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Joint association of diabetes mellitus and inflammation status with biological ageing acceleration and premature mortality

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ABSTRACT

Background: We aimed to investigate the associations of diabetes mellitus (DM) and C-reactive protein (CRP) with biological ageing acceleration and mortality risk.
Methods: We analyzed data from 41,634 adults with CRP and DM at baseline. Subjects were categorized into high CRP (>3 mg/L) and low CRP (≤3 mg/L) groups. The cross-sectional endpoints of the study were biological ageing indicators Klemera-Doubal method BioAge acceleration (KDMAccel) and Phenotypic age acceleration (PhenoAgeAccel), and the follow-up endpoints were all-cause mortality and cardiovascular mortality.
Results: In adults with high CRP, compared with those without DM, PhenoAgeAccel increased by 1.66 years (95 % CI: 1.38–1.93), and 8.74 years (95 % CI: 8.25–9.22) in adults with prediabetes and DM, respectively (p for interaction <0.001). Using the CRP_{low}/non-DM group as a reference, adults in the CRP_{high}/non-DM, CRP_{low}/DM, and CRP_{high}/DM groups had significantly advanced biological ageing. Compared to adults without DM, low CRP, and no ageing acceleration, the multivariable-adjusted HRs (95%CIs) of all-cause and cardiovascular mortality in those with DM, CRP, and ageing acceleration were 3.22 (2.79–3.72), and 3.57 (2.81–4.54), respectively.
Conclusions: These findings suggest that the joint presence of low-grade inflammation and DM might be associated with higher odds of biological ageing acceleration and premature mortality.

1. Introduction

Ageing stands out as a primary contributor to chronic diseases, emerging as a significant public health burden [1]. However, individuals of the same age exhibit significant heterogeneity in terms of age-related diseases and mortality risk, indicating variations in underlying biological ageing processes [2,3]. An increasing body of literature suggests that compared with chronological age, biological age, and ageing acceleration are more effective at predicting morbidity and mortality risks [4]. Many indicators of biological ageing have been identified, including DNA methylation age [5], and algorithms that integrate information from epigenetic, proteomic, and metabolomic profiling, such as the Klemera Biological Age [6] and Phenotypic Age [7].

Type 2 Diabetes (T2D) impacts more than 30 million adults in the United States, with an additional 88 million individuals estimated to have prediabetes [8]. Elevated C-reactive protein (CRP) serves as a sensitive biomarker indicating systemic inflammation. Low-grade inflammation is commonly observed in people with diabetes mellitus (DM) and is partly mediated by adipose tissue dysfunction and insulin resistance [9,10]. High CRP levels pose a higher risk of poor prognosis in individuals with both diabetes and non-diabetes than in individuals with normal CRP levels [11,12]. Chronic metabolic control/diabetic chronic vascular complications are associated with CRP levels in the people with DM [13]. However, population evidence linking inflammation and diabetes to biological ageing acceleration remains unclear. In mice induced to become obese through diet or genetic factors, treatment strategies aimed at decreasing the burden of senescent cells, either through genetic targeting or the administration of senolytic drugs,

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ameliorate diabetic phenotypes, resulting in improvements in glucose tolerance and insulin sensitivity [14]. The anti-ageing drug *ABT263* reverses hyperglycemia and restores normal *β-cell* gene expression profiles in mice with insulin resistance induced by the insulin receptor antagonist *S961* [15]. Recently, it has been demonstrated that inflammation contributes to chronic diseases associated with ageing [16]. In mouse models of progeria, interventions to reduce inflammation delay the onset of age-related characteristics and extend lifespan [17]. A low-level elevated inflammatory phenotype in *NF-κB* gain-of-function mice is associated with a shorter lifespan and signs of biological ageing acceleration, supporting a causal relationship between chronic inflammation and age-related tissue dysfunction [18].

However, little research has been conducted on the joint association between cumulative exposure to biological ageing and future mortality risk. Therefore, this study aimed to investigate the relationships between different diabetic states (no diabetes, prediabetes, and diabetes), serum CRP levels, biological ageing acceleration, and mortality risk using the National Health and Nutrition Examination Survey (NHANES) data.

2. Methods

2.1. Study Population

The NHANES is a comprehensive cross-sectional survey conducted across the nation [19]. The survey stands out for combining interviews with comprehensive medical examinations. The overall sample of the survey was designed using a multistage stratified probability approach, ensuring its representativeness of the entire U.S. population. In this study, datasets from 11 two-year cycles (from 1999 to 2000 to 2017–2020) of the NHANES were selected. Additionally, the data from the 11 cycles underwent standardization and merging, employing interview weights in line with the National Center for Health Statistics recommendations.

Between 1999 and 2020, the NHANES program surveyed 116,876 participants. The exclusion criteria were as follows (Fig. 1): (1) participants aged less than 20 years (n = 52,563); (2) participants without available diabetes diagnostic data (n = 5572); (3) participants without available C-reactive protein data (n = 16,820); and (4) participants who lacked biological ageing data (n = 287). Finally, 41,634 participants were recruited for analysis of biological age acceleration (n = 41,634) and phenotypic age acceleration (n = 41,408). The study received approval and written informed consent was obtained from all participants. For detailed information on the ethical review, please refer to the NHANES website.

2.2. Diabetes mellitus and serum C-reactive protein

The diabetic states included no diabetes, prediabetes, and diabetes. As described in our prior studies [20,21], diabetes was characterized by a self-reported diagnosis from a physician, the use of antihyperglycemic medications, plasma HbA1c levels ≥6.5 % (≥48 mmol/mol), or fasting plasma glucose (FPG) levels ≥7.0 mmol/L (126 mg/dL); prediabetes was characterized by plasma HbA1c levels between 5.7 % and 6.4 % (39–46 mmol/mol) or FPG levels between 100 mg/dL and 125 mg/dL in adults without preexisting diabetes and antihyperglycemic agents. No diabetes was characterized by the absence of a prior diagnosis, no use of antihyperglycemic agents, HbA1c levels less than 5.7 %, or FPG levels less than 100 mg/dL. Blood samples were obtained during MEC visits using a standardized protocol [20].

CRP is considered a highly reliable indicator of the acute phase response to infectious diseases or other factors causing tissue injury and inflammation [22]. CRP levels were measured by latex-enhanced turbidimetry [22], and high-sensitivity c-reactive protein (hsCRP) concentrations were quantified by a Roche Cobas 6000 chemistry analyzer (Roche Diagnostics, Indianapolis, Indiana) using reagents and calibrators. The minimum reported level of CRP was approximately 0.2 mg/L (0.02 mg/dL), with slight variations depending on the calibrator lot. The lower limit of detection for hsCRP in the NHANES dataset was 0.15 mg/L. When the result is below the limit of detection, the variable's value is determined as the limit of detection divided by the square root of 2. For more detailed information on blood sample collection, storage, calibration, and quality control procedures for the determination of CRP, see the NHANES Laboratory Procedures Manual (CDC, 2020, 2021).

Elevated CRP is defined as >3 mg/L according to the recommendations [23]. All subjects were categorized into two groups: high CRP (>3

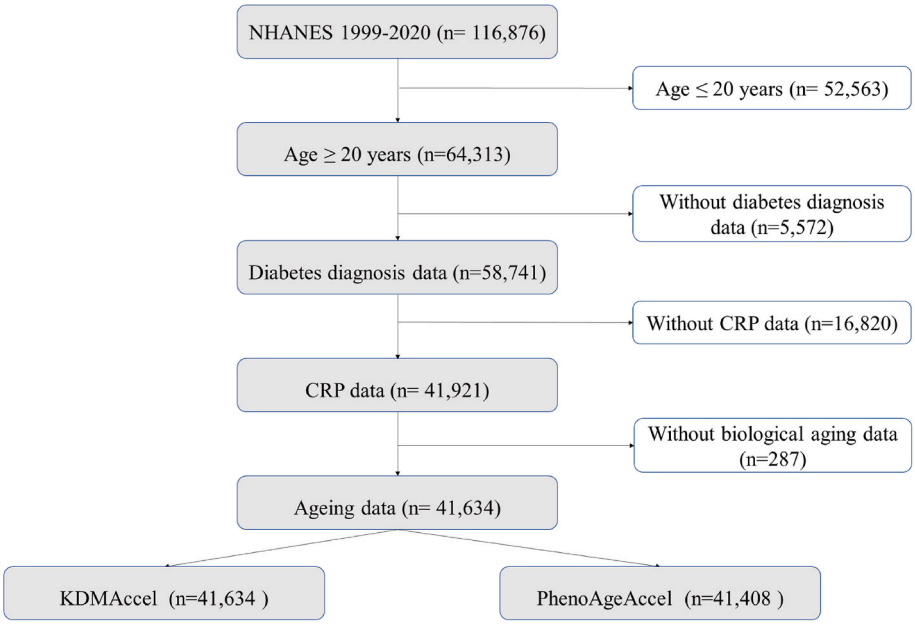

Fig. 1. Flow of study
AbbreviationsNHANES, the National Health and Nutrition Examination Survey; CRP, C-reactive protein; KDM, Klemera-Doubal method; KDM Accel, KDM Acceleration; PhenoageAccel, Phenotypic Age Acceleration.

mg/L) and low CRP (≤3 mg/L). Additionally, based on their CRP levels and diabetic states, participants were classified into four groups: CRP_{low}/non-DM, CRP_{high}/non-DM, CRP_{low}/DM, and CRP_{high}/DM.

2.3. Biological ageing indicators

Biological ageing was calculated using the R package BioAge package [24,24], including the Klemera-Doubal method BioAge (KDM BioAge) [6] and Phenotypic age (PhenoAge) [7]. We included biomarkers without CRP to calculate these 2 indicators of biological ageing. Biological ageing calculated using the biomarker algorithms without CRP correlates strongly with biological ageing measures estimated using the algorithms with CRP (r ranging from 0.9527 to 0.9996) [25]. To assess the biological acceleration of ageing, we computed residuals by regressing chronological age on biological age, called KDM BioAge acceleration (KDMAccel) and Phenotypic age acceleration (PhenoAgeAccel).

$$KDM\ BioAge = \frac{\sum_{i=1}^{n}(x_i - q_i)\frac{k_i}{s_i^2} + \frac{Chronological\ Age}{s_{BA}^2}}{\sum_{i=1}^{n}\left(\frac{k_i}{s_i}\right)^2 + \frac{1}{s_{BA}^2}}$$

$$KDMAccel = KDM\ BioAge - Chronological\ Age$$

Let x represent the value of the biomarker measured for the individual. For each biomarker i, the parameters k, q, and s are estimated through a regression analysis using the actual age of the biomarker in the reference sample. q, k, and s represent the regression intercept, slope, and root-mean-square error, respectively. The S_{BA} is a scaling factor that equals the square root of the variance of the parenchymal age explained by the biomarker, as established in the reference sample.

$$Phenotypic\ Age = 141.50 + \frac{\ln\left[-0.00553 \times \ln\left(\exp\left(\frac{-1.51714 \times \exp(xb)}{0.0076927}\right)\right)\right]}{0.09165}$$

$$PhenoAgeAccel = Phenotypic\ Age - Chronological\ Age$$

where:

$$\begin{aligned} xb = {} & -19.907 - 0.0336 \times Albumin + 0.0095 \times Creatinine + 0.1953 \\ & \times Glucose + 0.0954 \times LnCRP - 0.0120 \times Lymphocyte\ Percent \\ & + 0.0268 \times Mean\ Cell\ Volume + 0.3306 \times Red\ Cell\ Distribution\ Width \\ & + 0.00188 \times Alkaline\ Phosphatase + 0.0554 \times White\ Blood\ Cell\ Count \\ & + 0.0804 \times Chronological\ Age \end{aligned}$$

In our study, PhenoAge advancement was calculated as the disparity between the predicted biological age and the chronological age [26]. PhenoAge advancement was normalized to have a mean of 0 and a standard deviation of 1. A positive value signifies advanced states of biological ageing, whereas a negative value indicates delayed biological ageing. Additionally based on their CRP levels and diabetic states and PhenoAge advancement, the participants were classified into eight groups: CRP_{low}/non-DM/non-Ageing acceleration, CRP_{low}/non-DM/Ageing acceleration, CRP_{low}/DM/non-Ageing acceleration, CRP_{low}/DM/Ageing acceleration, CRP_{high}/non-DM/non-Ageing acceleration, CRP_{high}/non-DM/Ageing acceleration, CRP_{high}/DM/non-Ageing acceleration, and CRP_{high}/DM/Ageing acceleration.

2.4. All-cause and cardiovascular mortality

The follow-up endpoints of this study were all-cause and cardiovascular mortality. Mortality data for NHANES participants were obtained by linking to the National Death Index. According to the International Classification of Diseases, Tenth Revision (ICD-10) [27], we evaluated all-cause mortality and cardiovascular mortality. For each participant, the follow-up duration was calculated starting from their participation date until either death or December 31, 2019.

2.5. Other variables

We included sociodemographic variables, lifestyle factors, and health-related factors as covariates. The sociodemographic variables included age, sex (male/female), race/ethnicity (Mexican-American, other race, non-Hispanic white, non-Hispanic black), education level (less than high school, high school graduate, more than high school), marital status (married/cohabitating, divorced/widowed/separated, never married), and poverty income ratio (PIR, <1.3, 1.3–3.5, >3.5). Body mass index (BMI) was classified as normal (<25.0 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese (≥30.0 kg/m^2). Lifestyle variables encompassed smoking status (never, former, current) heavy alcohol consumption (male ≥20 g/day, female ≥10 g/day), and physical activity (moderate-equivalent exercise: at least 150 min per week to meet the recommended target). Blood tests are performed in specialized laboratories using standard procedures. High-density lipoprotein (HDL) cholesterol and triglycerides are also present. Health-related factors included cardiovascular disease (CVD) and hypertension, which were defined based on a previous NHANES study [27].

2.6. Statistical analysis

As mentioned earlier, we performed all analyses according to the NHANES analysis guidelines [27]. Weighted means (standard errors [SE]) and percentages were employed for both continuous and categorical variables. Due to the small amount of missing covariates data (<5 %), we used multivariate interpolation with chained equations to deal with missing data [19].

The relationship between diabetes mellitus and biological ageing indicators (KDMAccel, PhenoAgeAccel) in different CRP levels, as well as the association between the combination of CRP (≤ or > 3 mg/L) and diabetes (no or yes) with biological ageing indicators, were investigated using survey-weighted multivariate linear regression models assessing the regression coefficients (β) and 95 % confidence intervals (CIs) and the significance of the interaction effect was assessed using the survey-weighted Wald test. Hazard ratios (HRs) and 95 % CIs were assessed by investigating the association of CRP, diabetes, and biological ageing status with all-cause mortality and cardiovascular mortality by weighted Cox proportional risk regression modeling. Two models were constructed to account for traditional risk factors: Model 1 adjusted for age, sex, and race/ethnicity. Model 2 built upon Model 1 by incorporating adjustments for education level, marital status, PIR, BMI, physical activity, alcohol consumption, smoking, HDL cholesterol, triglycerides, hypertension, and CVD.

Sensitivity analyses were conducted to mitigate reverse causality and bolster result stability. (1) Guidelines recommended [28] a threshold value of ≤1 mg/L for low levels of CRP, and we investigated the relationship between diabetes mellitus and biological ageing indicators at a cutoff level of 1 mg/L for CRP; (2) excluded adults with CRP ≥10 mg/L, as abnormally high CRP levels may indicate active infections rather than chronic inflammation [29]; and (3) among persons with diagnosed DM, we evaluated trends in glycemic control defined by HbA1c <7.0 % or FBG 80–130 mg/dL [30] and investigated the relationship between glycemic control and biological ageing indicators; (4) hierarchical analysis by sex, age, hypertension and CVD.

Statistical analyses were performed using R (version 4.1.2) and STATA (version 15.1), and two-sided p values less than 0.05 indicated statistical significance.

3. Results

3.1. Participant characteristics

The study involved 41,634 participants aged 20 years or older. The characteristics of the participants were grouped according to CRP levels (Table 1). The weighted mean (SE) age was 48.7 [0.2] years, 39.1 % were men, and 16,488 (39.6 %) participants had serum CRP >3 mg/L. Participants with higher serum CRP were more likely to have a higher chronological age (48.7 years), KDM BioAge (49.5 years), and Phenotypic Age (49.6 years). Participants with elevated CRP had greater proportions of individuals with prediabetes, diabetes, CVD, and hypertension than did those with low CRP.

3.2. Relationship between diabetes mellitus, serum CRP, and biological ageing indicators

As expected, both diabetes mellitus and serum CRP levels were significantly associated with biological ageing (Table S1). After full adjustment for potential confounding factors, compared with those with no diabetes, KDMAccel increased by 0.86 years (95 % CI: 0.78–0.95), and 3.65 years (95 % CI: 3.43–3.87) in adults with prediabetes and diabetes, respectively, and PhenoAgeAccel increased by 1.60 years (95 % CI: 1.42–1.79), and 7.67 years (95 % CI: 7.30–8.04), respectively. KDMAccel increased by 0.63 years (95 % CI: 0.54–0.73) and the PhenoAgeAccel increased by 2.52 years (95 % CI: 2.32–2.71) in the high CRP group compared with the low CRP group.

Table 1
Baseline characteristics of participants in NHANES 1999–2020 by CRP levels.

Variables	CRP ≤3 mg/L	CRP >3 mg/L
Age, year	46.3 ± 0.2	48.7 ± 0.2
Male, %	14000 (53.4)	6442 (39.1)
Race/ethnicity, %		
Non-Hispanic White	11604 (69.5)	7088 (66.5)
Non-Hispanic Black	4648 (9.4)	3792 (12.8)
Mexican American	4438 (7.8)	3327 (8.8)
Other	4420 (13.3)	2281 (11.9)
Education, %		
Less than high school	6506 (15.6)	4999 (19.6)
High school	5774 (24.0)	3998 (26.7)
More than high school	12866 (60.5)	7491 (53.7)
Marital status, %		
Married/Living with a partner	14188 (59.6)	8942 (58.3)
Widowed/Divorced/Separated	6008 (23.8)	3358 (20.5)
Never Married	4950 (16.7)	4188 (21.2)
Poverty income ratio, %		
≤1.30	8489 (16.7)	6564 (22.0)
1.31–3.5	8786 (32.5)	5840 (34.2)
>3.5	7811 (43.0)	4084 (35.8)
Body mass index, %		
>25 kg/m^2	9662 (40.0)	2887 (16.9)
25–30 kg/m^2	9345 (36.1)	4645 (27.4)
≥30 kg/m^2	6139 (24.0)	8956 (55.8)
Smoking status, %		
Never	18138 (55.1)	8601 (50.8)
Former	6252 (24.9)	4257 (26)
Current	5013 (20)	3630 (23.2)
Physical activity, %	9476 (43.8)	4298 (30.1)
Heavy drinking, g/d	1779 (8.5)	988 (7.3)
Diabetes, %		
Non-diabetes	14851 (65.1)	7443 (49.7)
Prediabetes	6906 (25.2)	5305 (31.9)
Diabetes	3389 (9.7)	3740 (18.4)
CVD, %	2255 (6.7)	2069 (10.6)
Hypertension, %	9384 (32.0)	8110 (45.2)
KDM Age, year	46.3 ± 0.2	49.5 ± 0.2
Phenotypic Age, year	43.7 ± 0.3	49.6 ± 0.2
Triglycerides, mmol/L	1.56 ± 0.1	1.87 ± 0.2
HDL-Cholesterol, mmol/L	1.42 ± 0.1	1.30 ± 0.1

Percentage, means and standard errors are adjusted for NHANES sampling weights. The observed numbers for categorical variables were unweighted.
Abbreviations: KDM, Klemera-Doubal method; CVD, cardiovascular diseases; High-density Lipoprotein-Cholesterol, HDL-Cholesterol.

According to unadjusted and adjusted logistic regression models, the association between diabetes mellitus and biological ageing indicators KDMAccel or PhenoAgeAccel was especially significant in adults with elevated CRP levels compared to those with low CRP (Fig. 2). After adjustment for sociodemographic variables, among adults with serum CRP ≤3 mg/L, KDMAccel increased by 0.93 years (95 % CI: 0.82–1.05), and 3.35 years (95 % CI: 3.04–3.65) in adults with prediabetes and diabetes, respectively; and PhenoAgeAccel increased by 1.63 years (95 % CI. 1.42–1.84), and 7.15 years (95 % CI: 6.59–7.71) respectively, compared with those with no diabetes. Among adults with CRP >3 mg/L, KDMAccel increased by 0.89 years (95 % CI: 0.73–1.05), and 4.54 years (95 % CI: 4.23–4.84) in adults with prediabetes and diabetes, respectively; and PhenoAgeAccel increased by 1.96 years (95 % CI: 1.67–2.26), and 9.66 years (95 % CI: 9.20–10.13), respectively, compared with those with no diabetes. According to the fully adjusted models, compared with those with no diabetes and CRP ≤3 mg/L, KDMAccel increased by 0.86 years (95 % CI: 0.75–0.98), and 3.11 years (95 % CI: 2.80–3.41) in adults with CRP ≤3 mg/L and prediabetes and diabetes, respectively, and PhenoAgeAccel increased by 1.43 years (95 % CI: 1.23–1.64) and 6.38 years (95 % CI: 5.83–6.92), respectively. Among adults with high CRP levels, compared with those with no diabetes, KDMAccel increased by 0.82 years (95 % CI: 0.67–0.98), and 4.08 years (95 % CI: 3.78–4.39), in adults with prediabetes and diabetes, respectively; and PhenoAgeAccel was increased by 1.65 years (95 % CI: 1.36–1.93), and 8.65 years (95 % CI: 8.16–9.14), respectively. A significant interaction effect was found between CRP and diabetes status on biological ageing acceleration in both the adjusted and unadjusted models (p for interaction <0.001).

We further investigated the relationships between the combinations of CRP and diabetes and biological ageing (Fig. 3). The population with diabetes and high CRP levels had the most severe biological ageing acceleration. In the fully adjusted models, using the CRP_{low}/non-DM group as a reference, adults in CRP_{high}/non-DM, CRP_{low}/DM, and CRP_{high}/DM groups had significantly advanced biological ageing with KDMAccel increasing by 0.40 years (95%CI: 0.30–0.49), 2.65 years (95%CI: 2.37–2.93) and 4.13 years (95%CI: 3.89–4.38), respectively; and PhenoAgeAccel increasing by 2.03 years (95%CI: 1.86–2.21), 5.66 years (95%CI: 5.14–6.18) and 9.88 years (95%CI: 9.46–10.13), respectively.

3.3. The associations between CRP, diabetes, biological ageing acceleration, and mortality risk

There were 6534 deaths in the study population during a median of 11.9 years of follow-up. We calculated the unadjusted and adjusted HRs and 95%CIs for all-cause and cardiovascular mortality risk across the combination of binary CRP and diabetes (Fig. S1). According to the fully adjusted models, using the CRP_{low}/non-DM group as a reference, adults in CRP_{high}/non-DM, CRP_{low}/DM, and CRP_{high}/DM groups were significantly associated with all-cause mortality with HR 1.38(95%CI: 1.28–1.49), 1.69(95%CI: 1.53–1.86) and 2.15(95%CI: 1.93–2.39), respectively, and increased risk of cardiovascular mortality with HR 1.23(95%CI: 1.08–1.40), 1.72(95%CI: 1.45–2.04) and 2.20(95%CI: 1.85–2.63), respectively. We further considered the association of biological ageing acceleration with mortality risk (Fig. 4). We stratified binary CRP (low/high), DM (no/yes), and PhenoAge advancement (no/yes) to yield 8 combinations. Compared to adults with non-DM, low CRP, and no ageing acceleration, the multivariable-adjusted HRs (95% CIs) of all-cause and cardiovascular mortality in those with DM, CRP, and ageing acceleration were 3.22 (2.79–3.72), and 3.57 (2.81–4.54), respectively.

3.4. Additional analysis

We repeated the analysis for the association between diabetes and

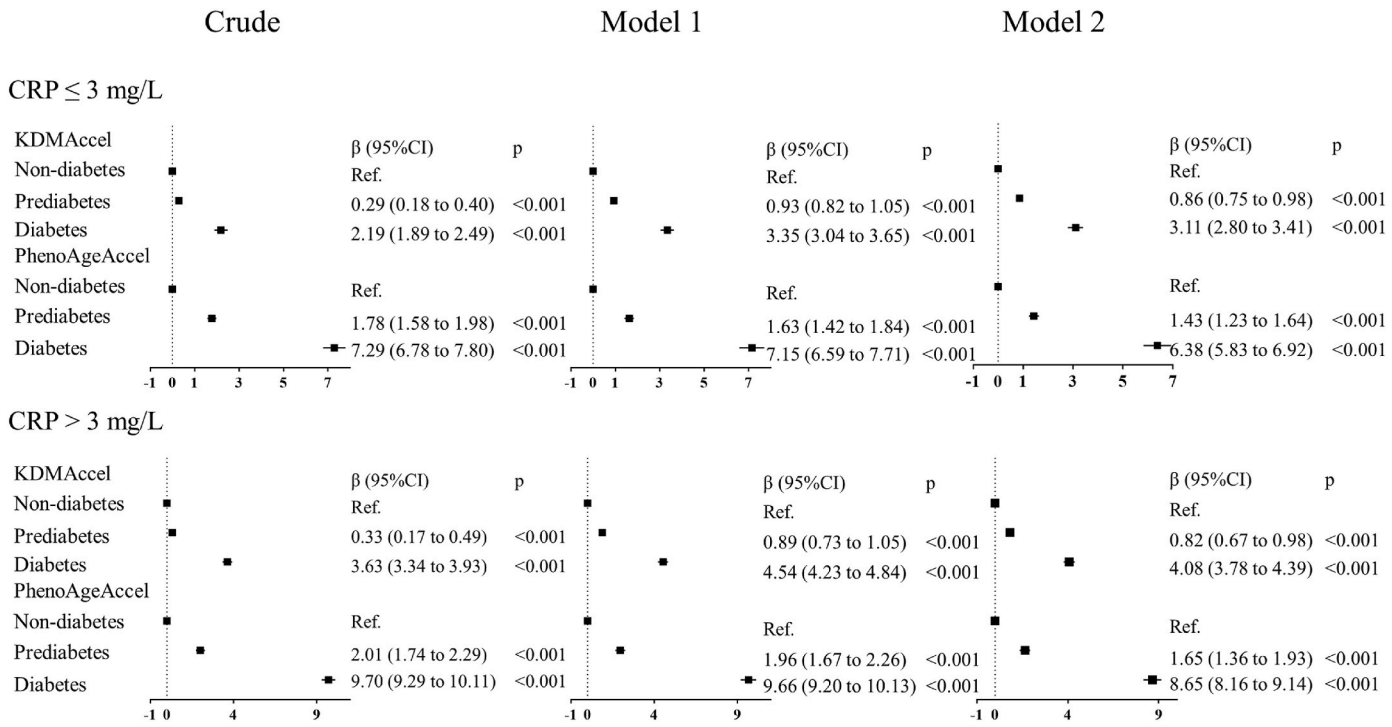

Fig. 2. The relationship between diabetes mellitus and biological ageing acceleration in different C-reactive protein levels
We found a significant interaction between CRP and diabetes status on biological ageing acceleration in both adjusted and unadjusted models (p for interaction <0.001).
Abbreviations: CRP, C-reactive protein; KDM, Klemera-Doubal method; KDMAccel, KDM Age acceleration; PhenoAgeAccel, Phenotypic Age acceleration.
Model 1: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black).
Model 2: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black), education level (less than high school, high school, more than high school), marital status (married/cohabitating, divorced/widowed/separated, never married), Poverty income ratio (<1.3, 1.3–3.5, >3.5). body mass index (<25.0 kg/m^2, 25.0–29.9 kg/m^2, ≥30.0 kg/m^2), smoking status (never, former, current), heavy alcohol consumption (male ≥20g/day, female ≥10g/day), physical activity (moderate-equivalent exercise: at least 150 min per week to meet the recommended target), Triglycerides (continuous), HDL-Cholesterol (continuous), hypertension (Yes/no) and cardiovascular diseases (Yes/no).

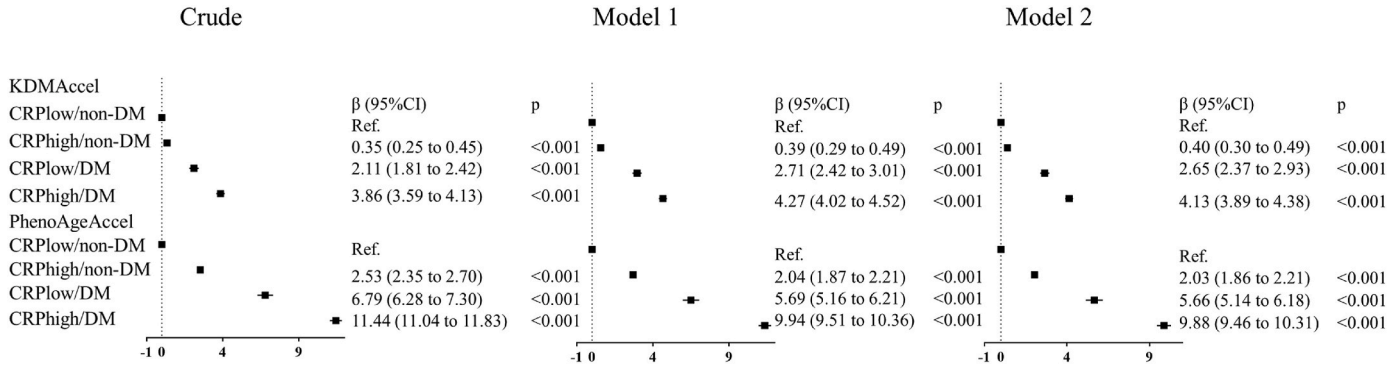

Fig. 3. The relationship between the combination of diabetes mellitus and different CRP levels and biological ageing indicators
Abbreviations: CRP, C-reactive protein; DM, diabetes mellitus; KDM, Klemera-Doubal method; KDMAccel, KDM Age acceleration; PhenoAgeAccel, Phenotypic Age acceleration.
Model 1: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black).
Model 2: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black), education level (less than high school, high school, more than high school), marital status (married/cohabitating, divorced/widowed/separated, never married), Poverty income ratio (<1.3, 1.3–3.5, >3.5). body mass index (<25.0 kg/m^2, 25.0–29.9 kg/m^2, ≥30.0 kg/m^2), smoking status (never, former, current), heavy alcohol consumption (male ≥20g/day, female ≥10g/day), physical activity (moderate-equivalent exercise: at least 150 min per week to meet the recommended target), Triglycerides (continuous), HDL-Cholesterol (continuous), hypertension (Yes/no) and cardiovascular diseases (Yes/no).

biological ageing acceleration stratified by serum CRP with the new threshold (≤1 mg/L and >1 mg/L, Table S2). Consistently, biological ageing acceleration associated with diabetes was more significant in adults with CRP >1 mg/L compared to those with CRP ≤1 mg/L (PhenoAgeAccel 7.98 versus 6.13 years). After excluding individuals with CRP ≥10 mg/L the findings were consistent with the primary analysis (Table S3). Taking the best glycemic control as a reference, after adjusting for confounders, KDMAccel increased by at least 2.20 years among adults with poorer glycemic control (Table S4). Subgroup analyses by age, sex, hypertension, and CVD were performed to further

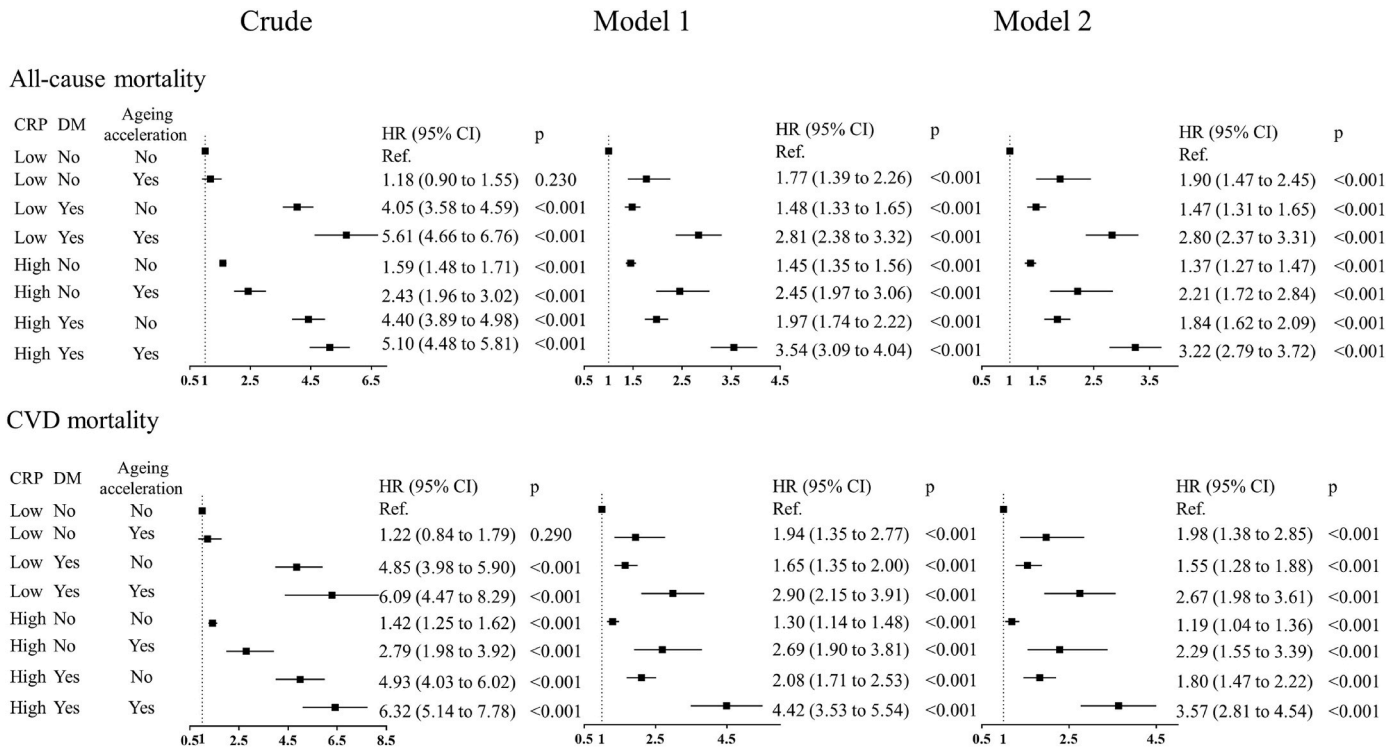

Fig. 4. The relationship between the combinations of diabetes mellitus, different CRP levels, and ageing state and mortality risk
Abbreviations: CRP, C-reactive protein; DM, diabetes mellitus; CVD, cardiovascular diseases.
Model 1: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black).
Model 2: age (continuous), sex (female, male), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black), education level (less than high school, high school, more than high school), marital status (married/cohabitating, divorced/widowed/separated, never married), Poverty income ratio (<1.3, 1.3–3.5, >3.5). body mass index (<25.0 kg/m^2, 25.0–29.9 kg/m^2, ≥30.0 kg/m^2), smoking status (never, former, current), heavy alcohol consumption (male ≥20g/day, female ≥10g/day), physical activity (moderate-equivalent exercise: at least 150 min per week to meet the recommended target), hypertension (Yes/no) and cardiovascular diseases (Yes/no).
*Additionally based on binary CRP (low/high), DM (no/yes), and PhenoAge advancement (no/yes) to yield 8 combinations: CRP_{low}/non-DM/non-Ageing acceleration, CRP_{low}/non-DM/Ageing acceleration, CRP_{low}/DM/non-Ageing acceleration, CRP_{low}/DM/Ageing acceleration, CRP_{high}/non-DM/non-Ageing acceleration, CRP_{high}/non-DM/Ageing acceleration, CRP_{high}/DM/non-Ageing acceleration, CRP_{high}/DM/Ageing acceleration.

explore the potential association of confounding factors on the association between diabetes and biological ageing indicators across various CRP levels (Tables S5–S8). The association between diabetes status and biological ageing was more significant among adults with aged less than 60 years and males.

4. Discussion

This study is the first to investigate the joint association of inflammation and diabetes mellitus on biological ageing in 41,634 US nationally representative participants. Our findings indicated that the association between diabetic status and accelerated biological ageing was more significant in adults with elevated CRP levels compared to those with low CRP. Notably, an accelerated rate of biological ageing was also observed in adults with prediabetes regardless of CRP levels, suggesting that biological ageing acceleration might be associated with impaired glycemic metabolism status before diabetes is diagnosed. Compared to the CRP_{low}/non-DM group, the CRP_{high}/DM group had the fastest rate of biological ageing and the highest risk of all-cause and cardiovascular mortality. The ageing acceleration phenotype might be increased the risk of mortality associated with diabetes, which was further amplified in populations with high CRP. Unexpectedly, the severity of biological ageing acceleration in populations with both diabetes and inflammation is more severe than that in those with diabetes or inflammation alone. Our finding underlined the importance of dual management of residual inflammation and blood glucose control which may substantially contribute to the improvement in healthy ageing.

Compared to young individuals, the number of *SA-β-gal* cells in isolated islets from healthy elderly individuals increases. Furthermore, compared to people with non-diabetes, individuals with T2D exhibit a further increase in the number of islets. This suggests that β-cell ageing may contribute to the pathogenesis of T2D [15]. Another model of chronic inflammation is based on the functional loss of the anti-inflammatory cytokine interleukin 10. In this scenario, the loss of anti-inflammatory signals leads to a state of low-grade proinflammatory status, accompanied by signs of accelerated ageing in middle age [31]. The research found that biological ageing is positively correlated with mortality in people with diabetes [32,33]. Our results support for the first time the population-based evidence to support the joint presence of low-grade inflammation and diabetes mellitus might be associated with an increased likelihood of biological ageing acceleration and premature mortality. Cribb et al. explored the association between inflammation and epigenetic ageing and found that the strongest associations were between CRP and GrimAgeAccel [34]. CRP-measured inflammation accounts for nearly one-third of the relationship between metabolic syndrome and GrimAge age acceleration [35]. Chiurazzi et al. reported that intermittent lipopolysaccharide-induced inflammation resulted in cognitive deficits [36].

Inflammation and immune-mediated injury are thought to play pathogenic roles in many diseases. Elevated CRP levels in plasma are associated with inflammation, injury, or bacterial infection [37]. Previous research has demonstrated an association between low-grade inflammation and the development of diabetes, cardiovascular disease, and malignant neoplasms [22,38]. Elevated CRP is linked to the

accelerated development of diabetes mellitus, which is attributed to the biological mechanisms of low-grade chronic inflammation in glucose metabolism disorders [39]. Beyond elevating the risk of diabetes development, heightened inflammation may worsen the progression of diabetes by inducing the destruction of pancreatic tissue through inflammation-induced amyloid deposits [40,41]. In addition, increased inflammation (e.g., elevated CRP levels), including diabetic retinopathy [42,43]and cardiovascular disease [10], may increase diabetic end-organ damage. Our study further demonstrated the significant interaction of CRP and diabetes status on biological ageing acceleration and premature mortality. Although the mechanisms underlying the role of chronic inflammation in diabetes development remain unknown, the observation that adipose tissue synthesizes significant proinflammatory cytokines such as tumor necrosis factor, interleukins-1, and -6 and that inflammatory biomarkers correlate with body fat quantity indicates that activated innate immunity and inflammation are crucial biological factors in the pathogenesis of diabetes and diabetes mellitus [44].

Diabetes is a risk factor associated with CVD [45,46]. A mechanistic study in diabetic rats revealed that throughout the progression of diabetes, there are gradual changes in cardiac function that are closely associated with alterations in two groups of proteins, neurotrophic cascade proteins (NTF4) and electron transport chain cascade proteins (ETFBs) [47]. Multiple epidemiological studies have explored increased mortality associated with cardiovascular disease in individuals with diabetes. Evidence from the Japanese Observational Cohort Cardiovascular Prevention Study suggested that diabetes mellitus represents a significant risk factor for all-cause mortality and deaths specific to CVD, with a 2- to 4-fold increased risk of cardiovascular death [48]. A meta-analysis revealed a nonlinear association between CRP and both all-cause mortality and CVD mortality [49]. Previous studies demonstrated the predictive value of CRP for the risk of both all-cause and cardiovascular mortality [50,50], and a study conducted using NHANES data from 1999 to 2011 demonstrated that elevated CRP levels were associated with reduced overall and CVD survival [51]. In people with diabetes [52], CRP might be associated with all-cause mortality, cardiovascular mortality, and malignant neoplasm mortality, and the results are similar in nondiabetic, but risk levels differ. Our study revealed that the risk of all-cause and cardiovascular death was greater in the CRP_{high}/DM group than in the CRP_{low}/non-DM group. These findings highlight the need for joint management of inflammation and blood glucose to prevent ageing acceleration and premature death.

5. Clinical relevance

The clinical implications of our study lie in assessing the association of different inflammatory states and diabetes with biological ageing and premature death. It was found that diabetes might accelerate biological ageing in a low-grade inflammatory state, and the severity of accelerated biological ageing in the population with diabetes and inflammation is more severe than that in the population with diabetes or inflammation. Our findings highlight the importance of dual management of residual inflammation and glycemic control toward improving healthy ageing in future practice.

6. Strengths

There are several strengths to this study. First, utilizing a nationally representative dataset with a large sample size (41,634) facilitates the generalization of the findings to a large population and enhances the external validity of our results. Second, utilizing rigorous statistical methods, we controlled for potential confounding variables, thereby enhancing the internal validity of the findings. Finally, multiple chained interpolations were employed to fill in missing covariate data, thereby enhancing the statistical efficiency.

7. Limitations

This study has limitations. First, as a cross-sectional study, it cannot establish causal conclusions because of inherent flaws. Therefore, a well-designed cohort study is essential to address this limitation. Second, in exploring the relationship between glycemic status and biological ageing across different CRP levels in the general population, special populations such as children were excluded from this study. In future research, we intend to incorporate specific populations for further analysis. Third, a definition of distinguishing Type 1 Diabetes and T2D was lacking in this study. Fourth, although we collected data from 11 cycles of the NHANES from 1999 to 2000 to 2017–2020, we did not track the data of the same individuals. Therefore, it is difficult to conduct longitudinal research or trajectory analysis on NHANES data. Further longitudinal research is needed in the future. Finally, CRP measurements and analyses were conducted at different stages of the NHANES employing various analytical methods. Despite efforts to statistically harmonize, the results do not match the precision of simultaneous using consistent experimental methods.

8. Conclusions

These findings suggest that the joint presence of low-grade inflammation and dysfunctional glycemic metabolism conditions might be associated with higher odds of biological ageing acceleration and premature mortality. The dual management of residual inflammation and blood glucose in the people with diabetes may contribute to improvements in healthy ageing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2024.103050.

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