The Journal of Nutrition, Health and Aging 28 (2024) 100203

Contents lists available at ScienceDirect

The Journal of Nutrition, Health and Aging

journal homepage: www.elsevier.com/locate/jnha

Original Article

Heterogeneity of aging and mortality risk among individuals with hypertension: Insights from phenotypic age and phenotypic age acceleration

Yuntao Feng [1], Hao Lin [1], Hongwei Tan *, Xuebo Liu *

Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China

ARTICLE INFO

Keywords:
Phenotypic age
Phenotypic age acceleration
Hypertension
Risk factors
Mendelian randomization

ABSTRACT

Objectives: Hypertension, a key contributor to mortality, is impacted by biological aging. We investigated the relationship between novel biological aging metrics - Phenotypic Age (PA) and Phenotypic Age Acceleration (PAA) - and mortality in individuals with hypertension, exploring the mediating effects of arterial stiffness (estimated Pulse Wave Velocity, ePWV), and Heart/Vascular Age (HVA).

Methods: Using data from 62,160 National Health and Nutrition Examination Survey (NHANES) participants (1999–2010), we selected 4,228 individuals with hypertension and computed PA, PAA, HVA, and ePWV. Weighted, multivariable Cox regression analysis yielded Hazard Ratios (HRs) relating PA, PAA to mortality, and mediation roles of ePWV, PAA, HVA were evaluated. Mendelian randomization (MR) analysis was employed to investigate causality between genetically inferred PAA and hypertension.

Results: Over a 12-year median follow-up, PA and PAA were tied to increased mortality risks in individuals with hypertension. All-cause mortality hazard ratios per 10-year PA and PAA increments were 1.96 (95% CI, 1.81–2.11) and 1.67 (95% CI, 1.52–1.85), respectively. Cardiovascular mortality HRs were 2.32 (95% CI, 1.97–2.73) and 1.93 (95% CI, 1.65–2.26) for PA and PAA, respectively. ePWV, PAA, and HVA mediated 42%, 30.3%, and 6.9% of PA's impact on mortality, respectively. Mendelian randomization highlighted a causal link between PAA genetics and hypertension (OR = 1.002; 95% CI, 1.000–1.003).

Conclusion: PA and PAA, enhancing cardiovascular risk scores by integrating diverse biomarkers, offer vital insights for aging and mortality evaluation in individuals with hypertension, suggesting avenues for intensified aging mitigation and cardiovascular issue prevention. Validations in varied populations and explorations of underlying mechanisms are warranted.

© 2024 The Author(s). Published by Elsevier Masson SAS on behalf of SERDI Publisher. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Hypertension is a major preventable risk factor for cardiovascular diseases (CVD), stroke, renal failure, and cognitive impairment [1]. Over the past 30 years, the number of adults (ages 30–79) with hypertension has almost doubled, affecting approximately 1.28 billion people worldwide and causing approximately 10 million deaths annually [1,2]. The age-standardized prevalence rate of hypertension rises from 7.3% at ages 30–34 to 58.2% at ages 75–79, suggesting that hypertension is more common in the older adults population [3]. Therefore, despite the complex and yet unclear etiology of hypertension, the inevitable biological aging is increasingly recognized as a key factor in the onset of hypertension.

Biological aging may participate in blood pressure regulation through pathways such as oxidative stress, inflammation, endothelial dysfunction and vascular remodeling [4,5]. However, aging is not a uniform phenomenon, and individuals of the same chronological age can exhibit different rates of biological aging and different health outcomes [6]. In order to better reflect the degree of aging and health status of individuals, researchers have calculated the biological age based on molecular-level biomarkers such as epigenetics, metabolomics and transcriptomics or clinical or functional indicators [7], which is capable of independently reflecting a person's rate of aging and potential lifespan, regardless of their chronological age [8,9]. However, there are some drawbacks in these calculated biological ages, such as methylation clocks being limited by tissue and cell types, and telomere length being influenced by

* Corresponding authors.
E-mail addresses: tanhongweikoui@163.com (H. Tan), liuxuebo70@hotmail.com (X. Liu).
[1] These authors contributed equally to this work.

http://doi.org/10.1016/j.jnha.2024.100203
Received 1 November 2023; Received in revised form 20 February 2024; Accepted 21 February 2024
Available online 8 March 2024
1279-7707/© 2024 The Author(s). Published by Elsevier Masson SAS on behalf of SERDI Publisher. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

environmental factors and genetic variations [10]. Therefore, a new, reliable and robust biological aging measurement is of practical importance for the stratified assessment and health management of hypertension.

Recently, phenotypic age (PA) is a novel indicator of biological aging, which has been proven to highly predict mortality even in different stratifications [11]. PA is calculated based on a combination of chronological age and nine clinical chemistry biomarkers that represent the expected age within the population that corresponds to a person's estimated mortality risk. Phenotypic age acceleration (PAA) is the residual of the regression between PA and chronological age, which reflects the relative aging level of an individual compared to their peers and degree of biological aging [12]. However, the PA and PAA's predictive value in patients with hypertension, a group vulnerable to cardiovascular complications, remains to be clarified.

The changes in arterial stiffness and cardiovascular age are closely associated with the inevitable process of biological aging [13,14]. Alterations in vascular structure and function caused by biological aging result in physiological maladaptation such as impaired vascular reactivity, increased vascular thickness, and stiffness [15,16], thereby increasing the risk of arteriosclerosis and cardiovascular age. The estimated pulse wave velocity (PWV) is a method to measure arterial stiffness, derived from easily obtainable data (age and blood pressure) using a standardized formula [4]. Based on the Framingham Risk Score (FRS) the heart age/vascular age (HVA) changes can be calculated [17]. In this study, we aim to explore whether ePWA and HVA can mediate or modify the association between PA, PAA, and mortality risk in the individuals with hypertension, and whether they can provide additional information for assessing and managing biological aging and hypertension.

Our study aims to explore the relationship between PA and PAA and mortality risk in hypertension, and to evaluate their potential value in hypertension management. Additionally, we seek to provide a more nuanced understanding of the relationship among aging phenotypes and hypertension and mortality outcomes in individuals with hypertension by mediation analysis.

2. Method

2.1. Study population

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey conducted in two-year cycles, and its results can be weighted to provide health and nutritional status for non-institutionalized adults and children in the United States. The data used in this study come from six NHANES survey cycles conducted between 1999 and 2018, with follow-up until the end of December 2019. A total of 62,160 participants were included in the data, of which 13,505 patients with hypertension were selected. After excluding participants with missing clinically relevant information, and mortality outcome data, uncertain medical history, and insufficient information for calculating PA, statistical analysis included 4,228 participants finally (Supplementary Fig. S1).

The study plan and research procedures were approved by the Ethics Review Committee of the National Center for Health Statistics and all adult participants provided written informed consent before the start. For more detailed information, please visit https://wwwn.cdc.gov/Nchs/Nhanes/.

2.2. Blood pressure assessment and hypertension classification

Blood pressure was measured by trained professionals through a standard sphygmomanometer, and participants were asked to rest quietly for 5 min before testing. The blood pressure value is the average of at least three measurements. Detailed information on quality assurance and quality control processes can be found in the physicians' section of the MEC operational manual [18].

The diagnosis of hypertension in participants includes being informed or diagnosed with hypertension by a doctor, having a systolic blood pressure (SBP) $\geq$140 mm Hg and/or diastolic blood pressure (DBP) $\geq$90 mm Hg, or reporting the use of antihypertensive medications.

2.3. PA and PAA

PA is a new method of calculating biological age proposed by Levine et al. [11], which can serve as a marker for tracking the aging process prior to the appearance of clinical symptoms of disease [19]. The calculation of PA involves nine biomarkers, including albumin, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count, as well as chronological age. PAA is the regression residual of chronological age on biological age, with its negative value indicating that an individual has a younger phenotypic age [20]. Supplementary Methods provides detailed calculation formulas for PA and PAA.

To minimize the impact of collinearity between PA and PAA on the results, we employed a grouping strategy. The individuals with hypertension was divided into high PA and low PA groups based on the median of PA and into high physiological age risk and low physiological age risk groups based on the positive or negative value of PAA.

2.4. FRS and HVA

The FRS is calculated specifically in different genders according to the method described by D'Agostino et al., incorporating factors such as age, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, arm systolic pressure, ongoing hypertension treatment, smoking, and diabetes status [17]. In addition, after excluding other risk factors, the HVA corresponding to people with the same predicted risk obtained through FRS can be calculated [17]. The cardiovascular disease risk of a person is converted to the age of a person with the same risk but all other risk factors are at normal levels. In the calculated HVA, for the mathematical calculations, we used the ages of 80 and 30 to replace the descriptions of "$>$80" and "$<$30".

Based on the comparison of HVA with chronological age, we classify patients whose HVA is greater than their chronological age into the high-risk group for age-associated disease, and the rest are classified into the low-risk group for age-associated disease.

2.5. Arterial stiffness

The degree of arterial stiffness is determined by calculating ePWV, which is a new non-invasive method of calculation [4]. The level of ePWV is also related to arterial aging, and when ePWV exceeds 10 m/s, the risk of death correspondingly increases [21,22]. Detailed formulas for ePWV calculation are shown in Supplementary Methods.

Based on the ePWV level at 10 m/s, the population is classified into a high arterial stiffness group and a low arterial stiffness group.

2.6. Outcome: mortality

The National Center for Health Statistics has publicly released national mortality index data. Based on this, the participants' vital status (all-cause mortality and cause-specific mortality) was determined by matching with the national mortality rate index up to the end of December 2019. The follow-up time was calculated as the time from baseline to death or censoring date. According to International Classification of Diseases, 10th Revision (ICD-10), there are nine cause-specific mortality rates. The ICD-10 codes for the 9 cause-specific mortality can be seen in the Supplementary Table S1.

2.7. Other variables

Covariates were selected based on established correlations with aging, mortality, and cardiovascular health in theindividuals with hypertension to control for potential confounding factors that could influence the relationship between PA/PAA and mortality risk.

Standardized questionnaires and computer-assisted personal interview systems were used to collect demographic information, including age, gender, ethnicity, education level, marital status, Family poverty-to-income (FamilyPIR) ratio, waist, body mass index (BMI), smoking and drinking status, history of Chronic Obstructive Pulmonary Disease (COPD), history of arthritis, and use of medication. The levels of total triglycerides (TG), TC, low-density lipoprotein cholesterol (LDL), HDL, serum creatinine (CR) and glycated hemoglobinA1c (HbAc1) were assessed using standard biochemical techniques by trained technicians in NHANES. More details can be found in the NHANES Laboratory/Medical Technicians Procedures Manual [23].

Estimated glomerular filtration rate (eGFR) is calculated through serum CR, with Charlson Comorbidity Index (CCI) determined by health status [24]. eGFR levels are categorized into quartiles to address covariance with CR. Diabetes mellitus (DM) encompasses self-reported cases, medication use, or fasting glucose $\geq$126 mg/dL. Prediabetes is defined by HbA1c (5.7%–6.4%), impaired fasting glucose (IFG, 100–125 mg/dL), or impaired glucose tolerance (IGT, 140–199 mg/dL). Combined heart failure, coronary artery disease, angina, myocardial infarction, and stroke are termed CVD [25]. Chronic kidney disease (CKD) identification aligns with Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. Hyperlipidemia criteria include lipid-lowering medication use, specific LDL, HDL, or TG levels (LDL levels $\geq$140 mg/dL, or male HDL $<$40 mg/dL or female HDL $<$50 mg/dL) [26]. The frailty index (FI) spans from 0 to 1, with frailty thresholds set at $>$0.25 for frail, 0.16–0.25 for pre-frail, and $<$0.16 for non-frail. The frailty index covers multiple systems including 49 deficiencies, whose individual variables and their scores are shown in Supplementary Table S2.

2.8. Mendelian randomization

2.8.1. Data source

Instrumental variants (IVs) for genetically inferred PAA were sourced from a genome-wide association study (GWAS) meta-analysis [27]. Summary-level data were also obtained from a GWAS of hypertension, encompassing 54,358 patients and 408,652 controls. To mitigate bias from population stratification in our MR analysis, the GWAS data comprised individuals of European ancestry, both male and female [28] (Supplementary Table S3).

2.8.2. IV selection

Three criteria for Mendelian randomization studies need to be implemented: a significant association between IVs and exposure; no IVs linkage with confounders related to outcomes; and IVs affecting outcomes only through exposure variables [29]. SNPs related to PAA ($p < 5e-08$) were selected. Clustering SNPs in line with the 1000 Genomes Project's LD reference, the most significant SNPs were retained. Phenoscanner was employed to eliminate SNPs possibly infringing MR analysis independence [30]. The proportion of variance explained by each SNP was evaluated to assess the strength of each SNP, and the F-statistic computed (Supplementary Methods). Supplementary materials (Supplementary Fig. S2 and Supplementary Table S4) detail the IVs selection process and summary.

2.8.3. Analysis

The main MR analysis utilized inverse variance weighted (IVW) meta-analysis [31]. Sensitivity analysis, comprising weighted median and MR-PRESSO, was conducted to offset polymorphic effects' bias [32]. Cochran's Q assessed IV heterogeneity, while MR-PRESSO detected pleiotropy [33,34]. A sensitivity analysis, cML-MA-BIC, was executed for possible instrumental variable hypothesis breaches [35]. The Bonferroni test adjusted Mendelian randomization for multiple testing.

2.9. Statistical analysis

Due to the complex sampling survey design of NHANES, we utilized relevant survey weights (MEC2yr) to generate nationally representative estimates for the data from 1999 to 2010. In the baseline information, continuous variables and categorical variables were displayed as mean $\pm$ sd and percentages, with their group differences compared through weighted analysis of variance tests and weighted Chi-square tests. Survival probabilities for people with hypertension with varying PA levels were assessed with standardized Kaplan–Meier curves and log-rank tests. The relationship (hazard ratio, HR, and 95% confidence intervals, CIs) between PA and PAA with all-cause mortality and cause-specific mortality was evaluated using univariate weighted Cox proportional hazards models and multivariate weighted Cox proportional hazards models. The models' proportional hazard assumption was verified with Schoenfeld residuals (Supplementary Fig. S3).

To mitigate confounding influences, covariates were adjusted across models (Models 1–4). Restricted cubic spline curves (RCS) depicted the relationship between PA, PAA, and both all-cause and cause-specific mortality in patients with hypertension. The predictive value of PA and PAA for mortality in individuals with hypertension was assessed using the ROC curve and AUC, compared to the FRS. The DeLong test determined the statistical significance of AUC differences. Causal mediation analysis was conducted to determine if the association between PA (X) and both all-cause and cardiovascular disease mortality (Y) is mediated through arterial stiffness, cardiac/vascular age, and PAA (M) [36]. A mediating effect is acknowledged only when there's a significant relationship between X and M and a significant connection between M and Y [37]. We also compared the estimates between models with and without the hypothesized mediators. Subgroup analyses assessed the consistency of associations between PA, PAA, and mortality, visualized using forest plots.

All statistical analyses were conducted using R software (Version 4.2.1, The R Foundation; http://www.R-project.org) and EmpowerStats software (Version 5.0, X&Y Solutions, Inc., Boston, MA; http://www.empowerstats.com). A p-value of less than 0.05 was deemed statistically significant.

3. Result

3.1. Characteristics

The 4228 patients with hypertension screened in NHANES represent 15 million non-institutionalized residents in the United States, among which 50.09% are female, 53.83% are white individuals, and the weighted average age is 56.24 years (Supplementary Table S5). The PA and PAA of the participants were 53.11 $\pm$ 0.42 and -3.12 $\pm$ 0.17, respectively.

Based on the PAA level, the patients are divided into high physiological age risk and low physiological age risk groups, and their baseline characteristics are shown in Table 1. Those who were former heavy drinkers, had high BMI levels, had histories of cardiovascular disease, chronic kidney disease, and diabetes, or were in pre-diabetic stages were more likely to be in the high physiological age risk group (Table 1).

3.2. Relationship between PA or PAA and all-cause mortality

During a median follow-up period of 12 years, a total of 1,469 deaths were recorded. Furthermore, for each additional 10 years of PA, the risk of mortality in patients with hypertension increased by 75% (HR = 1.75, 95% CI = 1.63–1.87, $P < 0.0001$). After multi-factor adjustments, the association between PA and the risk of death in the individuals with

Table 1
Baseline characteristics of study population in high physiological age risk and low physiological age risk groups.

Variable	High physiological age risk (1212, 24.18%)	Low physiological age risk (3016, 75.82%)
Age	58.18 (0.57)	55.62 (0.39)
Gender		
Female	538 (45.84)	1580 (52.11)
Male	674 (54.16)	1436 (47.89)
Race/ethnicity		
Black	332 (17.37)	570 (9.78)
Mexican	177 (5.25)	520 (4.86)
Other	97 (6.75)	256 (8.09)
White	606 (70.63)	1670 (77.27)
Smoke		
former	406 (31.57)	1024 (33.48)
never	507 (41.56)	1554 (50.86)
now	299 (26.87)	438 (15.67)
Drinking status		
former	382 (28.56)	718 (19.86)
heavy	174 (14.32)	408 (14.30)
mild	368 (32.87)	1076 (39.82)
moderate	102 (10.13)	363 (13.99)
never	186 (14.12)	451 (12.03)
Maritalstatus		
Divorced	161 (12.66)	320 (9.90)
Living with partner	74 (5.53)	128 (5.02)
Married	614 (54.01)	1811 (65.17)
Never married	122 (11.22)	238 (7.96)
Separated	42 (3.03)	72 (1.71)
Widowed	199 (13.54)	447 (10.24)
Education		
College graduate or above	140 (13.63)	588 (24.63)
High school or less	773 (57.70)	1666 (47.16)
Some college	299 (28.67)	762 (28.21)
Frailty		
Non-frailty	333 (28.75)	1790 (63.55)
Pre-Frailty	399 (34.72)	781 (24.41)
Frailty	480 (36.52)	445 (12.05)
Diastolic blood pressure	71.69 (0.63)	74.35 (0.40)
Systolic blood pressure	134.18 (0.60)	132.97 (0.41)
Family poverty income ratio	2.64 (0.06)	3.20 (0.04)
Waist	110.77 (0.64)	101.12 (0.38)
Body mass index	32.98 (0.29)	29.47 (0.16)
Total cholesterol	190.89 (1.75)	203.03 (0.92)
High density lipoprotein	49.14 (0.55)	54.26 (0.37)
Low density lipoprotein	111.32 (1.49)	120.68 (0.85)
Total triglycerides	152.21 (2.41)	140.45 (1.50)
Creatinine	1.13 (0.02)	0.87 (0.00)
HbA1c	6.37 (0.05)	5.51 (0.01)
eGFR	77.58 (0.98)	87.33 (0.48)
PhenoAge	65.34 (0.70)	49.21 (0.39)
ePWV	9.64 (0.07)	9.36 (0.05)
HVA	69.11 (0.52)	62.12 (0.41)
Antihypertensive drugs		
NO	332 (28.50)	1222 (42.95)
YES	880 (71.50)	1794 (57.05)
Lipid-lowering drugs		
NO	786 (65.68)	2222 (74.48)
YES	426 (34.32)	794 (25.52)
Glucose-lowering drugs		
NO	830 (73.91)	2759 (93.89)
YES	382 (26.09)	257 (6.11)
Cardiovascular diseases		
NO	852 (73.32)	2539 (86.56)
YES	360 (26.68)	477 (13.44)
Hyperlipidemia		
NO	196 (15.74)	508 (16.38)
YES	1016 (84.26)	2508 (83.62)
Diabetes mellitus		
No	155 (15.25)	1101 (41.85)
Prediabete	406 (36.14)	1388 (44.39)
Diabetes	651 (48.61)	527 (13.76)
Chronic kidney diseases		
NO	589 (54.98)	2337 (83.13)
YES	623 (45.02)	679 (16.87)
Chronic obstructive pulmonary disease		
NO	1108 (91.07)	2833 (93.83)
YES	104 (8.93)	183 (6.17)

Table 1 ***(continued)***

Variable	High physiological age risk (1212, 24.18%)	Low physiological age risk (3016, 75.82%)
Arthritis		
NO	639 (52.41)	1820 (62.59)
YES	573 (47.59)	1196 (37.41)

Continuous variables are presented as the weighted mean (Standard error, SE), category variables are presented as the counts (weighted %).

Table 2
Cox regression analysis results examining the association of Phenotypic age on all-cause and cause-specific mortality in Hypertension population from NHANES 1999 to 2010.

PhenoAge	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)	Adjusted Model 2 HR (95% CI)	Adjusted Model 3 HR (95% CI)	Adjusted Model 4 HR (95% CI)
All-cause mortality	1.75 (1.63,1.87)	1.64 (1.55,1.73)	1.57 (1.49,1.66)	2.03 (1.87,2.20)	1.96 (1.81,2.11)
Cardiovascular disease	2.03 (1.86,2.21)	1.89 (1.73,2.06)	1.82 (1.66,2.00)	2.49 (2.14,2.90)	2.32 (2.01,2.69)
Cerebrovascular disease	2.20 (1.94,2.51)	2.19 (1.88, 2.56)	2.30 (1.92, 2.77)	2.85 (2.18,3.71)	2.55 (1.85, 3.53)
Respiratory disease	2.03 (1.82,2.27)	1.88 (1.65, 2.13)	2.03 (1.69, 2.43)	3.05 (2.19, 4.23)	3.16 (2.15, 4.63)
Diabetes mellitus	2.06 (1.78,2.39)	2.03 (1.71, 2.41)	2.01 (1.60, 2.51)	1.54 (0.98, 2.42)	1.59 (1.05, 2.43)
Renal disease	2.44 (2.05,2.91)	2.61 (1.83, 3.73)	3.21 (2.48, 4.17)	3.51 (2.78, 4.45)	5.04 (3.97, 6.40)
Caner	1.92 (1.74,2.12)	1.78 (1.60,1.98)	1.77 (1.60,1.97)	2.22 (1.92,2.57)	2.21 (1.91,2.56)
Influenza and pneumonia	2.08 (1.77,2.45)	2.04 (1.68, 2.48)	2.38 (1.78, 3.19)	5.12 (2.69, 9.74)	5.04 (2.13,11.92)
Alzheimer's disease	2.17 (1.93,2.45)	1.81 (1.44,2.26)	1.97 (1.54,2.51)	2.80 (1.75,4.46)	2.58 (1.60,4.16)
Accidents	1.13 (0.80,1.60)	1.21 (0.76,1.95)	1.25 (0.81, 1.94)	1.64 (0.88, 3.05)	1.55 (0.88, 2.71)
Residual (all other causes)	1.82 (1.66,1.99)	1.79 (1.64, 1.95)	1.74 (1.61, 1.89)	2.47 (2.10,2.89)	2.49 (2.12,2.91)

Cerebrovascular disease indicates all deaths resulting from conditions affecting the blood vessels in the brain, such as stroke and transient ischemic attack (TIA).
Respiratory diseases indicated all deaths from chronic lower respiratory diseases.
Renal disease indicated all deaths from nephritis, nephrotic syndrome, and nephrosis.
HR per 10 years increase in Phenotypic age with 95% CIs.
Model 1: age (20–44, 45–64, >65 years), SBP (continuous), DBP (continuous).
Model 2: Model 1 + gender, race/ethnicity, smoke, alcohol user, FamilyPIR, education, BMI (continuous).
Model 3: Model 2 + HbA1c (continuous), CCI, TC (continuous), TG (continuous), CR (continuous), eGFR (continuous), lipid-lowering drug (yes/no), glucose-lowering drug (yes/no).
Model 4: Model 3 + CVD (yes/no), CKD (yes/no), DM (yes/no), hyperlipidemia (yes/no), arthritis (yes/no), COPD (yes/no), waist (yes/no), Marital status (yes/no).
HR, Hazard ratio; CI, confidence interval; BMI, body mass index; HbA1c, Glycated hemoglobin; TC, total cholesterol; CR, creating; TG, triglyceride; DBP, diastolic blood pressure; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; DM, Diabetes mellitus; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; PA, Phenotypic age.

hypertension still existed (Model 1: HR = 1.64, 95% CI = 1.55–1.73, P < 0.0001; Model 2: HR = 1.57, 95% CI = 1.49–1.66, P < 0.0001; Model 3: HR = 2.03, 95% CI = 1.87–2.20, P < 0.0001). Furthermore, after full adjustment in Model 4, for each additional 10 years of PA, the all-cause mortality risk increased by 96% for patients with hypertension (HR = 1.96, 95% CI = 1.81–2.11, P < 0.0001, Table 2).

Additionally, PAA can also serve as an independent predictive factor for the risk of death in patients with hypertension. For each additional 10 years of PAA, the risk of death in patients with hypertension increases by 39% (HR = 1.39, 95% CI = 1.30–1.49, P < 0.0001). After full multi-factor adjustments, for each additional 10 years of PAA, the risk of death in patients with hypertension increases by 67% (Model 4: HR = 1.67, 95% CI = 1.51–1.84, P < 0.0001, Table 3).

The individuals with hypertension was divided into high and low groups based on the median of PA, to explore the relationship between PA and survival rate in the individuals with hypertension. The Kaplan–Meier curve shows that patients with hypertension and low levels of PA have a higher survival rate (Supplementary Fig. S4). Moreover, in the unadjusted model (High vs Low: HR = 7.76, 95% CI = 6.70–8.98, P < 0.0001) and multi-factor adjusted model (High vs Low: HR = 2.21, 95% CI = 1.73–2.81, P < 0.0001), patients with hypertension and high PA levels have a higher risk of death (Supplementary Fig. S4A). Similar results were observed in the stratification of PAA, with the low physiological age risk group having a higher survival rate and lower risk of death (high physiological age risk vs low physiological age risk: HR = 1.36, 95% CI = 1.18–1.57, P < 0.0001; Supplementary Fig. S4B).

3.3. Relationship between PA or PAA and cause-specific mortality

Among the 1,469 deaths recorded in patients with hypertension, 411 patients died from cardiovascular diseases. For each additional 10 years of PA or PAA in patients with hypertension, the risk of death from CVD increases by 103% (P < 0.0001, Table 2) and 54% (P < 0.0001, Table 3) respectively. After full multivariate adjustments, with the increase in PA and PAA, the risk of death from CVD in patients with hypertension eventually increases by 132% (P < 0.0001, Table 2) and 93% (P < 0.0001, Table 3). Furthermore, patients with hypertension and low PA levels have a higher survival rate in terms of CVD mortality. After multivariate adjustment, compared to the low PA group, the high PA group had a 1.57-fold increased risk of CVD mortality (High vs Low: HR = 2.57, 95% CI = 1.71–3.87, P < 0.0001; Supplementary Fig. S4C). Similar results were also observed in the stratification of PAA, where the high physiological age risk group had a higher risk of CVD mortality (high physiological age risk vs low physiological age risk: HR = 1.60, 95% CI = 1.20–2.15, P < 0.0001; Supplementary Fig. S4D).

Moreover, in the final adjusted model, for each additional 10 years of PA and PAA in patients with hypertension, the risk of death from other cause specific mortality increases by 59%–404% (excluding Accidents, P > 0.05, Table 2) and 53%–289% (excluding Accidents and Alzheimer's disease, P > 0.05, Table 3) respectively. The restricted cubic splines (RCS) model was used to analyze the dose-response association between PA and PAA and both all-cause mortality and cardiovascular disease mortality, which demonstrated a non-linear relationship of them (non-linear P > 0.05, Supplementary Fig. S5).

Table 3
Cox regression analysis results examining the association of Phenotypic age acceleration on all-cause and cause-specific mortality in Hypertension population from NHANES 1999 to 2010.

Phenotypic age acceleration	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)	Adjusted Model 2 HR (95% CI)	Adjusted Model 3 HR (95% CI)	Adjusted Model 4 HR (95% CI)
All-cause mortality	1.39 (1.30,1.49)	1.53 (1.45, 1.62)	1.46 (1.38, 1.54)	1.74 (1.58, 1.92)	1.67 (1.51, 1.84)
Cardiovascular disease	1.54 (1.38,1.73)	1.70 (1.56, 1.86)	1.63 (1.48, 1.79)	2.03 (1.67, 2.48)	1.93 (1.58, 2.36)
Cerebrovascular disease	1.37 (1.19,1.58)	1.65 (1.37, 2.00)	1.85 (1.53, 2.23)	2.29 (1.59, 3.30)	2.24 (1.49, 3.38)
Respiratory disease	1.44 (1.24,1.67)	1.64 (1.39, 1.94)	1.68 (1.31, 2.15)	2.48 (1.56, 3.94)	2.58 (1.50, 4.44)
Diabetes mellitus	1.67 (1.45,1.92)	1.97 (1.70, 2.29)	2.00 (1.64, 2.44)	1.57 (1.11, 2.23)	1.53 (1.06, 2.20)
Renal disease	1.79 (1.54,2.09)	2.33 (1.70, 3.19)	2.91 (2.49, 3.41)	3.73 (3.10, 4.49)	3.89 (3.23, 4.69)
Caner	1.44 (1.28,1.61)	1.59 (1.43, 1.77)	1.57 (1.40, 1.75)	1.89 (1.57, 2.28)	1.87 (1.55, 2.26)
Influenza and pneumonia	1.55 (1.33,1.82)	1.75 (1.45, 2.11)	1.85 (1.45, 2.35)	3.72 (1.65, 8.40)	3.64 (1.38, 9.60)
Alzheimer's disease	0.71 (0.43,1.18)	0.78 (0.49,1.23)	1.03 (0.62, 1.73)	1.17 (0.54, 2.54)	1.17 (0.54, 2.48)
Accidents	1.30 (1.07,1.59)	1.31 (1.04,1.63)	1.32 (1.01, 1.72)	1.90 (1.00, 3.61)	1.77 (0.95, 3.32)
Residual (all other causes)	1.41 (1.32,1.50)	1.63 (1.51,1.76)	1.60 (1.49,1.71)	1.85 (1.48,2.31)	1.80 (1.43,2.26)

Cerebrovascular disease indicates all deaths resulting from conditions affecting the blood vessels in the brain, such as stroke and transient ischemic attack (TIA).
Respiratory diseases indicated all deaths from chronic lower respiratory diseases.
Renal disease indicated all deaths from nephritis, nephrotic syndrome, and nephrosis.
HR per 10 years increase in Phenotypic age acceleration with 95% CIs.
Model 1: age (20–44, 45–64, >65 years), SBP (continuous), DBP (continuous).
Model 2: Model 1 + gender, race/ethnicity, smoke, alcohol user, FamilyPIR, education, BMI (continuous).
Model 3: Model 2 + HbA1c (continuous), CCI, TC (continuous), TG (continuous), CR (continuous), eGFR (continuous), lipid-lowering drug (yes/no), glucose-lowering drug (yes/no).
Model 4: Model 3 + CVD (yes/no), CKD (yes/no), DM (yes/no), hyperlipidemia (yes/no), arthritis (yes/no), COPD (yes/no), waist (yes/no), Marital status (yes/no).
HR, Hazard ratio; CI, confidence interval; BMI, body mass index; HbA1c, Glycated hemoglobin; TC, total cholesterol; CR, creating; TG, triglyceride; DBP, diastolic blood pressure; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; DM, Diabetes mellitus; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease, low density lipoprotein.

3.4. Predictive value of PA and PAA

ROC analysis was used to evaluate the predictive value of PA and PAA for all-cause mortality and CVD mortality in patients with hypertension. The results indicate that PA and PAA have an independent predictive role for all-cause mortality (AUC = 0.836, 95% CI 0.8234–0.8486, $P < 0.001$; AUC = 0.763, 95% CI 0.7466–0.7793, $P < 0.001$) and cardiovascular disease mortality (AUC = 0.851, 95% CI 0.8313– 0.8709, $P < 0.001$; AUC = 0.786, 95% CI 0.7610–0.8112, $P < 0.001$). Compared to the FRS (AUC for all-cause mortality = 0.763, 95% CI 0.7466–0.7793, $P < 0.001$; AUC for CVD = 0.786, 95% CI 0.7610–0.8112, $P < 0.001$), PA has a higher predictive value for the prognostic risk in patients with hypertension, whereas PAA might be slightly less effective (Supplementary Fig. S6).

3.5. Mediation analysis

Causal mediation analysis elucidated whether and to what extent arterial stiffness (ePWV), HVA, and PAA mediate the association between PA and all-cause and cardiovascular disease mortality risks in patients with hypertension (Fig. 1; Supplementary Table S6).

In models unadjusted for arterial stiffness (ePWV), HVA, and PAA, hypertension patients with high PA levels had a 1.21-fold increased risk of all-cause mortality (HR = 2.21, 95% CI = 1.73–2.81, $P < 0.0001$) and a 1.57-fold increased risk of cardiovascular disease mortality (HR = 2.57, 95% CI = 1.71–3.87, $P < 0.0001$) respectively, compared to those with low PA levels. After separately adjusting for ePWV, HVA, and PAA, the hazard ratios for all-cause and CVD mortality when comparing patients with hypertension and high PA to those with low PA were respectively reduced to 1.64 (95% CI = 1.27–2.09, $P < 0.001$) /1.66 (95%CI = 1.06–2.59, $P < 0.0001$), 2.10 (95% CI = 1.64–2.68, $P < 0.0001$)/2.35 (95% CI = 1.55–3.57, $P < 0.0001$) and 1.75 (95% CI = 1.37–2.24, $P < 0.0001$) /1.88 (95% CI = 1.24–2.86, $P < 0.0001$). Consequently, the proportions of all-cause and CVD mortality effects mediated were 42.0% and 48.7% ($P < 0.0001$) by ePWV, 6.9% and 11.2% ($P < 0.0001$) by HVA, and 30.3% and 33.3% ($P < 0.0001$) by PAA respectively.

However, when comparing the high physiological age risk group with the low physiological age risk group, the proportions mediated by ePWV and HVA were not statistically significant ($P > 0.05$, Supplementary Table S6).

3.6. MR analysis of the causal relationship between genetically inferred PAA and hypertension

In the GWAS data, 10 SNPs were identified (Table S4). SNPs without genome-wide significant associations or overlaps with the outcome trait were included in the analysis as genetic instruments. Upon adjusting for confounding SNPs, the MR effect was gauged using a fixed-effect model. Inverse Variance Weighted (IVW) analysis identified a tight link between PAA and hypertension incidence [Odds Ratio (OR) = 1.002; 95% Confidence Interval (CI) 1.000–1.003; $P = 0.023$; Supplementary Fig. S7], suggesting a potential positive causal relationship. Similar associations persisted in MR-PRESSO and cML-MA-BIC analyses after outlier elimination, though no significant link was seen in the weighted median model (OR = 1.001, 95% CI 0.999–1.004, $P = 0.067$) and MR Egger Model (OR = 1.000, 95% CI 0.996–1.004, $P = 0.984$). The Cochran Q test reported no notable heterogeneity (Cochran's $Q = 10.515713$, $p = 0.310$), and no pleiotropic bias was found in sensitivity analysis concerning genetic prediction of PAA. Additionally, Leave-One-Out (LOO) analysis assured that the results were not influenced by any single SNP. The MR-Egger regression intercept showed no apparent horizontal pleiotropy (intercept = 0.001, $p = 0.319$).

3.7. Subgroup analysis

PA and PAA are positively associated with the overall mortality and CVD mortality risk in patients with hypertension across different genders, ages, races, and stratifications of various chronic diseases including COPD, CKD, and hyperlipidemia (Fig. 2 and Supplementary Fig. S8). This suggests that different demographic characteristics and disease statuses do not cause bias in the predictive effects of PA and PAA on mortality. Interestingly, among males with hypertension, black individuals, or those with obesity (high BMI), the predictive capacity of PA or PAA for all-cause or CVD mortality appeared less pronounced, despite non-significant differences (interaction $P > 0.05$). Therefore, these factors should be taken into account when assessing and

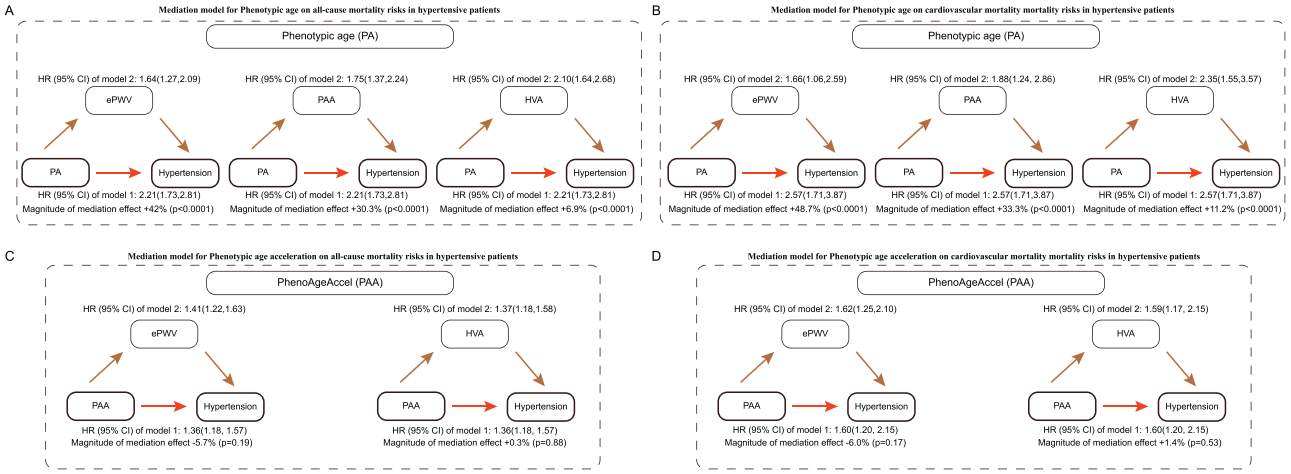

Fig. 1. Mediation analysis. (A) Represents mediation model for PA on all-cause mortality risks in patients with hypertension: hypertension as the dependent variable (Y), the PA as the independent variable (X), and the ePWV, PAA and HVA respectively as the mediator (M); (B) Represents mediation model for PA on cardiovascular mortality mortality risks in patients with hypertension: hypertension as the dependent variable (Y), the PA as the independent variable (X), and the ePWV, PAA and HVA respectively as the mediator (M); (C) Represents mediation model for PAA on all-cause mortality risks in patients with hypertension: hypertension as the dependent variable (Y), the PA as the independent variable (X), and the ePWV and HVA respectively as the mediator (M); (D) Represents mediation model for PAA on cardiovascular mortality mortality risks in patients with hypertension: hypertension as the dependent variable (Y), the PA as the independent variable (X), and the ePWV and HVA respectively as the mediator (M). Note: PA: Phenotypic age; PAA: Phenotypic age acceleration; ePWV, estimated pulse wave velocity; HVA, heart age/vascular age; X: independent variable; Y: dependent variable. "+" represents a positive mediating effect and "–" represents a negative mediating effect. P □ 0.05 indicate that there is no mediating effect.

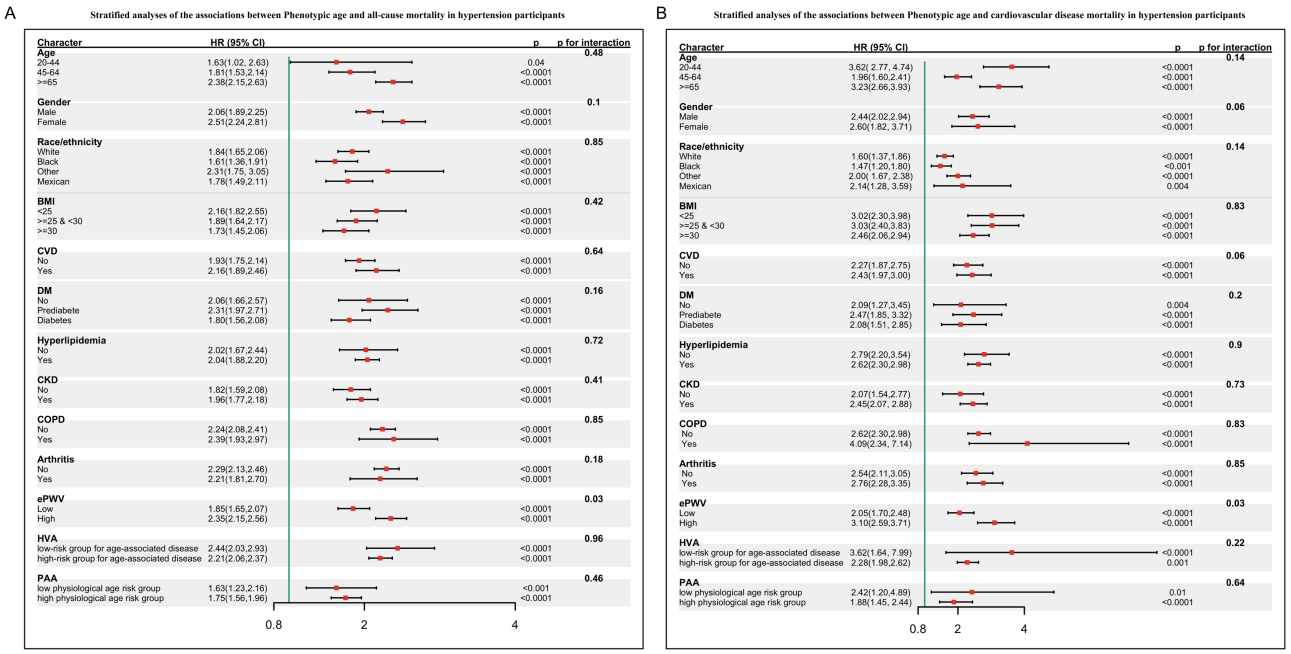

Fig. 2. A. Stratified analyses of the associations (hazard ratios, 95% CIs) between Phenotypic age and all-cause mortality in hypertension participants. B. Stratified analyses of the associations (hazard ratios, 95% CIs) between Phenotypic age and cardiovascular disease mortality in hypertension participants. The adjustment of HRs involves the following covariates (excluding the stratified variables): gender, age, race, SBP, DBP, BMI, smoking status, drinking status, FamilyPIR, education level, TC, TG, CR, eGFR, HbA1c, frailty, Antihypertensive drugs, lipid-lowering drugs, hypoglycemic drugs, CVD, DM, hyperlipidemia, CKD, COPD, arthritis, ePWV, HVA and PAA. HR, Hazard ratio; CI, confidence interval; BMI, body mass index; HbA1c, Glycated hemoglobin; TC, total cholesterol; CR, creating; TG, triglyceride; DBP, diastolic blood pressure; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; DM, Diabetes mellitus; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; ePWV, estimated pulse wave velocity; HVA, heart age/vascular age; PAA, Phenotypic age acceleration.

managing the biological aging and mortality risks in the individuals with hypertension.

Further analysis was conducted to investigate the predictive role of PA on overall mortality and CVD mortality in patients with hypertension among different levels of arterial stiffness, HVA, and PAA populations with hypertension. It was found that only in the high arterial stiffness group, the predictive effect of PA on overall mortality (interaction P = 0.03, Fig. 2A) and CVD mortality (interaction P = 0.03, Fig. 2B) among patients with hypertension was different. However, no significant differences were observed in the predictive effects of PAA on overall mortality and CVD mortality among different levels of arterial stiffness, HVA populations (Supplementary Fig. S8).

4. Discussion

Our study examined the link between biological aging metrics (PA and PAA) and mortality in people with hypertension, focusing on arterial stiffness (ePWV) and HVA mediation. PA and PAA emerged as independent mortality predictors. ePWV and PAA predominantly mediated the PA-mortality association. Genetically inferred PAA causally related to hypertension progression was identified. These results underscore the significance of integrating age-associated risk measures and vascular health in hypertension mortality management.

PA is a composite biomarker that reflects the cumulative effects of aging on multiple biological systems [11]. PAA is the difference between PA and chronological age, indicating the deviation from normal aging process. Both PA and PAA can capture the heterogeneity of aging among individuals with the same chronological age, and provide more accurate estimation of mortality risk than chronological age alone [12].

However, the hazard ratios of PA and PAA remain consistent across adjusted models. This stems from complex interactions between aging, hypertension, and mortality, with each model capturing different facets of these variables, affecting risk ratios. Additionally, nonlinear relationships of some covariates with outcomes may cause inconsistencies in risk ratios compared to unadjusted models. Furthermore, unmeasured confounders or varying impacts of measured confounders in different models might also contribute to this variability. And, in complex diseases, the hazard ratios exhibit similar variations when different sets of covariates are included in the model [38,39].

The study illuminates ePWV, an arterial stiffness marker, as central in PA's association with mortality risk in individuals with hypertension, corroborating prior findings linking arterial stiffness to elevated physiological age and mortality [40–42]. People with hypertension stratified by ePWV demonstrated its mediation role, accounting for 42.0% of PA's influence on mortality, which underscores arterial stiffness's critical role in high-PA people with hypertension. However, ePWV did not fully account for the effect of PA, indicating that other mechanisms may also be involved.

HVA represents heart and vascular ages, linked to cardiovascular risk. In patients with hypertension, HVA was found to mediate 13.2% of mortality risk, reflecting its complex influence. HVA includes physiological changes like arterial stiffness and endothelial dysfunction, variably affecting mortality risk [43]. The complexity partly explains its limited mediating effect. The interplay of HVA with demographic factors, lifestyle behaviors, and comorbidities also impacts mortality risk [44]. Additionally, HVA's scope, focusing on risk factors such as age, sex, smoking, HDL cholesterol, and systolic pressure, may exclude other crucial elements like BMI, diabetes, and lifestyle factors, and its calculation could introduce measurement errors. These results emphasize the necessity for further research to understand HVA's comprehensive role and investigate other mortality factors in individuals with hypertension.

PAA causally relates to hypertension onset and progression, potentially reflecting biological phenomena like epigenetic alterations, telomere shortening, oxidative stress, and inflammation [12,45,46], which may not be captured by PA. These processes may also contribute to the development and progression of hypertension and its complications [47,48]. Apart from ePWV and HVA, PAA mediated 30.3% of the association between PA and mortality risk in patients with hypertension, indicating that aging-related mechanisms not captured by PA also significantly contribute to the relationship between PA and mortality risk in patients with hypertension. Therefore, measuring PAA may provide additional information for assessing and managing mortality risk in individuals with hypertension. However, due to selection bias in the NHANES population or limitations of cross-sectional data, further studies are needed to explore the role of PAA.

Besides, although the interaction was not statistically different, the impact of PA or PAA on mortality appears diminished in obese people with hypertension compared to those with healthy BMI, suggesting an "obesity paradox". This phenomenon might result from elevated baseline mortality risk in obese people with hypertension and the metabolic reserves of overweight individuals against frailty [49,50]. Other confounders, like diet or medication, may affect PA or PAA's predictive power [51]. Women with hypertension and higher PA or PAA showed elevated cardiovascular and all-cause mortality risks than men, possibly due to increased arterial stiffness and vascular aging markers. Gender differences in CVD risk, such as increased myocardial infarction risk in women with hypertension and post-menopausal hormonal changes affecting systems like RAAS, underscore the need to understand gender-specific hypertension effects [52,53]. Notably, black people with hypertension displayed a lower PA-related mortality risk than white individuals, potentially due to differences in biological aging and societal health determinants [12,54]. Future research should explore these variances and integrate comprehensive health determinants.

In summary, PA and PAA effectively assess biological aging and mortality risk in patients with hypertension, which assist in gauging patients' biological aging and mortality risk, enabling more personalized and effective prevention and treatment strategies aimed at decelerating biological aging and reducing mortality risk.

5. Strengths and limitations

Our study highlights aging and mortality risk heterogeneity in people with hypertension, introducing PA and PAA as aging biomarkers linked to heightened mortality. The associations varied by the levels of ePWV and HVA, signifiers of arterial stiffness. Notably, people with hypertension with elevated ePWV showed a stronger link between PA, PAA, and mortality. These insights underscore the amplified risk for people with hypertension with advanced physiological age and arterial stiffness, necessitating intensified monitoring. Benefiting from the extensive NHANES database ensured robust analysis and wider relevance. A subsequent MR study further verified PAA's linkage to hypertension and expanded insights to European demographics.

We must also acknowledge that our study has some limitations. First, we mainly focus on the mortality risk of diseases, however, most clinical physiological indicators are mainly used for the prediction and diagnosis of diseases. We cannot infer that the occurrence risk of diseases also has the same linear relationship. Meanwhile, the use of ePWV, derived from age and blood pressure may introduce variability compared to direct PWV measurements and not highlight regional arterial differences impacting cardiovascular health. PA's biological significance is unclear, possibly reflecting cardiovascular metabolic health rather than cellular aging processes. The use of NHANES data may introduce potential selection bias, unadjusted confounding, and measurement inaccuracies, possibly obscuring relationships between PA, PAA, and mortality. Our study only employed a cross-sectional measurement for phenotypic age, phenotypic age acceleration, ePWV and HVA, which may not reflect the dynamic changes of these biomarkers over time. NHANES, primarily a social survey, lacks details like hospitalization, inhibiting morbidity outcome analyses. Additionally, MR analysis has inherent constraints: potential genetic variant limitations, population stratification, and variable genetic impacts on outcomes. While we adopted measures to counter these biases and employed a sizable sample for robustness, residual biases could persist. Our MR findings, based on European data, might not be globally generalizable, underscoring the need for broader studies. Furthermore, MR analysis was constrained by the availability of instrumental variables. While significant SNPs for PAA were identified, these instrumental variables were not available for PA, limiting our ability to conduct MR analysis on PA.

6. Conclusions

The study reveals that PA and PAA are potent indicators of biological aging and mortality risk in people with hypertension. Arterial stiffness

and HVA mediate the relationship between phenotypic age and mortality, suggesting potential mechanisms and targets for intervention. Mendelian randomization evidences a causal association between PAA and hypertension progression. These results underscore the importance of combining biological aging and vascular health metrics in hypertension assessment and management.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 82170346), Grant of Shanghai Science and Technology Committee (NO. 19XD1403300 and 19411963200), Shanghai Municipal Health Commission (NO. 2019LJ10) and the Natural Science Foundation of Shanghai (20ZR1451300).

Contributions

HL and YF designed the manuscript and HT and XL revised the manuscript. All authors contributed to the article and approved the submitted version.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of the article

Availability of data and materials

The data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

Competing interests

All authors have no conflict of interest to declare.

Acknowledgements

We are grateful to the US participants of NHANES database who generously contributed their data for this study. In addition, thanks for the EmpowerStats software (Version 5.0, X&Y Solutions, Inc., Boston, MA; http://www.empowerstats.com) providing support for the mainly analysis in this study. And thanks for https://www.mrbase.org/ providing a convenient and efficient online MR analysis platform.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jnha.2024.100203.

References

[1] Topic WHOJH. More than 700 million people with untreated hypertension. 2021.
[2] Chen H, Wu W, Fang W, Chen Z, Yan X, Chen Y, et al. Does an increase in estimated pulse wave velocity increase the incidence of hypertension? J Hypertens 2021;39(12):2388–94, doi:http://dx.doi.org/10.1097/hjh.0000000000002945.
[3] Lu Y, Lan T. Global, regional, and national burden of hypertensive heart disease during 1990–2019: an analysis of the global burden of disease study 2019. BMC Public Health 2022;22(1):841, doi:http://dx.doi.org/10.1186/s12889-022-13271-0.
[4] Vlachopoulos C, Terentes-Printzios D, Laurent S, Nilsson PM, Protogerou AD, Aznaouridis K, et al. Association of estimated pulse wave velocity with survival: a secondary analysis of SPRINT. JAMA Netw Open 2019;2(10):e1912831, doi:http://dx.doi.org/10.1001/jamanetworkopen.2019.12831.
[5] Afsar B, Afsar RE. Hypertension and cellular senescence. Biogerontology 2023;24(4):457–78, doi:http://dx.doi.org/10.1007/s10522-023-10031-4.
[6] Wu JW, Yaqub A, Ma Y, Koudstaal W, Hofman A, Ikram MA, et al. Biological age in healthy elderly predicts aging-related diseases including dementia. Sci Rep 2021;11(1):15929, doi:http://dx.doi.org/10.1038/s41598-021-95425-5.
[7] Guerville F, De Souto Barreto P, Ader I, Andrieu S, Casteilla L, Dray C, et al. Revisiting the hallmarks of aging to identify markers of biological age. J Prev Alzheimer Dis 2020;7(1):56–64, doi:http://dx.doi.org/10.14283/jpad.2019.50.
[8] Kozlov MJN. 'Inflammation clock'can reveal body's biological age. 2021.
[9] Stephan Y, Sutin AR, Terracciano A. How old do you feel? The role of age discrimination and biological aging in subjective age. PLoS One 2015;10(3):e0119293, doi:http://dx.doi.org/10.1371/journal.pone.0119293.
[10] Rutledge J, Oh H, Wyss-Coray T. Measuring biological age using omics data. Nat Rev Genet 2022;23(12):715–27, doi:http://dx.doi.org/10.1038/s41576-022-00511-7.
[11] Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging 2018;10(4):573–91, doi:http://dx.doi.org/10.18632/aging.101414.
[12] Liu Z, Kuo PL, Horvath S, Crimmins E, Ferrucci L, Levine M. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: a cohort study. PLoS Med 2018;15(12):e1002718, doi:http://dx.doi.org/10.1371/journal.pmed.1002718.
[13] Vatner SF, Zhang J, Vyzas C, Mishra K, Graham RM, Vatner DE. Vascular stiffness in aging and disease. Front Physiol 2021;12:762437, doi:http://dx.doi.org/10.3389/fphys.2021.762437.
[14] Nie C, Li Y, Li R, Yan Y, Zhang D, Li T, et al. Distinct biological ages of organs and systems identified from a multi-omics study. Cell Rep 2022;38(10):110459, doi:http://dx.doi.org/10.1016/j.celrep.2022.110459.
[15] Pierce GL, Coutinho TA, DuBose LE, Donato AJ. Is it good to have a stiff aorta with aging? Causes and consequences. Physiology (Bethesda) 2022;37(3):154–73, doi:http://dx.doi.org/10.1152/physiol.00035.2021.
[16] Seals DR, Moreau KL, Gates PE, Eskurza I. Modulatory influences on ageing of the vasculature in healthy humans. Exp Gerontol 2006;41(5):501–7, doi:http://dx.doi.org/10.1016/j.exger.2006.01.001.
[17] D'Agostino Sr RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117(6):743–53.
[18] Control CfD. Prevention %J Atlanta GCfDC, Prevention. NHANES laboratory/medical technologists procedures manual. 2001.
[19] Liu Z, Kuo P-L, Horvath S, Crimmins E, Ferrucci L, Levine M. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: a cohort study. PLoS Med 2018;15(12):e1002718.
[20] Zhang R, Wu M, Zhang W, Liu X, Pu J, Wei T, et al. Association between life's essential 8 and biological ageing among US adults. J Transl Med 2023;21(1):622, doi:http://dx.doi.org/10.1186/s12967-023-04495-8.
[21] Heffernan KS, Stoner L, London AS, Augustine JA, Lefferts WK. Estimated pulse wave velocity as a measure of vascular aging. PLoS One 2023;18(1):e0280896, doi:http://dx.doi.org/10.1371/journal.pone.0280896.
[22] Heffernan KS, Jae SY, Loprinzi PD. Estimated pulse wave velocity is associated with residual-specific mortality: findings from the National Health and Nutrition Examination Survey. J Hypertens 2021;39(4):698–702, doi:http://dx.doi.org/10.1097/hjh.0000000000002691.
[23] Borrud LG. National Health and Nutrition Examination Survey: national youth fitness survey plan, operations, and analysis, 2012, vol 163. US Department of Health and Human Services, Centers for Disease Control and . . . ; 2014.
[24] Zhao H, Pan Y, Wang C, Guo Y, Yao N, Wang H, et al. The effects of metal exposures on Charlson comorbidity index using zero-inflated negative binomial regression model: NHANES 2011-2016. Biol Trace Element Res 2021;199(6):2104–11, doi:http://dx.doi.org/10.1007/s12011-020-02331-4.
[25] KDIGO 2021. Clinical practice guideline for the management of glomerular diseases. Kidney Int 2021;100(4s):S1–s276, doi:http://dx.doi.org/10.1016/j.kint.2021.05.021.
[26] Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106(25):3143–421.
[27] McCartney DL, Min JL, Richmond RC, Lu AT, Sobczyk MK, Davies G, et al. Genome-wide association studies identify 137 genetic loci for DNA methylation biomarkers of aging. Genome Biol 2021;22(1):194, doi:http://dx.doi.org/10.1186/s13059-021-02398-9.
[28] Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations. Wellcome Open Res 2019;4:186, doi:http://dx.doi.org/10.12688/wellcomeopenres.15555.2.
[29] Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: The STROBE-MR statement. JAMA 2021;326(16):1614–21, doi:http://dx.doi.org/10.1001/jama.2021.18236.
[30] Staley JR, Blackshaw J, Kamat MA, Ellis S, Surendran P, Sun BB, et al. PhenoScanner: a database of human genotype-phenotype associations. Bioinformatics 2016;32(20):3207–9, doi:http://dx.doi.org/10.1093/bioinformatics/btw373.
[31] Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Stat Med 2017;36(11):1783–802, doi:http://dx.doi.org/10.1002/sim.7221.
[32] Lee K, Lim CY. Mendelian randomization analysis in observational epidemiology. J Lipid Atheroscler 2019;8(2):67–77, doi:http://dx.doi.org/10.12997/jla.2019.8.2.67.
[33] Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40(4):304–14, doi:http://dx.doi.org/10.1002/gepi.21965.

[34] Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50(5):693–8, doi:http://dx.doi.org/10.1038/s41588-018-0099-7.
[35] Xue H, Shen X, Pan W. Constrained maximum likelihood-based Mendelian randomization robust to both correlated and uncorrelated pleiotropic effects. Am J Hum Genet 2021;108(7):1251–69, doi:http://dx.doi.org/10.1016/j.ajhg.2021.05.014.
[36] Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. Psychol Methods 2010;15(4):309–34, doi:http://dx.doi.org/10.1037/a0020761.
[37] Khosravi M. The mediating role of alexithymia in the association between attachment styles and borderline personality symptomatology. Health Psychol Res 2020;8(2):8894, doi:http://dx.doi.org/10.4081/hpr.2020.8894.
[38] Cheng W, Xu W, Luan S, Wen G, Kong F. Predictive value of estimated pulse wave velocity with all-cause and cause-specific mortality in the hypertensive population: the National Health and Nutrition Examination Surveys 1999-2014. J Hypertens 2023;41(8):1313–22, doi:http://dx.doi.org/10.1097/hjh.0000000000003469.
[39] Li L, Wang Y, Yang C, Huang C, Duan L, Zhou J, et al. Frailty in hypertensive population and its association with all-cause mortality: data from the National Health and Nutrition Examination Survey. Front Cardiovasc Med 2023;10:945468, doi:http://dx.doi.org/10.3389/fcvm.2023.945468.
[40] Mitchell GF. Arterial stiffness in aging: does it have a place in clinical practice?: Recent advances in hypertension. Hypertension 2021;77(3):768–80, doi:http://dx.doi.org/10.1161/hypertensionaha.120.14515.
[41] Lacolley P, Regnault V, Avolio AP. Smooth muscle cell and arterial aging: basic and clinical aspects. Cardiovasc Res 2018;114(4):513–28, doi:http://dx.doi.org/10.1093/cvr/cvy009.
[42] Ma Q, Li BL, Yang L, Zhang M, Feng XX, Li Q, et al. Association between phenotypic age and mortality in patients with multivessel coronary artery disease. Dis Markers 2022;2022:4524032, doi:http://dx.doi.org/10.1155/2022/4524032.
[43] González LDM, Romero-Orjuela SP, Rabeya FJ, Del Castillo V, Echeverri D. Age and vascular aging: an unexplored frontier. Front Cardiovasc Med 2023;10:1278795, doi:http://dx.doi.org/10.3389/fcvm.2023.1278795.
[44] Tabaei BP, Chamany S, Perlman S, Thorpe L, Bartley K, Wu WY. Heart age, cardiovascular disease risk, and disparities by sex and race/ethnicity among New York city adults. Public Health Rep (Washington, DC: 1974) 2019;134(4):404–16, doi:http://dx.doi.org/10.1177/0033354919849881.
[45] Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai PC, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY) 2016;8(9):1844–65, doi:http://dx.doi.org/10.18632/aging.101020.
[46] Kresovich JK, Sandler DP, Taylor JA. Methylation-based biological age and hypertension prevalence and incidence. Hypertension (Dallas, Tex: 1979) 2023;80(6):1213–22, doi:http://dx.doi.org/10.1161/hypertensionaha.122.20796.
[47] Nilsson PM, Boutouyrie P, Laurent S. Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension 2009;54(1):3–10, doi:http://dx.doi.org/10.1161/hypertensionaha.109.129114.
[48] Nilsson PM, Boutouyrie P, Cunha P, Kotsis V, Narkiewicz K, Parati G, et al. Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. J Hypertens 2013;31(8):1517–26, doi:http://dx.doi.org/10.1097/HJH.0b013e328361e4bd.
[49] Cleven L, Krell-Roesch J, Nigg CR, Woll A. The association between physical activity with incident obesity, coronary heart disease, diabetes and hypertension in adults: a systematic review of longitudinal studies published after 2012. BMC Public Health 2020;20(1):726, doi:http://dx.doi.org/10.1186/s12889-020-08715-4.
[50] Song R, Chen X, He K, Hu X, Bai K, Shi W, et al. Associations of BMI with all-cause mortality in normoglycemia, impaired fasting glucose and type 2 diabetes mellitus among an elderly Chinese population: a cohort study. BMC Geriatr 2022;22(1):690, doi:http://dx.doi.org/10.1186/s12877-022-03382-z.
[51] Kotchen TA. Obesity-related hypertension: epidemiology, pathophysiology, and clinical management. Am J Hypertens 2010;23(11):1170–8, doi:http://dx.doi.org/10.1038/ajh.2010.172.
[52] Chidambaram M, Duncan JA, Lai VS, Cattran DC, Floras JS, Scholey JW, et al. Variation in the renin angiotensin system throughout the normal menstrual cycle. J Am Soc Nephrol 2002;13(2):446–52, doi:http://dx.doi.org/10.1681/asn.V132446.
[53] Lu KT, Keen HL, Weatherford ET, Sequeira-Lopez ML, Gomez RA, Sigmund CD. Estrogen receptor α is required for maintaining baseline renin expression. Hypertension 2016;67(5):992–9, doi:http://dx.doi.org/10.1161/hypertensionaha.115.07082.
[54] Pérez-Stable EJ, Rodriquez EJ. Social determinants and differences in mortality by race/ethnicity. JAMA Netw Open 2020;3(2):e1921392, doi:http://dx.doi.org/10.1001/jamanetworkopen.2019.21392.