenoAge PGS group (greater than or equal to the median). The mean proportion of mediation was 12% (*P*=0.05) for this group. In participants with lower GrimAge PGS or PhenoAge PGS (less than the median), no significant mediation effect was observed for any of the outcomes for both GrimAge and PhenoAge (Table 3).

DISCUSSION

This analysis, conducted in a group of middle- to older-aged adults, showed a strong inverse relationship between CVH, captured using the LE8 score, and incident CVD, CVD-specific mortality, and all-cause mortality. Our observations on the potential mediating effects of epigenetic age on the relationships between LE8 and clinical outcomes suggest that more favorable CVH may reduce the risk of CVD and mortality via epigenetic effects. Furthermore, our results suggest the potential benefit of optimal CVH on the reduction of epigenetic burden is more noticeable in individuals who were genetically predisposed to an older epigenetic age. Taken together, our study emphasizes that improving CVH is a promising strategy to delay the new onset of CVD and promote healthy aging.

The LE8 has 2 domains, health behaviors (diet, physical activity, smoking, and sleep health) and health factors (BMI, blood lipids, blood glucose, and blood

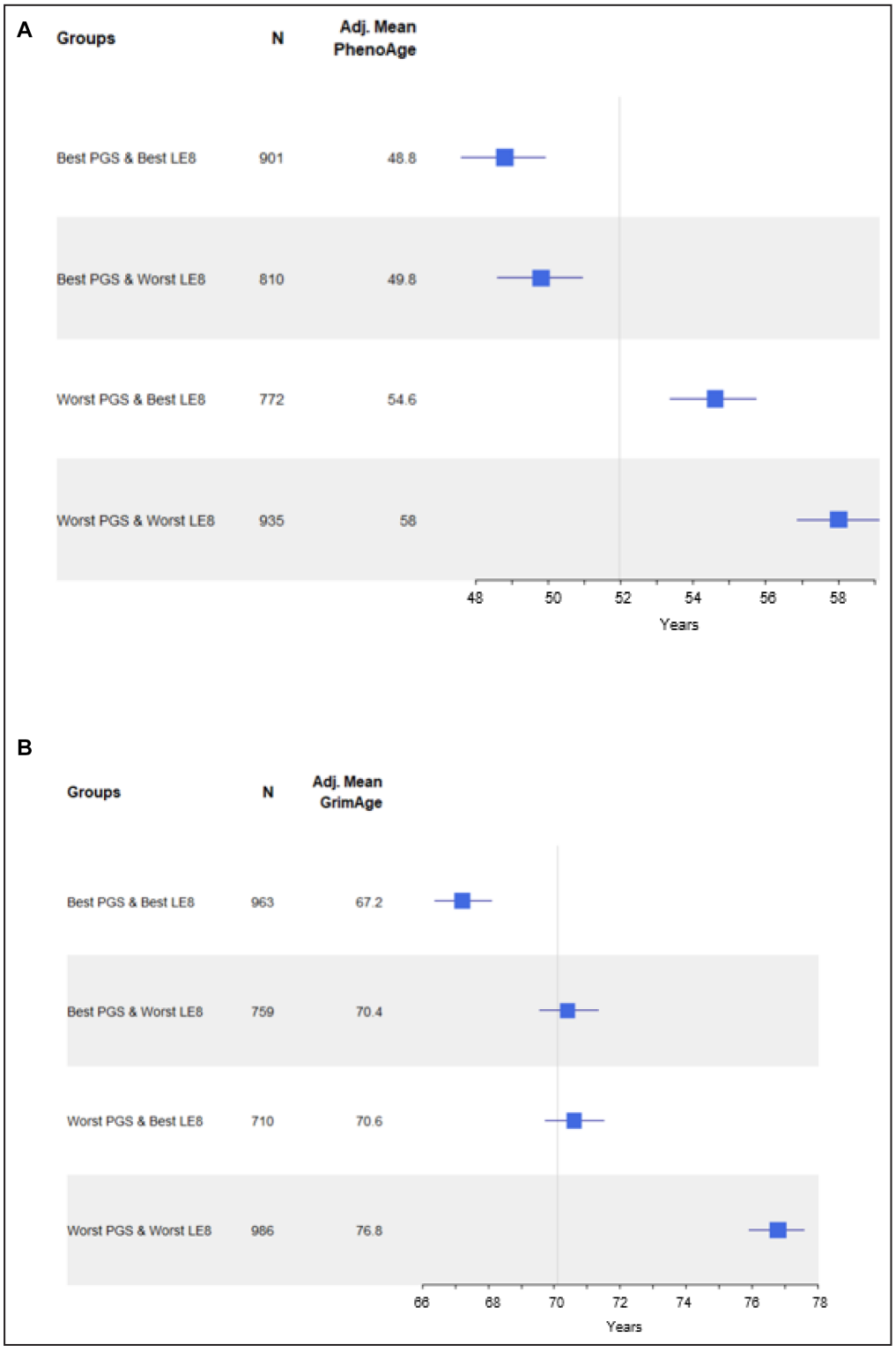

Figure 2. Stratified analysis for epigenetic age based on the dichotomized LE8 score and PGS. LE8 score and PGS were dichotomized by median. **A**, PhenoAge. **B**, GrimAge. Regressions were calculated controlling for sex, age, leukocyte composition, and family relatedness. Adj. indicates adjusted; LE8, Life's Essential 8; and PGS, polygenic score.

pressure).[2] There is a rich body of literature that addresses the link between these 2 domains (and their constituent components) and CVD and mortality.[14,45–51] A previous FHS publication based on an earlier version of the AHA's CVH metrics (ie, Life's Simple 7 [LS7]) showed that maintaining a high CVH over time

was associated with lower risk of CVD and all-cause mortality.[52] Compared with the LS7, the LE8 is enhanced by including sleep quality and an upgraded algorithm to quantify CVH.[2] Comprehensive evaluation of whether or not LE8 score outperforms the LS7 is beyond the scope of the present study. Nonetheless, we observed that, independent of other LE8 components, sleep health score was inversely associated with all-cause mortality but not with CVD phenotypes. This observation suggests that a more refined sleep health parameter may be considered to further improve disease prediction of the LE8 score.[53] Consistent with our observations, several recent studies showed the inverse association between the LE8 score and CVD-specific and all-cause mortality in populations with different ethnic backgrounds.[12,13,54] For example, based on National Health and Nutrition Examination Surveys data from 2005 to 2014, Yi et al demonstrated a dose-response relationship between the LE8 score and mortality.[13]

Relationships between LS7 and epigenetic age scores have also been examined by several studies.[27,55,56] For example, a study demonstrated an inverse association between LS7 and GrimAge residuals that was observed in the CARDIA (Coronary Artery Risk Development in Young Adults) study and replicated in the FHS.[27] The present study expanded the literature by using the AHA's latest CVH metrics and demonstrating the potential mediation effects of the epigenetic age scores on the association between the LE8 score and clinical outcomes. Using PGSs that describe epigenetic age and are computed by a novel high-dimensional Bayesian regression framework, we demonstrated that participants with a higher PGS had older epigenetic age (ie, higher epigenetic burden). Furthermore, based on the association and mediation analyses, we speculate that optimal CVH may lower the risk of developing new-onset CVD and mortality by reducing the high epigenetic burden in participants with higher epigenetic age PGS. Thus, these findings highlight the importance of promoting CVH in the general population, particularly in those with a genetic predisposition to older epigenetic age.

In the present study, we observed that the GrimAge and DunedinPACE scores had stronger mediating effect sizes on the association of LE8 with clinical outcomes compared with the PhenoAge and DNAmTL scores, suggesting that the CpGs used to calculate the former 2 epigenetic scores may be more relevant to the biological mechanisms underlying the associations of the LE8 score with CVD and mortality. We also observed that the mean proportion of mediation was larger in the analysis for all-cause mortality compared with that for CVD analysis. This observation suggests that the epigenetic age scores also represent DNA methylation related mechanisms linking the LE8 score with diseases other than CVD. Nonetheless, to better understand the extent to which epigenetic age scores are involved in different LE8-associated diseases, analyses including additional cohorts are warranted.

A study using data from CARDIA and FHS has examined individual LS7-associated CpGs.[15] These CpGs have been implicated in pathways involved with lipid metabolism and biosynthesis, proinflammatory cytokines signaling, and serine biosynthesis pathways.[15] Individual CpGs have also been associated with CVD risk factors, CVD, and mortality.[14,16–18,30] To better characterize CpGs where DNA methylation plays the most significant mediating role, future large-scale studies with diverse study populations are needed to comprehensively examine the association of LE8 scores with individual CpGs.

Strengths of this study include the use of comprehensive lifestyle, genetic, DNA methylation, and clinical data collected in a well-established community-based cohort. With respect to limitations, given that the study participants were of predominantly European ancestry, our findings, particularly the LE8 score–genotype interaction, might not be generalizable to other populations. Additionally, FHS has contributed DNA methylation and other data in the process of developing or validating the 4 epigenetic age scores that were analyzed in the present study. This raised a concern of overfitting in the present analysis, which may lead to an overestimation for the potential mediation effect. Replication of these results in larger and more diverse populations would improve the generalizability of our findings. Factors used to select CpGs to build the epigenetic age scores may overlap with the LE8 score components (eg, smoking pack-years that were used to select CpGs for the GrimAge is an overlapping factor with the smoking component of the LE8). The correlation among the components of health factors was generally stronger than the correlation among the components of health behaviors. The components in the latter LE8 domain were scored based on self-reported data, which may be biased with measurement errors. The temporal sequence of data collection for exposure, mediator, and outcome variables allows greater latitude in investigating the effects over time and causal direction in mediation analysis. However, we used DNA methylation data collected at a single time point, which limits validity of the mediation analyses. Large-scale longitudinal studies with repeated DNA methylation measurements evaluating change in epigenetic age scores or at individual CpGs over time may provide stronger causal inference. DNA methylation-based age scores may be improved by integrating information such as specific aging-related biological pathways, cell- and tissue-specific DNA methylation profiles, and functional epigenetic biomarkers. In addition, a recent study suggested that, compared with

the method using a subset of individual CpGs to build age scores, those calculated based on principal components of CpG-level data may reduce the impact of technical noise and improve score reliability.[57] Future analyses using these more advanced age scores may facilitate a better understanding of epigenetic mechanisms linking CVH components and the risk of CVD and mortality.

In conclusion, we demonstrated that DNA methylation-based epigenetic age scores partially mediate the association between the LE8 score and CVD, CVD-specific mortality, and all-cause mortality. In addition, our data suggest that, in individuals with higher genetic predisposition for older epigenetic age scores, an optimal CVH may be beneficial by decreasing their epigenetic burden. Future studies are needed to investigate this potential mediation effect with longitudinal DNA methylation measurements in large and diverse populations.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1

REFERENCES

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, et al. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation*. 2020;141:e139–e596. doi: 10.1161/CIR.0000000000000757
2. Lloyd-Jones DM, Allen NB, Anderson CAM, Black T, Brewer LC, Foraker RE, Grandner MA, Lavretsky H, Perak AM, Sharma G, et al. Life's essential 8: updating and enhancing the American Heart Association's construct of cardiovascular health: a presidential advisory from the American Heart Association. *Circulation*. 2022;146:e18–e43. doi: 10.1161/CIR.0000000000001078
3. Ogunmoroti O, Allen NB, Cushman M, Michos ED, Rundek T, Rana JS, Blankstein R, Blumenthal RS, Blaha MJ, Veledar E, et al. Association between Life's simple 7 and noncardiovascular disease: the multi-ethnic study of atherosclerosis. *J Am Heart Assoc*. 2016;5:5. doi: 10.1161/JAHA.116.003954
4. Guo J, Brickman AM, Manly JJ, Reitz C, Schupf N, Mayeux RP, Gu Y. Association of Life's simple 7 with incident dementia and its modification by the apolipoprotein E genotype. *Alzheimers Dement*. 2021;17:1905–1913. doi: 10.1002/alz.12359
5. Wei J, Wang L, Kulshreshtha A, Xu H. Adherence to life's simple 7 and cognitive function among older adults: the National Health and nutrition examination survey 2011 to 2014. *J Am Heart Assoc*. 2022;11:e022959. doi: 10.1161/JAHA.121.022959
6. Tin A, Bressler J, Simino J, Sullivan KJ, Mei H, Windham BG, Griswold M, Gottesman RF, Boerwinkle E, Fornage M, et al. Genetic risk, mid-life life's simple 7, and incident dementia in the atherosclerosis risk in communities study. *Neurology*. 2022;99:e154–e163. doi: 10.1212/WNL.0000000000200520
7. Hasbani NR, Ligthart S, Brown MR, Heath AS, Bebo A, Ashley KE, Boerwinkle E, Morrison AC, Folsom AR, Aguilar D, et al. American Heart Association's Life's simple 7: lifestyle recommendations, polygenic risk, and lifetime risk of coronary heart disease. *Circulation*. 2022;145:808–818. doi: 10.1161/CIRCULATIONAHA.121.053730
8. Fang N, Jiang M, Fan Y. Ideal cardiovascular health metrics and risk of cardiovascular disease or mortality: a meta-analysis. *Int J Cardiol*. 2016;214:279–283. doi: 10.1016/j.ijcard.2016.03.210
9. Li X, Ma H, Wang X, Feng H, Qi L. Life's essential 8, genetic susceptibility, and incident cardiovascular disease: a prospective study. *Arterioscler Thromb Vasc Biol*. 2023;43:1324–1333. doi: 10.1161/ATVBAHA.123.319290
10. Hernandez-Martinez A, Duarte-Junior MA, Sotos-Prieto M, Ortola R, Banegas JR, Rodriguez-Artalejo F, Soriano-Maldonado A, Martinez-Gomez D. Cardiovascular health in Spain based on the Life's essential 8 and its association with all-cause and cardiovascular mortality: the ENRICA cohort. *Rev Esp Cardiol (Engl ed)*. 2023;23:S1885-5857(23)00258-X. doi: 10.1016/j.rec.2023.09.001
11. Rempakos A, Prescott B, Mitchell GF, Vasan RS, Xanthakis V. Association of life's essential 8 with cardiovascular disease and mortality: the Framingham heart study. *J Am Heart Assoc*. 2023;12:e030764. doi: 10.1161/JAHA.123.030764
12. Isiozor NM, Kunutsor SK, Voutilainen A, Laukkanen JA. Life's essential 8 and the risk of cardiovascular disease death and all-cause mortality in Finnish men. *Eur J Prev Cardiol*. 2023;30:658–667. doi: 10.1093/eurjpc/zwad040
13. Yi J, Wang L, Guo X, Ren X. Association of life's essential 8 with all-cause and cardiovascular mortality among US adults: a prospective cohort study from the NHANES 2005-2014. *Nutr Metab Cardiovasc Dis*. 2023;33:1134–1143. doi: 10.1016/j.numecd.2023.01.021
14. Ma J, Rebholz CM, Braun KVE, Reynolds LM, Aslibekyan S, Xia R, Biligowda NG, Huan T, Liu C, Mendelson MM, et al. Whole blood DNA methylation signatures of diet are associated with cardiovascular disease risk factors and all-cause mortality. *Circ Genom Precis Med*. 2020;13:e002766. doi: 10.1161/CIRCGEN.119.002766

15. Zheng Y, Joyce BT, Hwang SJ, Ma J, Liu L, Allen NB, Krefman AE, Wang J, Gao T, Nannini DR, et al. Association of Cardiovascular Health through Young Adulthood with Genome-Wide DNA methylation patterns in midlife: the CARDIA study. *Circulation*. 2022;146:94–109. doi: 10.1161/CIRCULATIONAHA.121.055484
16. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, Guan W, Xu T, Elks CE, Aslibekyan S, et al. Epigenetic signatures of cigarette smoking. *Circ Cardiovasc Genet*. 2016;9:436–447. doi: 10.1161/CIRCGENETICS.116.001506
17. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman AK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C, et al. Association of body mass index with DNA methylation and gene expression in blood cells and relations to cardiometabolic disease: a mendelian randomization approach. *PLoS Med*. 2017;14:e1002215. doi: 10.1371/journal.pmed.1002215
18. Richard MA, Huan T, Ligthart S, Gondalia R, Jhun MA, Brody JA, Irvin MR, Marioni R, Shen J, Tsai PC, et al. DNA methylation analysis identifies loci for blood pressure regulation. *Am J Hum Genet*. 2017;101:888–902. doi: 10.1016/j.ajhg.2017.09.028
19. Agha G, Mendelson MM, Ward-Caviness CK, Joehanes R, Huan T, Gondalia R, Salfati E, Brody JA, Fiorito G, Bressler J, et al. Blood leukocyte DNA methylation predicts risk of future myocardial infarction and coronary heart disease. *Circulation*. 2019;140:645–657. doi: 10.1161/CIRCULATIONAHA.118.039357
20. Battram T, Yousefi P, Crawford G, Prince C, Sheikhali Babaei M, Sharp G, Hatcher C, Vega-Salas MJ, Khodabakhsh S, Whitehurst O, et al. The EWAS catalog: a database of epigenome-wide association studies. *Wellcome Open Res*. 2022;7:41. doi: 10.12688/wellcomeopenres.17598.2
21. Huan T, Joehanes R, Song C, Peng F, Guo Y, Mendelson M, Yao C, Liu C, Ma J, Richard M, et al. Genome-wide identification of DNA methylation QTLs in whole blood highlights pathways for cardiovascular disease. *Nat Commun*. 2019;10:4267. doi: 10.1038/s41467-019-12228-z
22. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan JB, Gao Y, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. 2013;49:359–367. doi: 10.1016/j.molcel.2012.10.016
23. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)*. 2018;10:573–591. doi: 10.18632/aging.101414
24. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY)*. 2019;11:303–327. doi: 10.18632/aging.101684
25. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14:R115. doi: 10.1186/gb-2013-14-10-r115
26. Belsky DW, Caspi A, Corcoran DL, Sugden K, Poulton R, Arseneault L, Baccarelli A, Chamarti K, Gao X, Hannon E, et al. DunedinPACE, a DNA methylation biomarker of the pace of aging. *Elife*. 2022;11:11. doi: 10.7554/eLife.73420
27. Joyce BT, Gao T, Zheng Y, Ma J, Hwang SJ, Liu L, Nannini D, Horvath S, Lu AT, Bai Allen N, et al. Epigenetic age acceleration reflects long-term cardiovascular health. *Circ Res*. 2021;129:770–781. doi: 10.1161/CIRCRESAHA.121.318965
28. Lo YH, Lin WY. Cardiovascular health and four epigenetic clocks. *Clin Epigenetics*. 2022;14:73. doi: 10.1186/s13148-022-01295-7
29. Kim Y, Huan T, Joehanes R, McKeown NM, Horvath S, Levy D, Ma J. Higher diet quality relates to decelerated epigenetic aging. *Am J Clin Nutr*. 2022;115:163–170. doi: 10.1093/ajcn/nqab201
30. Huan T, Nguyen S, Colicino E, Ochoa-Rosales C, Hill WD, Brody JA, Soerensen M, Zhang Y, Baldassari A, Elhadad MA, et al. Integrative analysis of clinical and epigenetic biomarkers of mortality. *Aging Cell*. 2022;21:e13608. doi: 10.1111/acel.13608
31. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol*. 1979;110:281–290. doi: 10.1093/oxfordjournals.aje.a112813
32. Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham study. *Arch Intern Med*. 1979;139:857–861. doi: 10.1001/archinte.1979.03630450011006
33. Lu AT, Seeboth A, Tsai PC, Sun D, Quach A, Reiner AP, Kooperberg C, Ferrucci L, Hou L, Baccarelli AA, et al. DNA methylation-based estimator of telomere length. *Aging (Albany NY)*. 2019;11:5895–5923. doi: 10.18632/aging.102173
34. Lu AT, Binder AM, Zhang J, Yan Q, Reiner AP, Cox SR, Corley J, Harris SE, Kuo PL, Moore AZ, et al. DNA methylation GrimAge version 2. *Aging (Albany NY)*. 2022;14:9484–9549. doi: 10.18632/aging.204434
35. Pelegi-Siso D, de Prado P, Ronkainen J, Bustamante M, Gonzalez JR. Methylclock: a Bioconductor package to estimate DNA methylation age. *Bioinformatics*. 2021;37:1759–1760. doi: 10.1093/bioinformatics/btaa825
36. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13:86. doi: 10.1186/1471-2105-13-86
37. Ge T, Chen CY, Ni Y, Feng YA, Smoller JW. Polygenic prediction via Bayesian regression and continuous shrinkage priors. *Nat Commun*. 2019;10:1776. doi: 10.1038/s41467-019-09718-5
38. McCartney DL, Min JL, Richmond RC, Lu AT, Sobczyk MK, Davies G, Broer L, Guo X, Jeong A, Jung J, et al. Genome-wide association studies identify 137 genetic loci for DNA methylation biomarkers of aging. *Genome Biol*. 2021;22:194. doi: 10.1186/s13059-021-02398-9
39. Ma J, Yang Q, Hwang SJ, Fox CS, Chu AY. Genetic risk score and risk of stage 3 chronic kidney disease. *BMC Nephrol*. 2017;18:32. doi: 10.1186/s12882-017-0439-3
40. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, et al. A global reference for human genetic variation. *Nature*. 2015;526:68–74. doi: 10.1038/nature15393
41. Huang YT, Yang HI. Causal mediation analysis of survival outcome with multiple mediators. *Epidemiology*. 2017;28:370–378. doi: 10.1097/EDE.0000000000000651
42. Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychol Methods*. 2010;15:309–334. doi: 10.1037/a0020761
43. Imai K, Keele L, Yamamoto T. Identification, inference and sensitivity analysis for causal mediation effects. *Statist Sci*. 2010;25:51–71. doi: 10.1214/10-STS321
44. Imai K, Tingley D, Yamamoto T. Experimental designs for identifying causal mechanisms. *Journal of the Royal Statistical Society Series A: Statistics in Society*. 2013;176:5–51. doi: 10.1111/j.1467-985X.2012.01032.x
45. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA*. 2013;309:71–82. doi: 10.1001/jama.2012.113905
46. Lariscy JT, Hummer RA, Rogers RG. Cigarette smoking and all-cause and cause-specific adult mortality in the United States. *Demography*. 2018;55:1855–1885. doi: 10.1007/s13524-018-0707-2
47. Kim JK, Crimmins EM. Blood pressure and mortality: joint effect of blood pressure measures. *J Clin Cardiol Cardiovasc Ther*. 2020;2:2. doi: 10.31546/2633-7916.1009
48. Kris-Etherton P, Eckel RH, Howard BV, St Jeor S, Bazzarre TL. Nutrition committee population science C, clinical science Committee of the American Heart a. AHA science advisory: Lyon diet heart study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association step I dietary pattern on cardiovascular disease. *Circulation*. 2001;103:1823–1825. doi: 10.1161/01.cir.103.13.1823
49. Khoramdad M, Vahedian-Azimi A, Karimi L, Rahimi-Bashar F, Amini H, Sahebkar A. Association between passive smoking and cardiovascular disease: a systematic review and meta-analysis. *IUBMB Life*. 2020;72:677–686. doi: 10.1002/iub.2207
50. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, Sweis RN, Lloyd-Jones DM. Association of Body Mass Index with Lifetime Risk of cardiovascular disease and compression of morbidity. *JAMA Cardiol*. 2018;3:280–287. doi: 10.1001/jamacardio.2018.0022
51. Fuchs FD, Whelton PK. High blood pressure and cardiovascular disease. *Hypertension*. 2020;75:285–292. doi: 10.1161/HYPERTENSIONAHA.119.14240
52. Enserro DM, Vasan RS, Xanthakis V. Twenty-year trends in the American Heart Association cardiovascular health score and impact on subclinical and clinical cardiovascular disease: the Framingham offspring study. *J Am Heart Assoc*. 2018;7:7. doi: 10.1161/JAHA.118.008741
53. Makarem N, Castro-Diehl C, St-Onge MP, Redline S, Shea S, Lloyd-Jones D, Ning H, Aggarwal B. Redefining cardiovascular health to include sleep: prospective associations with cardiovascular disease in the MESA sleep study. *J Am Heart Assoc*. 2022;11:e025252. doi: 10.1161/JAHA.122.025252

54. Sun J, Li Y, Zhao M, Yu X, Zhang C, Magnussen CG, Xi B. Association of the American Heart Association's new "Life's essential 8" with all-cause and cardiovascular disease-specific mortality: prospective cohort study. *BMC Med*. 2023;21:116. doi: 10.1186/s12916-023-02824-8
55. Pottinger TD, Khan SS, Zheng Y, Zhang W, Tindle HA, Allison M, Wells G, Shadyab AH, Nassir R, Martin LW, et al. Association of cardiovascular health and epigenetic age acceleration. *Clin Epigenetics*. 2021;13:42. doi: 10.1186/s13148-021-01028-2
56. Lemke E, Vetter VM, Berger N, Banszerus VL, Konig M, Demuth I. Cardiovascular health is associated with the epigenetic clock in the Berlin aging study II (BASE-II). *Mech Ageing Dev*. 2022;201:111616. doi: 10.1016/j.mad.2021.111616
57. Higgins-Chen AT, Thrush KL, Wang Y, Minteer CJ, Kuo PL, Wang M, Niimi P, Sturm G, Lin J, Moore AZ, et al. A computational solution for bolstering reliability of epigenetic clocks: implications for clinical trials and longitudinal tracking. *Nat Aging*. 2022;2:644–661. doi: 10.1038/s43587-022-00248-2