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Synergistic impact of 25-hydroxyvitamin D concentrations and physical activity on delaying aging

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ABSTRACT

Objective: Our study aims to examine the independent and combined associations of serum 25-hydroxyvitamin D [25(OH)D] concentrations and physical activity (PA) status with phenotypic age (PhenoAge).
Method: The analysis included 18,738 participants from the NHANES 2007–2010 & 2015–2018. Phenotypic Age Acceleration (PhenoAgeAccel) was calculated as the residuals from regressing PhenoAge on chronological age. Weighted multivariable logistic regression models were used to analysis the relationship between 25(OH)D and PA with PhenoAgeAccel. Population attributable fraction (PAF) was used to estimate the proportion of PhenoAgeAccel which could be avoided if exposure were eliminated.
Results: The multivariate-adjusted OR (95%CI) for PhenoAgeAccel with high 25(OH)D and adequate PA were 0.657 (0.549,0.787) ($p < 0.001$) for all, 0.663 (0.538,0.818) ($p < 0.001$) for participants whose age ≤65years old. Furthermore, there was multiplicative interaction between 25(OH)D and PA in age ≤65 years old group (0.729 (0.542,0.979), $p = 0.036$). High 25(OH)D level and adequate PA reduced the risk of PhenoAgeAccel by 14.3 % and 14.2 %, respectively. Notably, 30.7 % decrease was attributable to both high 25(OH)D level and engaging in adequate PA concurrently. Combining 25(OH)D above 80.4 nmol/l with PA decreased PhenoAge by 1.291 years ($p < 0.001$).
Conclusion: Higher 25(OH)D level was associated with lower risk of biological ageing. Combining 25(OH)D and PA demonstrated enhanced protective effects, especially in middle or young adults. These findings underscore the importance of outdoor PA in slowing down the aging process.

1. Introduction

Vitamin D, a steroid hormone that classically regulates bone and mineral metabolism, is also linked to overall human health [1,2]. It displays a complex metabolism and acts as a hormone on many extra-skeletal targets since vitamin D receptor (VDR) and vitamin D metabolic enzymes are widely expressed [1,3]. In immune system, many studies showed that vitamin D supplementation of deficient populations in preventing infections [1]. A trial proved that vitamin D supplementation reduced the risk of seasonal influenza infections [4]. There is also accumulating evidence suggesting potential benefits for the prevention and treatment of Coronavirus Disease 2019 (COVID-19) [2,5]. In cardiovascular system, a meta-analysis of 19 prospective studies demonstrated an inverse relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and risk of cardiovascular disease (CVD) [6]. Furthermore, studies found higher mortality rates in persons with the lowest vitamin D (<50–60 nmol/L) [7,8]. A recent study also showed that higher 25(OH)D levels were significantly associated with lower all-cause and CVD mortality and highlighted the importance of maintaining adequate vitamin D status in diabetes [9].

To prevent and treat vitamin D deficiency, a healthy lifestyle including regular physical activity (PA), moderate sunlight exposure and a healthy diet is highly recommended [2]. A protective factor against insufficiency and deficiency was active leisure-time PA (≥150 min/week) [10]. Furthermore, research suggested that an increase in moderate and vigorous PA and a decrease in body fat percentage could be strategies to increase 25(OH)D levels in older adults [11]. PA also contribute to overall human health. It can delay onset age-related diseases, such as obesity, type 2 diabetes, CVD, cerebrovascular accidents and even certain types of cancer [12]. On the contrary, literature

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indicated that sustained sedentary behavior is correlated with a negative health outcome, such as risk of cancer [13,14]. From above mentioned factors, a research gap is found: the lack of studies exploring the combined effects of PA and vitamin D status on health outcome, especially effects on the aging process. This gap becomes even more significant because of the potential for outdoor PA behavior to increase vitamin D synthesis and bioavailability due to increased sunlight exposure.

Biological age can represent a person's aging status more appropriately than chronological age because chronological age is only a period of living and no related to person's health status [15]. Biological aging is thought to arise from the accumulation of molecular changes or "hallmarks" that undermine the functioning and resilience capacity of tissues and organs, ultimately leading to disease and death [16,17]. Studies on animals and humans indicate that biological aging is modifiable [17]. For example, a healthy lifestyle index consisting of non-drinking, non-smoking, a healthy diet, PA and healthy body mass index (BMI) was significantly associated with an aging measure derived from chemistry biomarkers [18,19]. Slowing the biological aging has the potential to prevent or delay the onset of multiple age-related diseases and potentially extend lifespan [17]. In this study, we hypothesize that there would be a synergistic effect of vitamin D status and PA on slowing biological aging.

To fill these knowledge gaps, we aimed to examine the associations of serum 25(OH)D concentrations and PA with phenotypic age (PhenoAge) independently and jointly among adults in U.S. population using nationally representative sample data from the National Health and Nutrition Examination Survey (NHANES).

2. Method

2.1. Data source

Data for this cohort study came from NHANES, a survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC), designed to evaluate health and nutritional status of a nationally representative sample of the U.S. civilian population using a multistage, stratified, clustered probabilistic design. The survey included questionnaire interviews, laboratory tests and physical examinations. This study synthesized the NHANES continuous datasets from 2007 to 2010 & 2015–2018 (n = 19,583). The NHANES study protocol was approved by the NCHS Ethical Review Board and written informed consent was obtained from all participants [20,21].

2.2. Study participants

In this study, from 1999 to 2018, data available for calculating PhenoAge was during 2007–2010 & 2015–2018, so the four-cycle data of NHANES are combined (N = 19,583). 637 subjects were ineligible or ≥250 nmol/L for 25(OH)D value [2] and 208 pregnant were excluded. 18,738 subjects were included in the final analysis. Fig. 1 illustrates the complete data integration process.

2.3. Measurement of PhenoAge

It comes to the fore that designing a novel PhenoAge, rather than using the chronological age alone, better predicts health-related outcomes. Referring to the definition of PhenoAge proposed by Morgan E. Levine et al. [22], we calculated the PhenoAge using ten aging related variables, including chronological age, albumin (liver), creatinine (kidney), glucose (metabolic), C-reactive protein (inflammation), lymphocyte percent (immune), mean cell volume (immune), red blood cell distribution width (immune), alkaline phosphatase (liver), and white blood cell count (immune). Blood samples were taken at the mobile examination center. These samples were collected in a standard way and stored in a secure facility. More information about blood samples collecting and processing was reported elsewhere [16,23]. The algorithm and detailed calculation methods have been described in the previous literature [22,24,25].

2.4. Measurement of PA patterns

PA was assessed using weekly physical activity participation information collected by the Global Physical Activity questionnaire which was created by the World Health Organization (https://wwwn.cdc.gov/nchs/nhanes) (World Health Organization. Global Physical Activity Questionnaire (GPAQ) Analysis Guide. Available online: https://www.who.int/ncds/surveillance/steps/resources/GPAQ_Analysis_Guide.pdf). Data were analyzed following the World Health Organization analysis guide(https://www.who.int/ncds/surveillance/steps/resources/GPAQ_Analysis_Guide.pdf). PA data was converted to metabolic equivalent minutes of moderate to vigorous physical activity per week

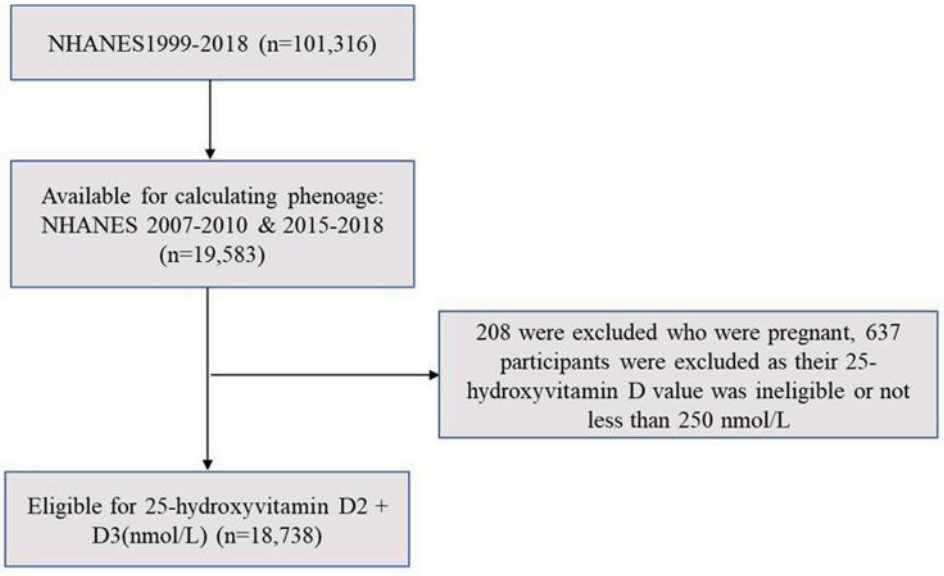

Fig. 1. Flow chart for subject selection.
NHANES: National Health and Nutrition Examination Survey.

(MET) (https://www.who.int/ncds/surveillance/steps/resources/GPAQ_Analysis_Guide.pdf). Respondents were classified based on the criterion of meeting MET (≥600 MET-minutes/week, equivalent to 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity physical activity) or not meeting the recommendation guidelines for adults (<600 MET-minutes/week) (The U.S. Department of Health and Human Services. Physical Activity Guidelines for Americans. Available online: https://health.gov/paguidelines/guidelines/chapter5.aspx).

2.5. Study variables

Demographic information included age, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other races), education (below high school, high school or equivalent, high school above), marital status (married/cohabiting with partner, widowed/divorced/separated, never married), household income-to-poverty ratio (PIR: ≤1, 1 < to ≤3, >3), at least 100 cigarettes in life (yes/no). The NHANES defined 1 alcohol-based drink as 12 ounces of beer, 4 ounces of wine, or 1 ounce of liquor. Drinking status was divided into 3 categories: nondrinkers were defined as participants who had consumed fewer than 12 alcohol-based drinks in the past year or lifetime; former drinkers were defined as participants who had consumed at least 12 drinks in their lifetime but not in the past year; and current drinkers were defined as participants who had at least 12 drinks in the past year and reported a non-zero number of drinks per week [26]. BMI is calculated as weight (Kg) divided by height squared (m^2). Hypertension, hyperlipidemia, coronary heart disease (CHD), stroke history and glycosylated hemoglobin were extracted from the database. Diabetes was defined based on self-reported diagnosis by doctors, use of insulin or oral hypoglycemic drugs, fasting blood glucose ≥7.0 mmol/L, 2-h glucose ≥11.1 mmol/L after an oral glucose tolerance test, or glycosylated hemoglobin A1c (HbA1c) ≥ 6.5 %. The detailed process of data collection is available from the NHANES website.

2.6. Statistical analysis

Based on the NHANES analytical guidelines, sampling weights were used for all analysis to interpret the complicated NHANES survey design. Twelve-year weights were calculated for 2007–2010 & 2015–2018 estimates by dividing the 2-year weights by four. Continuous variables were expressed as mean ± standard error (SE), and one-way ANOVA was used for comparison between groups. Data of categorical variables were expressed as numbers (%) and the Rao-Scott Chi-square was used to compare groups. Logistic regression model was used for joint association of serum 25(OH)D level and PA status with Phenotypic Age Acceleration (PhenoAgeAccel) (calculated as the residuals from regressing PhenoAge on chronological age) among participants. Logistic regression Model was adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, CHD, stroke and diabetes. Confounding variables were selected based on three criteria: clinical relevance, a P-value less than 0.05 in univariate analysis, and the availability of sufficient event data to construct a robust regression model. Restricted cubic spline method was used to assess nonlinear associations of 25(OH)D with PhenoAgeAccel (3 nodes, the 25th percentile as a reference point). In addition to the primary logistic regression models, interaction terms were also introduced to evaluate whether the combined effects of 25(OH)D and PA status exceeded the cumulative effects of the two in the additive and multiplicative scales. Specifically, for the additive scale interaction, we computed the relative excess risk due to interaction (RERI) following the guidelines proposed by Knol and VanderWeele [27]. Multiplicative interaction was assessed by comparing models with and without a cross-product interaction term of 25(OH)D and PA, using likelihood ratio tests. Then, we used the population attributable fraction (PAF) to estimate the proportion of PhenoAgeAccel which could be avoided if exposure (25(OH)D and/or PA) were eliminated. The PAF is calculated using the relative risk from 25(OH)D and/or PA (risk of PhenoAgeAccel for the exposed divided by risk for the non-exposed) and population prevalence of high 25(OH)D and/or PA exposure (proportion exposed) [28,29]. Finally, liner regression was conducted to analyze the association of 25(OH)D and PA with PhenoAge.

Statistical analysis was performed using STATA software (version Jun 14, 2021; STATA/MP 17.0, USA) and R studio 2022.07.1 (© 2009–2022 RStudio, PBC). P < 0.05 (2-tailed) was considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 18,738 US adults were included in the present analysis. The weighted mean (SE) age was 47.8 (0.2) years, and 49.09 % were males. Participants with or without PhenoAgeAccel exhibited certain distinguishing factors. Participants with PhenoAgeAccel were generally older, more likely to be male, Other-Hispanic, less educated, less married, had lower PIR, more likely to smoke, more likely to drink alcohol, more likely to have hypertension, hyperlipidemia, CHD or diabetes, had larger BMI, engaged in less PA, and had lower 25(OH)D levels ($p < 0.05$ or < 0.001) (Table 1).

3.2. Association of 25(OH)D levels and PA status with PhenoAgeAccel

The weighted multivariable logistic regression models were performed to investigate the association between 25(OH)D level and PA with PhenoAgeAccel. After fully adjusting for age, gender, ethnicity, BMI, PIR, education level, smoking, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, CHD, stroke and diabetes, lower 25(OH)D and less PA were significantly related to the risk of increased PhenoAgeAccel in both total (OR [95%CI]: 0.994 [0.991,0.997], $p < 0.001$ & 0.795[0.696, 0.907], $p = 0.001$) and people younger than or equal to 65 years old (0.994[0.990,0.997], $p = 0.001$ & 0.798[0.684,0.931], $p = 0.004$), while PA status was not related to the PhenoAgeAccel in people older than 65 years old ($p = 0.136$) (Table 2). Moreover, we used restricted Cubic Splines to analyze the dose response relationship between the serum 25(OH)D and PhenoAgeAccel. After multivariable adjustment, L-shaped relationships between 25(OH)D and PhenoAgeAccel with an infection point at about 80 nmol/L were found (non-linear $P = 0.018$ for total, 0.039 for younger than or equal to 65 years old and $P = 0.257$ for more than 65 years old) (Fig. 2).

3.3. Joint association and PAF of 25(OH)D levels and PA status with PhenoAgeAccel

In joint analyses, participants with vitamin D deficiency and inadequate PA had the highest risk of PhenoAgeAccel. Compared to the combination of vitamin D deficiency and inadequate PA, the OR for PhenoAgeAccel in the groups of vitamin D nondeficiency and PA were 0.657[0.549, 0.787] ($p < 0.001$) for total, 0.663[0.538, 0.818] ($p < 0.001$) for participants whose age ≤65years old and 0.678[0.476, 0.967] ($p = 0.032$) for participants whose age >65 years old, respectively (Table 3 & Fig. 3).

In addition, we conducted the interaction test to analysis the interaction between 25(OH)D and PA with PhenoAgeAccel. We found that there was multiplicative interaction between 25(OH)D and PA in subjects whose age ≤65 years old (OR [95 % CI]: 0.729[0.542,0.979], $p = 0.036$), while no additive interaction in total or multiplicative interaction in total and older than 65 years group were found (Table S1).

Furthermore, to estimates the proportion of participants from risk of PhenoAgeAccel which could be avoided if low 25(OH)D and/or PA deficiency were eliminated, we conducted the PAF analysis. We found that 14.3 % and 14.2 % of the reduced PhenoAgeAccel was attributable to high 25(OH)D level and adequate PA. However, 30.7 % of the reduced

Table 1
Characteristics of participants who were older or younger than phenotypic age.

	PhenoAge Deceleration/Stasis (n = 13,630)	PhenoAgeAccel (n = 5,108)	P value
Age (years)	47.38 ± 0.33	49.27 ± 0.38	**< 0.001**
Sex (men%)	5838(43.29)	3360(65.27)	**< 0.001**
Race (%)			**< 0.001**
Mexican American	5715(68.05)	2226(66.05)	
Other Hispanic	2349(9.18)	1226(13.63)	
Non-Hispanic White	2407(8.62)	747(8.32)	
Non-Hispanic Black	1566(5.83)	488(5.21)	
Other Race	1593(8.31)	421(6.81)	
Education level (%)			**< 0.001**
Less Than 12th Grade	3307(14.28)	1409(18.37)	
High School Grade or Equivalent	2997(22.07)	1362(29.89)	
Some College	3911(30.62)	1539(32.56)	
College Graduate or above	3401(33.03)	791(19.18)	
Marital status (%)			**< 0.001**
Married	7250(57.40)	2507(51.16)	
Widowed	1129(8.22)	444(9.20)	
Divorced	1448(9.79)	603(10.72)	
Separated	441(2.21)	183(2.96)	
Never married	1027(5.34)	469(6.49)	
Living with partner	2330(17.05)	899(19.49)	
PIR (%)			**< 0.001**
>1	2380(12.39)	1073(16.23)	
1 to 3	5267(34.58)	2139(39.96)	
≥ 3	4653(53.03)	1417(43.80)	
Smoking	5430(40.82)	2929(56.25)	**< 0.001**
Drinking status			**< 0.001**
No drinkers (%)	2064(14.32)	544(10.79)	
Former drinkers (%)	682(4.65)	253(5.79)	
Current drinkers (%)	8349(81.03)	3221(83.42)	
Physical activity (%)			**0.01**
No PA	5618(35.66)	2312(39.40)	
PA	8012(64.34)	2796(60.60)	
Hypertension (%)	4123(26.79)	2632(45.85)	**< 0.001**
Hyperlipidemia (%)	4367(36.09)	1943(41.15)	**< 0.001**
CHD (%)	410(2.57)	367(6.18)	**< 0.001**
Stroke (%)	1247(10.04)	595(10.98)	0.09
BMI (kg/m^2)	28.08 ± 0.10	32.62 ± 0.13	**< 0.001**
Diabetes (%)	1137(5.75)	1515(24.48)	**< 0.001**
25(OH)D (nmol/L)	71.65 ± 0.77	67.09 ± 0.83	**< 0.001**

Data are means ± standard deviations for continuous variables, and percentages for categorical variables. All estimates accounted for complex survey designs. Bold indicates P value < 0.05.
25(OH)D: 25-hydroxyvitamin D; PhenoAgeAccel: phenotypic age acceleration; PIR: ratio of family income to poverty; PA: physical activity – meeting MET (≥600 MET-minutes/week, equivalent to 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity physical activity); MET: metabolic equivalent minutes of moderate to vigorous physical activity per week; BMI: body mass index; CHD: coronary heart disease; HbA1c: glycated hemoglobin.

PhenoAgeAccel was attributable to both high 25(OH)D level and engaging in adequate PA concurrently. Notably, in participants less than or equal to 65 years old, there were 14.3 %, 14.6 % and 31.4 % of the reduction were attributable to high 25(OH)D level and adequate PA alone and both concurrently (p < 0.001). In contrast, in subjects older than 65 years, we found that 23.5 % of the reduced PhenoAgeAccel was attributable to high 25(OH)D level and adequate PA together (p = 0.038), while there was no significant PAF observed for 25(OH)D level or PA alone (p = 0.133 & p = 0.148) (Table 4).

Table 2
Association of 25(OH)D levels and PA status with PhenoAgeAccel among participants.

		Unadjusted Mode		Adjusted Mode	
		OR (95%CI)	P	OR (95%CI)	P
25 (OH) D	Total	0.994 (0.992,0.996)	**<0.001**	0.994 (0.991,0.997)	**<0.001**
	≤65 years	0.992 (0.990,0.994)	**<0.001**	0.994 (0.990,0.997)	**<0.001**
	>65 years	0.997 (0.994,1.000)	0.052	0.995 (0.991,1.000)	**0.027**
PA	Total	0.853 (0.780,0.933)	**<0.001**	0.795 (0.696,0.907)	**0.001**
	≤65 years	0.893 (0.803,0.993)	**0.037**	0.798 (0.684,0.931)	**0.004**
	>65 years	0.859 (0.719,1.026)	0.093	0.826 (0.642,1.062)	0.136

Data are expressed as odd ratio (OR) and 95%CI. Adjusted Mode: adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking every day, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, coronary heart disease, stroke and diabetes. All estimates accounted for complex survey designs. Bold indicates P value < 0.05.
25(OH)D: 25-hydroxyvitamin D; PhenoAgeAccel: phenotypic age acceleration; PA: physical activity – meeting MET (≥600 MET-minutes/week, equivalent to 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity physical activity).

3.4. Joint association of 25(OH)D levels and PA status with PhenoAge

Furthermore, to investigate the relationship between 25(OH)D and PA with PhenoAge, weighted multivariable liner regression was performed. We found that comparing with the first quartile group (25(OH)D less than 45 nmol/l and without PA), participants with the third and fourth quartile of 25(OH)D (between 62.2 and 80.4 nmol/l, or more than 80.4 nmol/l) together with PA gained 1.129 and 1.291 years decrease in the PhenoAge [β (95CI): -1.129(-1.546,-0.711) and -1.291 (-1.716,-0.866), p < 0.001] in the fully adjusted model (Table S2).

3.5. Stratified analyses and sensitivity analyses

Stratified analyses were conducted by age, gender, BMI, PIR, education level, smoking, drinking status, diagnosis of hypertension, hyperlipidemia, CHD, stroke and diabetes. The results consistently showed that higher 25(OH)D level and adequate PA were associated with a lower risk of PhenoAgeAccel across all subgroups. Notably, education, PIR, PA and drinking status had an interaction with 25(OH)D in relation to PhenoAgeAccel. Higher levels of 25(OH)D significantly decreased the risk of PhenoAgeAccel in high education, PIR and currently drinking groups, while this protective effect was found in both high and low PA groups. However, there was no significant interaction between 25(OH)D level and other factors (Table S3).

A sensitivity analysis was conducted to evaluate the robustness of our primary results. Upon excluding subjects whose age less than 30 years old in total, subjects that age more than 50 years old in ≤65 years old group and whose age less than 75 years old in the >65years old group, the findings remained consistent with our main results (Table S4). Considering of the inter-relationship of parathyroid hormone (PTH) and 25(OH)D status, we further adjusted for PTH levels in the participants (only available in the NHANES 2003–2006, n = 8,162). The results did not change when PTH was further adjusted for in this group of participants (OR [95 % CI]: 0.932[0.882,0.986], p = 0.014).

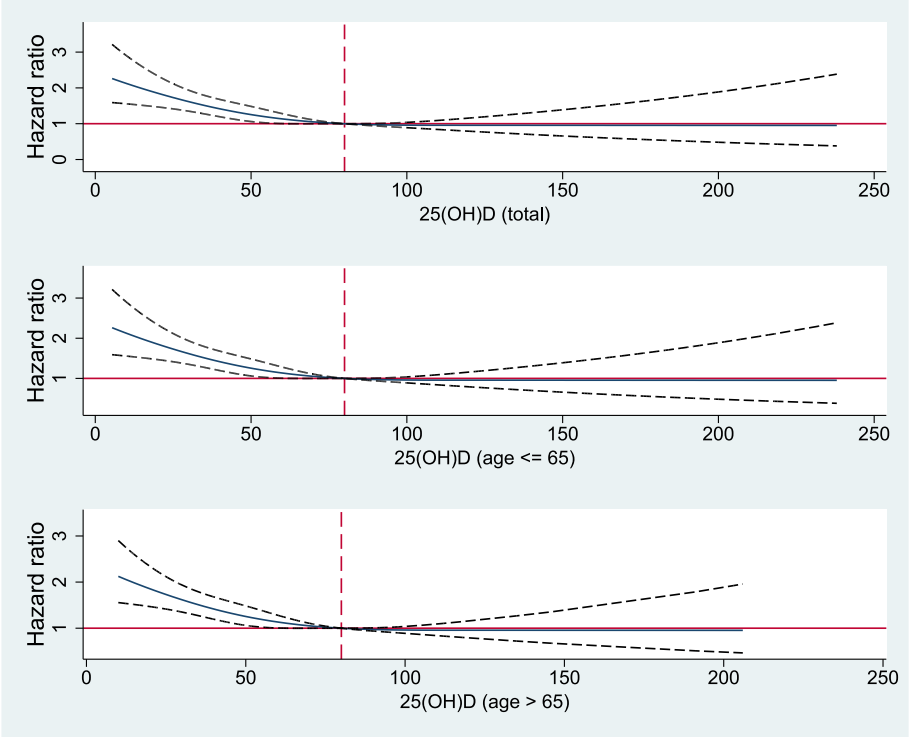

Fig. 2. Association of 25(OH)D levels with PhenoAgeAccel among participants by Restricted Cubic Splines. Upper: all subjects. Middle: subjects age less than or equal to 65 years old. Lower: subjects age more than 65 years old.
Data was adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking every day, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, coronary heart disease, stroke and diabetes. All estimates accounted for complex survey designs.
25(OH)D: 25-hydroxyvitamin D; PhenoAgeAccel: Phenotypic Age Acceleration.

Table 3
Joint association of 25(OH)D levels and PA status with PhenoAgeAccel among participants.

			Total		≤65 years		>65 years	
			OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
25(OH)D	**Low level**	**No PA**	1 [Reference]		1 [Reference]		1 [Reference]	
		PA	0.919(0.774,1.090)	0.333	0.955(0.789,1.155)	0.634	0.767(0.504,1.169)	0.217
	High level	**No PA**	0.909(0.746,1.107)	0.343	0.953(0.745,1.219)	0.702	0.784(0.571,1.076)	0.132
		PA	0.657(0.549,0.787)	**<0.001**	0.663(0.538,0.818)	**<0.001**	0.678(0.476,0.967)	**0.032**

Data are expressed as odd ratio (OR) and 95 % CI. Multivariable adjusted model additionally adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking every day, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, coronary heart disease, stroke and diabetes. All estimates accounted for complex survey designs. Bold indicates P value < 0.05.
25(OH)D: 25-hydroxyvitamin D; PhenoAgeAccel: phenotypic age acceleration; PA: physical activity – meeting MET (≥600 MET-minutes/week, equivalent to 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity physical activity).

4. Discussion

In this study, we found that serum 25(OH)D concentrations were significantly associated with a deceleration in PhenoAge independently. Furthermore, when combined with PA, it could significantly enhance the protective effects, especially among individuals aged 65 years or younger. Importantly, there was an interaction between serum 25(OH)D and PA. These correlations were found in a sample representative of the U.S. population and remained significant even after adjusting for multiple traditional risk factors such as smoking, hypertension and diabetes. To the best of our knowledge, this is the first study to evaluate the joint association of 25(OH)D and PA status with aging.

Accelerated biological aging was associated with an increased risk of various adverse outcomes, such as CVD, cancer, depression, and even mortality. It is important to identify people whose PhenoAgeAccel in order to facilitate timely interventions and delay the occurrence of diseases [30]. As a result, aging biomarkers are needed because it can provide a measurable outcome and does not require extremely long follow-up observation [22,31]. Studies showed that biological age measures derived from blood chemistry data demonstrate superior performance to the DNA methylation measures in prediction of healthy lifespan [31]. A study including 557,940 Koreans discovered that biological ages using 15 biomarkers measured in general health check-ups was a useful index to predict seventeen-year survival and mortality [15]. The PhenoAge algorithm is derived from multivariate analysis of mortality hazards. PhenoAge older than chronological age indicates an advanced state of biological aging and increased risk for disease [31]. Combining PhenoAge methods with biological and clinical markers offers an effective way to predict mortality risks, providing a more comprehensive assessment of an individual's overall health status [12].

Epidemiological studies have discovered that low 25(OH)D concentrations are associated with an increased risk of all-cause mortality and major acute and chronic diseases, such as infections, cancer, cardiovascular and autoimmune diseases [2]. The human VDR is extensive expressed in at least thirty tissues, involved in bone metabolism or in other extra-skeletal functions (heart, immune system, adipose tissues

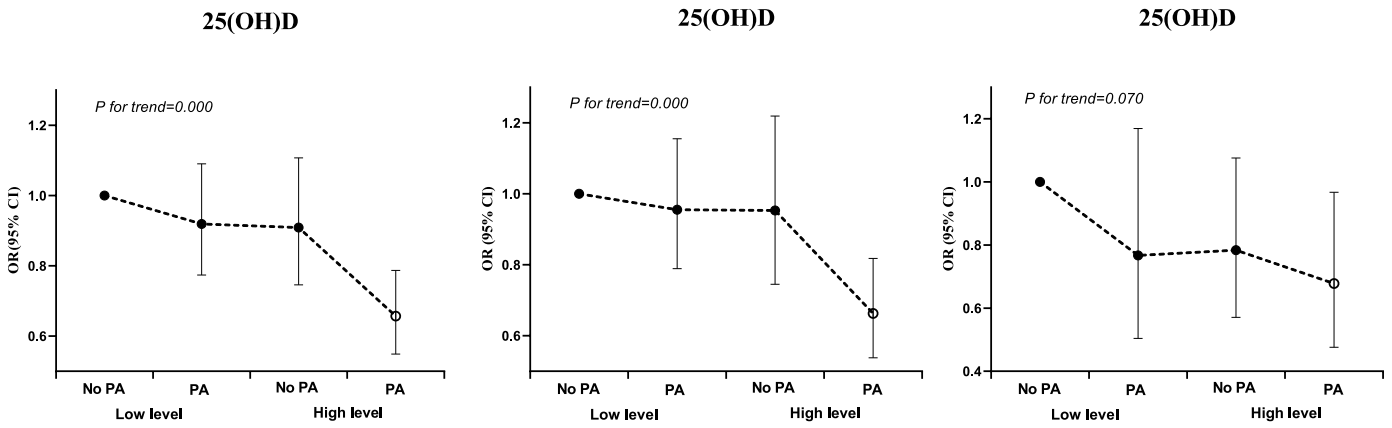

Fig. 3. Joint association of 25(OH)D levels and PA status with PhenoAgeAccel among participants. Left: all subjects. Middle: subjects age less than or equal to 65 years old. Right: subjects age more than 65 years old.
The round symbols and error bars represent the odd ratio (OR) and 95 % CI. Multivariable adjusted model additionally adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking every day, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, coronary heart disease, stroke and diabetes. All estimates accounted for complex survey designs. Hollow circle symbol indicates P value < 0.05.
25(OH)D: 25-hydroxyvitamin D; PA: physical activity; PhenoAgeAccel: Phenotypic Age Acceleration.

Table 4
PAF of 25(OH)D levels and PA status with PhenoAgeAccel among participants.

	Total		≤65 years		>65 years	
	PAF (95%CI)	P	PAF (95%CI)	P	PAF (95%CI)	P
25(OH)D	-0.143(-0.231, -0.062)	**<0.001**	-0.143(-0.241, -0.053)	**0.001**	-0.133(-0.334, 0.037)	0.133
PA	-0.142(-0.240, -0.052)	**0.002**	-0.146(-0.268, -0.035)	**0.009**	-0.090(-0.226,0.030)	0.148
25(OH)D & PA	-0.307(-0.451, -0.177)	**<0.001**	-0.314(-0.484, -0.164)	**<0.001**	-0.235(-0.509, -0.118)	**0.038**

Data are expressed as PAF and 95 % CI. Multivariable adjusted model additionally adjusted for age, gender, ethnicity, BMI, PIR, education level, smoking every day, alcohol status, marital status, diagnosis of hypertension, hyperlipidemia, coronary heart disease, stroke and diabetes. All estimates accounted for complex survey designs. Bold indicates P value < 0.05.
25(OH)D: 25-hydroxyvitamin D; PhenoAgeAccel: phenotypic age acceleration; PA: physical activity – meeting MET (≥600 MET-minutes/week, equivalent to 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity physical activity).

and many others) [3,32]. Vitamin D deficiency is strongly associated with increased risk of infections and autoimmune diseases [33]. 25(OH)D concentration is inversely related to pulmonary injuries such as pneumonia, acute respiratory distress syndrome (ARDS), sepsis and mortality from pulmonary infections [3,34]. Many studies have also shown a correlation between low 25(OH)D levels and chronic autoimmune thyroiditis-Hashimoto thyroiditis (HT), Graves' disease (GD) and postpartum thyroiditis (PPT) [35]. In cardiovascular system, low serum 25(OH)D levels have been associated with increased risk of CVD (hypertension, coronary artery disease, heart failure, stroke, and type 2 diabetes), cardiovascular endpoints and mortality [3]. A study from NHANES also showed that the multivariate-adjusted HRs for participants with 25(OH)D > 75 nmol/L were 0.59 for all-cause mortality (P = 0.003), 0.50 for CVD mortality (P = 0.02) compared with participants with 25(OH)D < 25 nmol/L(9). In 2022, A Central and Eastern European Expert Consensus Statement suggested that a 25(OH)D concentration of 30–50 ng/mL (75–125 nmol/L) is considered as vitamin D sufficiency [2]. Consistent with the aforementioned literature, we found that lower 25(OH)D were significantly related to the risk of PhenoAgeAccel after fully adjusting for confounding factors in the present study, and an L-shaped relationship between vitamin D concentrations and PhenoAgeAccel with an infection point at about 80 nmol/L.

Regular physical activity has been shown to have beneficial effects on the aging process. It can delay onset of several age-related diseases, such as CVD, cerebrovascular accidents, type 2 diabetes, and certain types of cancer, as well as enhance cognitive function and amplify mental health [36,37]. A study showed that replacing sedentary time with 30 min of moderate-to-vigorous PA daily was associated with a 1.9-year decrease in PhenoAge [12]. In present study, we found that participants with vitamin D deficiency and inadequate PA had the highest risk of PhenoAgeAccel (OR = 0.657); 14.3 % and 14.2 % of the reduced PhenoAgeAccel was attributable to high 25(OH)D levels and adequate PA respectively, and even 30.7 % of the reduction was attributable to both of them. Specifically, combing 25(OH)D of more than 80.4 nmol/l with PA decreased 1.291 years of PhenoAge. Furthermore, a multiplicative interaction between 25(OH)D and PA in subjects whose age less than or equal to 65 years old was found. This synergistic effect suggests that engaging in outdoor PA could help decelerate the aging process, especially for younger individuals. The joint analysis enables an examination of the distinct as well as synergistic impacts of each variable on PhenoAgeAccel, thereby offering a more holistic insight into the dynamics of aging [14].

Our study had some limitations. First, there is no gold standard for measuring biological aging. However, the PhenoAge was well validated as a predictor of age-related diseases in diverse populations [17]. Second, the cross-sectional design of this study can only assess associations at a single point in time. Therefore, we could not determine causality relationship between these variables. Third, the study relied on self-reported data for PA. It's hard to avoid biases such as recall bias. Finally, although we controlled for many possible confounders, such as age, gender, ethnicity, BMI, PIR, education level, hypertension and diabetes, the findings may be affected by other confounders which affect aging were not controlled.

In conclusion, this study found that high 25(OH)D level and engaging in PA was associated with lower risk of biological ageing. Furthermore, combining high 25(OH)D level and PA demonstrated enhanced protective effects, especially in middle or young adults. A synergistic effect in decelerating the aging process was demonstrated

among them. These findings underscore the importance of outdoor physical activity in slowing down the aging process, which may have significant public health implications.

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Ethics statement

The studies involving human participants were reviewed and approved by NHANES. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Disclosure Statement

The authors have nothing to disclose.

CRediT authorship contribution statement

Chang Liu: Writing – original draft, Formal analysis, Data curation. **Lin Hua:** Formal analysis. **Zhong Xin:** Writing – review & editing, Formal analysis, Conceptualization.

Declaration of competing interest

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Data availability

Data will be made available on request.

Abbreviations

VDR	vitamin D receptor
25(OH)D	25-hydroxyvitamin D
CVD	cardiovascular disease
PA	physical activity
PhenoAge	phenotypic age
NHANES	National Health and Nutrition Examination Survey
MET	metabolic equivalent minutes of moderate to vigorous physical activity per week
CHD	coronary heart disease
SE	standard error
PhenoAgeAccel	Phenotypic Age Acceleration
RERI	relative excess risk due to interaction
PAF	population attributable fraction
ARDS	Acute Respiratory Distress Syndrome
HT	Hashimoto thyroiditis
GD	Graves' disease
PPT	postpartum thyroiditis

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2024.103188.

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