The mixed effect of Endocrine-Disrupting chemicals on biological age Acceleration: Unveiling the mechanism and potential intervention target

Journal Article

Author(s):
Huang, Weichao; Zhang, Zilong; Colucci, Manuel; Deng, Linghui; Yang, Mi; Huang, Xinyi; Zhou, Xianghong; Jin, Yumin; Lazzarini, Edoardo; Balbi, Carolina; Oriol, Juanola; Valdata, Aurora; Bressan, Silvia; Zhan, Yu; Qi, Fang; Wei, Qiang; Yang, Lu; Zou, Xiaoli; Qiu, Shi

Publication date:
2024-02

Permanent link:
https://doi.org/10.3929/ethz-b-000657093

Originally published in:
Environment International 184, https://doi.org/10.1016/j.envint.2024.108447

Environment International 184 (2024) 108447

Contents lists available at ScienceDirect

Environment International

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

Full length article

The mixed effect of Endocrine-Disrupting chemicals on biological age Acceleration: Unveiling the mechanism and potential intervention target

Weichao Huang [a,c,1], Zilong Zhang [a,c,1], Manuel Colucci [b,e,f,1], Linghui Deng [c,d], Mi Yang [g], Xinyi Huang [g], Xianghong Zhou [a], Yumin Jin [a], Edoardo Lazzarini [h,i], Carolina Balbi [j,k], Oriol Juanola [l], Aurora Valdata [b,e,m], Silvia Bressan [b,e], Yu Zhan [n], Fang Qi [o], Qiang Wei [a], Lu Yang [a,*], Xiaoli Zou [g,*], Shi Qiu [a,b,*]

[a] Department of Urology and Institute of Urology, West China Hospital, Sichuan University, Chengdu 610041, China
[b] Institute of Oncology Research (IOR), Oncology Institute of Southern Switzerland (IOSI), CH6500 Bellinzona, Switzerland
[c] National Clinical Research Center of Geriatrics, The Center of Gerontology and Geriatrics, West China Hospital, Sichuan University, Chengdu, China
[d] Department of Gerontology, West China Hospital of Sichuan University, Chengdu, China
[e] Università della Svizzera Italiana, CH6900 Lugano, Switzerland
[f] Faculty of Biology and Medicine, University of Lausanne UNIL, CH1011 Lausanne, Switzerland
[g] Department of Sanitary Technology, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, China
[h] Laboratory for Cardiovascular Theranostics, Cardiocentro Ticino Institute, Ente Ospedaliero Cantonale Lugano, Switzerland
[i] Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano Switzerland
[j] Cellular and Molecular Cardiology, Istituto Cardiocentro Ticino, Laboratories for Translational Research, Ente Ospedaliero Cantonale, Bellinzona, Switzerland
[k] Center for Molecular Cardiology, Zurich, Switzerland
[l] Gastroenterology and Hepatology, Laboratories for Translational Research, Ente Ospedaliero Cantonale, Bellinzona, Switzerland
[m] Department of Health Sciences and Technology (D-HEST) ETH Zurich, Zurich, CH, Switzerland
[n] Department of Environmental Science and Engineering, Sichuan University, Chengdu, Sichuan 610065, China
[o] Department of Plastic Surgery, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou 563000, People's Republic of China

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Biological age
Endocrine-disrupting chemical
Phthalate
Paraben
AGE-RAGE signaling pathway

ABSTRACT

Introduction: Although previous studies investigated the potential adverse effects of endocrine-disrupting chemicals (EDCs) on biological age acceleration and aging-related diseases, the mixed effect of multiple types of EDCs on biological age acceleration, including its potential underlying mechanism, remains unclear.

Methods: Data from the National Health and Nutrition Examination Survey (NHANES) were used to analyze biological age measures, including Klemera-Doubal method biological age (KDM-BA), phenotypic age, and homeostatic dysregulation (HD). Weight quantile sum (WQS) regression was performed to screen biological age-related EDCs (BA-EDCs) and assess the mixed effect of BA-EDCs on biological age acceleration and aging-related disease. Targets of BA-EDCs were obtained from three databases, while heart aging-related genes were obtained from the Aging Anno database. Protein–protein interaction (PPI) network and MCODE algorithm were applied to identify potential interactions between BA-EDC targets and heart aging-related genes. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to identify related pathways.

Results: This cross-sectional study included 1,439 participants. A decile increase in BA-EDCs co-exposure was associated with 0.31 years and 0.17 years of KDM-BA and phenotypic age acceleration, respectively. The mixed effect of BA-EDCs was associated with an increased prevalence of atherosclerotic cardiovascular disease (ASCVD). Vitamins C and E demonstrated a significant interaction effect on the association between BA-EDCs and KDM-BA acceleration. PPI network and functional enrichment analysis indicated that the AGE-RAGE signaling pathway in diabetic complications was significantly enriched.

* Corresponding authors.
E-mail addresses: wycleflue@scu.edu.cn (L. Yang), zouxl_1113@163.com (X. Zou), qiushi@scu.edu.cn (S. Qiu).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.envint.2024.108447
Received 20 June 2023; Received in revised form 29 November 2023; Accepted 16 January 2024
Available online 17 January 2024

Conclusion: Our results showed that the co-exposure effect of BA-EDCs was associated with biological age acceleration and ASCVD, with the AGE-RAGE signaling pathway being the underlying mechanism. Vitamins C and E may also be an actionable target for preventing EDC-induced biological aging.

1. Introduction

Aging is a process characterized by time-related deterioration in body homeostasis, and it is a major risk factor for the development of various diseases, including cardiovascular disease. (Pietri and Stefanadis, 2021) Though aging is an inevitable process, there is an incongruence between chronological age and aging rate. (Ferrucci et al., 2018) Therefore, the concept of biological age has been introduced to reflect the actual state of aging for each individual. (Franceschi et al., 2018) Klemera-Doubal method biological age (KDM-BA), phenotypic age, homeostatic dysregulation (HD), epigenetic age, and telomere length are very commonly used biological age metrics that can effectively reflect the actual status and rate of individual aging, each with its own focus and advantages. Specifically, the first three of these biological aging metrics have been constructed using clinical biomarkers. For instance, KDM-BA corresponds to the chronological age at which the physiology of an individual would be approximately normal, (Kwon and Belsky, 2021) while phenotypic age corresponds to the chronological age at which individual mortality risk would be approximately normal in a reference population. (Kwon and Belsky, 2021) Furthermore, HD reflects individual differences in physiological status from a healthy reference. (Kwon and Belsky, 2021) Moreover, epigenetic age has been constructed to predict chronological age based on the methylation of CpG sites, (Li et al., 2022) while telomere length has been used as a biological age measure, especially since it is a well-known hallmark of both cellular senescence and organismal aging. (Vaiserman and Krasnienkov, 2020) Despite the fact that these two biological aging metrics can accurately reflect the state of aging at both cellular and molecular levels, their use is less convenient. Previous research studies on aging biology revealed that the high prevalence of multimorbidity in older people is due to the age-dependent accumulation of cellular and molecular changes acting to damage the integrity of tissues and organ systems throughout the body, giving rise to multiple chronic conditions. (López-Otín et al., 2013) Therefore, interventions to slow or reverse the accumulation of such alterations could prevent or delay the onset of many different diseases, ultimately extending a healthy lifespan. (Kennedy et al., 2014).

Endocrine-disrupting chemicals (EDCs) are defined as exogenous chemicals or mixtures of chemicals interfering with many aspects of the hormone action. (Gore, 2016; Gore et al., 2015) To date, EDC accounts for approximately 1000 synthesized chemicals. (Gore, 2016) Among those chemicals, the most characterized EDC classes are phenol, paraben, phthalate, and per- and poly-fluoroalkyl substances (PFAS), which can induce adverse effects on glucose homeostasis and diabetes, (Wang et al., 2012; Lin et al., 2011; Lang et al., 2008; Shankar and Teppala, 2011) reproduction, (Bloom et al., 2015; Upson et al., 2013) hormone-sensitive cancer, (Hu et al., 2012; Pupo et al., 2012) and cardiovascular disease. (Gore, 2016; Gore et al., 2015; Lang et al., 2008; Chang et al., 2021; Melzer et al., 2010; Melzer et al., 2012) The relationship between EDCs and aging is still controversial. (Khodasevich et al., 2023; Chaney and Wiley, 2023; Curtis et al., 2019) Some studies have suggested a negative correlation between EDC exposure and accelerated biological aging. Previous studies demonstrated that prenatal phthalate exposure and PFAS exposure were negatively associated with epigenetic age or biological age acceleration. (Khodasevich et al., 2023; Chaney and Wiley, 2023) Recently, Niemiec demonstrated that perfluorodecanoate (PFDA) was inversely associated with epigenetic age acceleration in umbilical cord blood. (Niemiec et al., 2023) The Harvard Epigenetic Birth Cohort showed that telomere length was 20 % shorter among boys in the highest quartile of maternal triclosan concentration compared to the lowest one. (Michels et al., 2020) However, longer telomere length was associated with increased gestational concentrations of mono-isobutyl phthalate, and, among boys, with increased concentrations of mono-2-ethylhexyl phthalate. (Michels et al., 2020) In addition polybrominated biphenyl was found to be positively associated with epigenetic age acceleration. (Curtis et al., 2019) Besides, bisphenol A (BPA) actively participates in accelerated cell aging mechanisms, affecting vascular endothelium and promoting cardiovascular diseases. (Moreno-Gómez-Toledano et al., 2021; Ribeiro-Varandas et al., 2014)Clarity *et al.* suggested a positive association between PFAS and telomere length in female workers. (Clarity et al., 2021) Interestingly, this effect was observed to be significantly stronger among firefighters compared to office workers. (Clarity et al., 2021) Most studies have attempted to explore the effect of either single chemicals or one category of EDCs on biological age. In reality, however, the average person is exposed to various chemicals simultaneously, possibly fostering interactions among these co-administered chemicals. (Zhang et al., 2019) As a result, it is essential to explore the mixed effect of EDCs on biological age. Since the relationship between EDC mixture exposure and biological age acceleration remains elusive, it is necessary to investigate the effect of simultaneous exposure to various EDCs on biological aging, and also assess the association between biological age-related EDCs (BA-EDCs) and aging-related diseases.

Aging-related diseases, including atherosclerotic cardiovascular disease (ASCVD), hypertension, diabetes, and chronic kidney disease (CKD), have also been related to exposure to EDCs. Atherosclerosis is widely acknowledged as the primary contributor to cardiovascular disease, the main cause of mortality worldwide, (Libby et al., 2016; Tsao et al., 2022) and it is characterized by the build-up of plaque in arterial walls, leading to arterial thickening. Mechanisms involved in the aging process were also found to be related to the pathogenesis of cardiovascular disease, including oxidative stress, activation of inflammation, and metabolic disorder. (Pietri and Stefanadis, 2021) Previous studies suggested that exposure to individual EDCs posed an adverse effect on the prognosis of cardiovascular disease and atherosclerosis. (Posnack, 2014; Wen et al., 2022; Lind and Lind, 2011) In addition, exposure to phthalate was related to elevated blood pressure across various age groups and populations. (Trasande and Attina, 2015; Karle et al., 1997; Zhang et al., 2018) Besides, EDCs exposure has also been considered a risk factor for the development of diabetes. (Lin et al., 2011; Shankar and Teppala, 2011) Priego *et al.* demonstrated that BPA may have a deleterious effect on the kidneys by means of deregulating autophagy flux and redox protective mechanisms. (Priego et al., 2021) Epidemiological study also suggested that phthalate and BPA are associated with decreased renal function. (Kang et al., 2021) As mentioned before, due to the complex exposure pattern, high correlation, and complicated interactions of chemicals, it is both reasonable and necessary to perform a mixture analysis to assess the co-exposure effect of EDCs. However, the mixture effect of multiple categories of EDCs on aging-related diseases is poorly understood.

Although the relationship between EDC exposure and accelerated biological aging, as well as age-related diseases, has been confirmed in many studies, the underlying mechanisms remain unclear. Exploring the potential mechanisms and pathways linking EDC-induced accelerated biological aging and age-related diseases can help identify intervention targets, which hold significantly positive public health implications for preventing or mitigating the adverse effects of EDCs.

Due to the negative effect of EDCs, it is urgent to explore possible interventions mitigating induced adverse health effects. Vitamins may serve as actionable prevention targets. For instance, vitamins C and E are

powerful antioxidants that mediate several beneficial effects on redox oxidative and mitochondrial pathways. (Padayatty et al., 2003; Naidu, 2003; Napolitano et al., 2019) Accumulating evidence indicates a rescuing role of vitamin C in premature aging, (Monacelli et al., 2017) while supplementation of vitamin C appears to mediate oxidative stress, telomere attrition, and excessive secretion of inflammatory factors, extending lifespan. (Monacelli et al., 2017) Interestingly, vitamin C was also found to positively modulate inflammaging and immunosenescence, two hallmarks of biological aging. (Monacelli et al., 2017) Moreover, it has been shown to epigenetically regulate genome integrity and stability, indicating a key role of targeted nutrition in healthy aging. (Monacelli et al., 2017) According to Zingg *et al.,* treatment with vitamin E analogs may counteract CD-36 mediating inflammatory, senescent, and atherosclerotic events in monocytes and macrophages. (Zingg et al.,

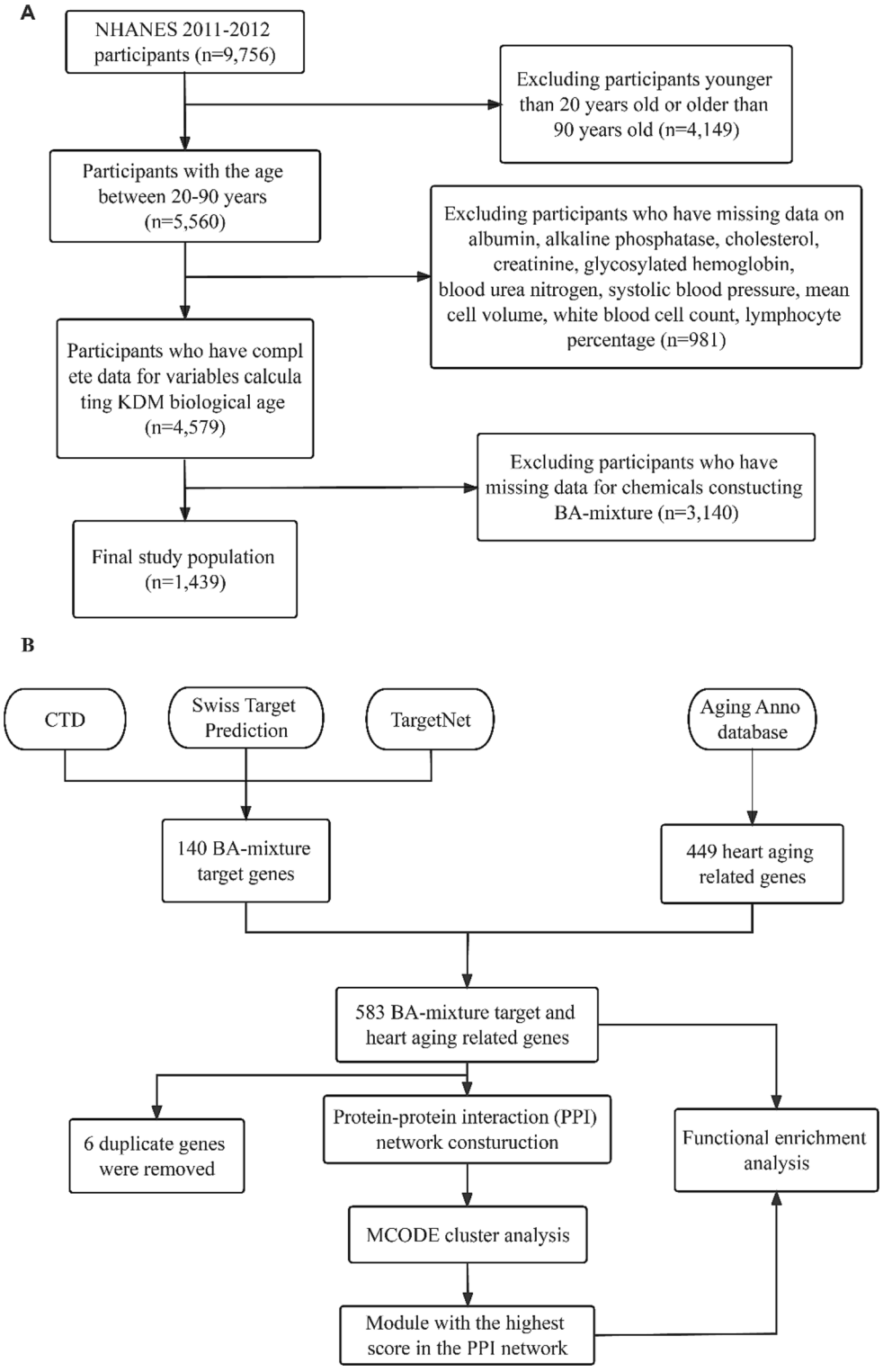

Fig. 1. Flowchart of our study. A) Inclusion and exclusion criteria of the study population in this cross-sectional study. B) Workflow of the network and functional enrichment analysis.

2022) Besides, vitamin E was suggested as an intervention for improving age-associated immune function. (Wu and Meydani, 2014) Therefore, both vitamins C and E may be a potential target for preventing biological aging.

Given their effect on biological age acceleration and aging-related disease, (Lang et al., 2008; Melzer et al., 2010; Melzer et al., 2012; Khodasevich et al., 2023; Chaney and Wiley, 2023; Curtis et al., 2019; Niemiec et al., 2023; Michels et al., 2020; Moreno-Gómez-Toledano et al., 2021; Ribeiro-Varandas et al., 2014; Clarity et al., 2021; Zhang et al., 2019; Posnack, 2014; Wen et al., 2022; Lind and Lind, 2011; Trasande and Attina, 2015; Karle et al., 1997; Zhang et al., 2018; Priego et al., 2021; Kang et al., 2021; Campisi et al., 2023; Oluwayiose et al., 2022; Rattan et al., 2022; Zhang et al., 2022; Moreno-Gomez-Toledano, 2022) we hypothesized that EDCs mixture exposure may disrupt metabolic process and lead to metabolic disorder, which accelerates biological age, and therefore promotes the development of aging-related diseases, including ASCVD, hypertension, diabetes, and CKD. To address this hypothesis, we combined epidemiological evidence with bioinformatic analysis to identify potential mechanisms of BA-EDCs-induced aging-related diseases and actionable targets for BA-EDCs-induced biological aging.

First, weighted quantile sum (WQS) regression was performed to screen EDCs that have non-negligible contribution to biological age acceleration, these EDCs were considered as biological age-related EDCs (BA-EDC). Further, we investigated both individual and mixed effects of BA-EDCs on biological age acceleration and aging-related diseases. Consequently, we tried to identify vitamins that could mitigate the adverse effects of BA-EDCs. Last, but not least, network pharmacology analysis methods were applied to explore potential mechanisms of BA-EDCs and aging-related diseases associated to BA-EDCs exposure.

2. Materials and methods

2.1. Study population, EDC targets, and heart aging-related genes

NHANES is a nationally representative survey of the non-institutionalized US population that is continuously conducted every other year by the Centers for Disease Control and Prevention (CDC). The study protocol was approved by the Institutional Review Board of the National Center for Health Statistics, and consent in written form was obtained from all participants (https://www.cdc.gov/nchs/nhanes/irba98.htm). A more detailed description of NHANES is available at the following address: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm.

In this study, we used the 2011–12 cycle of the NHANES data, since this is the only cycle measuring four types of endocrine-disrupting chemicals, namely phenols, parabens, phthalates, and poly-fluoroalkyl chemicals. To ensure consistency with the use of the BioAge R package, this study was limited to adults aged 20–90 years. After excluding participants without data on endocrine-disrupting chemicals or biological markers for calculating their biological age, 1,439 participants were finally included in this study (Fig. 1A).

We used the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/), Swiss Target Prediction (https://swisstargetprediction.ch/), and TargetNet (https://targetnet.scbdd.com/home/index/) to collect prediction targets for EDCs. These targets were obtained by intersecting targets from all three databases. Transcription or translation of these targets was regulated by EDCs, and was predicted to interact with EDCs. Aging-related differentially expressed genes were acquired from the AgeAnno (https://relab.xidian.edu.cn/AgeAnno/#/), a knowledge base of single-cell annotation of aging in humans, aiming to provide comprehensive characterizations for aging-related genes across diverse tissue-cell types by using single-cell RNA and ATAC sequencing data. Also, we limited the tissue to heart tissue in order to obtain the heart aging-related genes. Then, the BA-mixture target genes and heart aging-related genes were merged for further analysis (Fig. 1B).

2.2. Definition of ASCVD, CKD, diabetes, and hypertension

ASCVD was diagnosed based on the questionnaire data obtained from NHANES. According to the 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults, ASCVD was defined as having at least one diagnosis of coronary heart disease, angina, heart attack, and stroke. (Stone et al., 2013) Individuals told by a physician or other health professional they have the above disease was diagnosed with this disease, according to the answer to the question MCQ160c, MCQ160d, MCQ160e, MCQ160f in NHANES. Diabetes was diagnosed by self-report, or glycosylated hemoglobin (HbA1c) > 6.5 %, fasting glucose $>= 7.0$ mmol/L, or random blood glucose $>=11.1$ mmol/L, or two-hour oral glucose tolerance test blood glucose $>=11.1$ mmol/L, or using diabetes medication or insulin. The estimated glomerular filtration rate (eGFR) scores were calculated using the Chronic Kidney Disease Epidemiology Collaboration algorithm, (Levey et al., 2009) and urine albumin-to-creatinine (ACR) ≥ 30 mg/g and/or eGFR < 60 mL/min/ 1.73 m (Ferrucci et al., 2018) were used to diagnose CKD. (KDIGO, 2021) Hypertension was diagnosed by self-report, or use of the anti-hypertension medication, or systolic blood pressure > 140 mmHg, or diastolic blood pressure > 90 mmHg.

2.3. Measurements of endocrine-disrupting chemicals

Urinary concentrations of environmental phenols, parabens, phthalates, and serum concentration of poly-fluoroalkyl chemicals were measured in a randomly selected one-third subset of NHANES participants. In our study, chemicals were included based on the metabolite's detectable frequencies ≥ 50 %. The finally analyzed chemicals were three phenols (bisphenol A (ng/mL), benzophenone-3 (ng/mL), triclosan (ng/mL)), three parabens (ethyl paraben (ng/mL), methyl paraben (ng/mL), propyl paraben (ng/mL)), 13 phthalates (mono-n-butyl phthalate (ng/mL), mono-ethyl phthalate (ng/mL), mono-(2-ethyl)-hexyl phthalate (ng/mL), mono-isonoyl phthalate (ng/mL), mono-benzyl phthalate (ng/mL), mono-n-methyl phthalate (ng/mL), mono-(3-carboxypropyl) phthalate (ng/mL), mono(2-ethyl-5-hydroxyhexyl) phthalate (ng/mL), mono-(2-ethyl-5-oxohexyl) phthalate (ng/mL), mono-isobutyl phthalate (ng/mL), mono-2-ethyl-5-carboxypentyl phthalate (ng/mL), mono(carboxynonyl) phthalate (ng/mL), mono (carboxyoctyl) phthalate) (ng/mL), and seven polyfluoroakyl chemicals (perfluorooctanoic acid (ng/mL), perfluorooctane sulfonic acid (ng/mL), perfluorohexane sulfonic acid (ng/mL), 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid (ng/mL), perfluorodecanoic acid (ng/mL), perfluorononanoic acid (ng/mL), perfluoroundecanoic acid (ng/mL)). The urinary concentrations of BPA, BP-3, triclosan, and of the four parabens were measured by online solid phase extraction (SPE) coupled to HPLC and tandem mass spectrometry (HPLC-MS/MS). Phthalates concentration was measured by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). Online solid phase extraction coupled to high-performance liquid chromatography-turbo ionspray ionization-tandem mass spectrometry (online SPE-HPLC-TIS-MS/MS) was used to quantitatively measure serum polyfluoroakyl chemicals. The concentrations below the lower limit of detection (LLOD) were assigned a value equal to the LLOD/$\sqrt{2}$, as recommended by NHANES. The concentrations of chemicals measured in urine samples were all corrected by urinary creatinine for further analysis, and the chemical concentration was Ln-transformed for further analysis.

2.4. Measurement of dietary vitamins

Dietary data regarding vitamins intake were obtained via a precise list of all foods consumed by an individual within 24 h prior to examination. The 24-h recall method is most often used to determine dietary intake in large-scale surveys. The previous 24 h dietary information this study used was from a 24 h dietary recall interview collected in person in

the MEC. NHANES participants were asked whether they used any dietary supplements in the past 30 days during an in-house interview, and those who responded positively were asked about the product name, frequency, duration, and serving form. For each vitamin, the daily dose was calculated by combining intake frequency with product information, such as its ingredients, amount of ingredients per serving, and ingredient unit. Vitamin intake from each product was summed to estimate the total daily dose of each supplemental nutrient for each individual. (Chen et al., 2019).

2.5. Covariates

Covariates were selected based on previous research studies, (Chaney and Wiley, 2023; Zhang et al., 2019; Kresovich et al., 2021) and the variance inflation factor (VIF) of each covariate was calculated; subsequently, covariates with VIF < 5 were selected. The selected covariates were age, gender, race (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, other race), and education (less than 9th grade, 9–11th grade, High school graduate/GED or equivalent, college or AA degree, college graduate or above). In addition, the poverty to income ratio was obtained from the demographic file of the NHANES 2011–12 cycle. Body mass index (BMI) was calculated using height and weight data. Individuals smoking more than 100 cigarettes or those who smoked daily or at some days were identified as smokers. With respect to the alcohol user status, individuals who had < 12 drinks in lifetime were categorized as non-alcohol user, while individuals who had at least 12 drinks in one year and did not drink last year, or did not drink last year but drank at least 12 times in their lifetime were categorized as former alcohol users. Individual were defined as current heavy alcohol users when they had at least three drinks on a daily basis for females and four drinks per day for males, or were binge drinking five or more days per month. In contrast, individuals were defined as moderate alcohol users when they had at least two drinks and three drinks per day for females and males, respectively, or were binge drinking two or more days per month. Individuals drinking during the last year but who did not meet the standard described above were defined as mild current alcohol users. (Rattan et al., 2022).

2.6. Biological age measures

KDM-BA was validated to be an effective algorithm for assessing biological age by using blood-chemistry-derived measures. (Klemera and Doubal, 2006) An individual's KDM biological age prediction corresponds to the chronological age at which his/her physiology would be approximately normal.

KDM-BA is derived from a series of regressions of individual biomarkers on chronological age in a reference population. The equation takes information from an n number of regression lines of chronological age regressed on n biomarkers, according to the following formula:

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

where x is the value of the biomarker i measured for an individual. For each biomarker i, k, q, and s represent the regression intercept, slope, and root mean squared error, respectively. sBA is a scaling factor equal to the square root of the variance in chronological age explained by the biomarker set in the reference sample, while CA refers to the chronological age. In the BioAge package, the reference sample is NHANES III nonpregnant participants aged 30–75 years. Algorithm parameters were estimated separately for males and women. In our study, we used ten biomarkers based on previous studies, namely systolic blood pressure (SBP), albumin, alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, glycated hemoglobin (HbA1c), total cholesterol, lymphocyte percentage, white blood cell counts (WBC), and mean cell volume (MCV). We did not use force expiratory volume in one second (FEV1) and C-reactive protein since they were not measured in the 2011–12 cycle. However, we did perform a validation analysis to demonstrate that the selected set of biomarkers was comparable to Levine's study. (Levine, 2013)

Phenotypic age uses a multivariate analysis of mortality hazards based on a reference population to calculate an individual's phenotypic age. (Levine, 2013) Specifically, this measure uses an algorithm based on the parametrization of two Gompertz proportional hazard models, where the first model uses the ten biomarkers mentioned above and chronological age, while the second model uses only chronological age. (Levine, 2013) We used NHANES III as the reference population, and the individual's resulting phenotypic age reflected the chronological age of an individual having the same mortality risk in the reference population, in accordance with protocols published in previous studies. (Kwon and Belsky, 2021).

The resulting final equation for calculating phenotypic age Is as followed:

$$Phenotypicage=\frac{\mathrm{Ln}[-0.00553*\mathrm{Ln}(\frac{-1.51714*\exp(xb)}{0.0076927})]}{0.09165}$$

where xb = − 19.907—-0.0336*Albumin + 0.0095*Creatinine + 0.1953 × Glucose-0.0120 × Lymphocyte Percent + 0.0268 × Mean Cell Volume + 0.3306 × Red Cell Distribution Width + 0.00188 × Alkaline Phosphatase + 0.0554 × White Blood Cell Count + 0.0804 × Chronological Age. Biological aging was defined as the value of biological age that is greater than chronological age, and biological age acceleration was calculated as the difference between biological age estimation and chronological age. (Kwon and Belsky, 2021)

Homeostatic dysregulation (HD) was computed as the Mahalanobis distance based on a set of biomarkers related to a "healthy" reference, which does not include information on chronological age, and the individual's HD value quantifies how different their physiology is from this "healthy" reference sample. Here, the "healthy" reference sample was defined as NHANES III nonpregnant participants aged 20–30 years for whom all selected biomarkers fall within the clinically normal range. Since HD in our sample had skewed distribution, it was log-transformed for further analysis. (Kwon and Belsky, 2021).

The construction of KDM biological age, phenotypic age, and HD was performed using the R package BioAge (https://github.com/dayoonkwon/BioAge).

2.7. Selection of biological age acceleration-related EDCs (BA-EDCs)

The present study employed WQS regression to estimate the empirical weights for a weighted sum of concentrations that were most associated with health outcomes. (Carrico et al., 2015) This approach took all the measured chemicals into consideration, and chemicals included in this model were constrained to have the same effect direction for KDM biological age acceleration. By grouping different BA-EDCs into deciles, the WQS regression model calculated a weighted linear index that represented the overall body burden of all chemicals. The weight assigned to each chemical reflected its contribution to the WQS index. And weight of each particular chemicals were used to identify the predominant EDC exposure associated with biological age acceleration. (Carrico et al., 2015) EDCs with non-negligible weight, i.e., weights exceeding a selection threshold parameter of 0.038, were selected as biological age acceleration-related EDCs for further analysis. (Caporale et al., 2022).

2.8. Construction of the Protein-Protein interaction (PPI) network and MCODE module network

The combined genes obtained above were uploaded to the STRING 11.5 database (https://cn.string-db.org/), to identify their interactions

and construct the PPI network. (Szklarczyk et al., 2019) PPI is a comprehensive representation of protein interactions obtained by integrating multiple sources of experimental data, literature annotations, and computational predictions. (von Mering et al., 2003) These interactions encompass both direct physical interactions, such as the formation of protein complexes, and indirect functional associations, where proteins participate in the same biological processes. (von Mering et al., 2003) The list of genes is mapped to their corresponding protein products and added to the PPI network, and they are subsequently connected to the existing network based on their interactions with other proteins. This integration allows the identification of potential interacting partners for all genes of interest. The organism was limited to "Homo sapiens," and an interaction score with medium confidence (0.400) was set to analyze the protein–protein interaction.

Proteins with similar functions typically aggregate together to represent functional molecular biological units. Therefore, we can predict the exact protein function by using an algorithm to analyze the network containing proteins with known and unknown functions. (Altaf-Ul-Amin et al., 2006) Thus, we used MCODE, a plug-in of Cytoscape 3.9.1 to identify the density region of interaction in the PPI network, which was not affected by high false positives due to high flux technology.

2.9. Functional enrichment analysis

Functional enrichment analysis, including Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, is a computational approach used to gain insights into the biological functions, pathways, and processes associated with a set of genes or proteins. (Chen et al., 2017) Specifically, GO enrichment analysis categorizes genes or proteins into functional groups based on their molecular function, biological process, and cellular component. It provides a standardized vocabulary to describe gene functions, and it allows researchers to comprehend the functional landscape of a given set of genes or proteins. (Gene Ontology Consortium: going forward, 2015) KEGG enrichment analysis focuses on the biological pathways and networks in which genes or proteins are involved, and it provides information regarding potential interactions and relationships between genes and their involvement in specific biological processes, diseases, or signaling pathways. (Kanehisa et al., 2016) Functional enrichment analysis (GO and KEGG enrichment analysis) complements PPI analysis by providing a deeper understanding of the biological functions, pathways, and processes associated with the genes or proteins of interest. Consequently, this approach expands the scope of our analysis beyond physical interactions, and also helps researchers gain novel insights into the functional significance and implications of their findings. The R package ClusterProfiler was used to perform GO enrichment analysis and KEGG analysis in the R software.

2.10. Statistical analysis

In the descriptive analysis, chi-square tests were performed to compare categorical variables. Continuous variables were expressed as mean and standard deviation (SD) when normally distributed or median and interquartile range (IQR) when skewed. The One-Way ANOVA and Kruskal-Wallis test were used for comparison, as appropriate.

Three statistical models, including generalized linear regression model, WQS regression, and Bayesian kernel machine regression (BKMR) were performed to evaluate the individual or mixed effect of BA-EDCs on biological age acceleration and aging-related diseases.

2.11. Generalized linear regression model

First, we used multivariable generalized linear regression model to examine the association between individual BA-EDCs and biological age acceleration or aging-related disease, including diabetes, hypertension, ASCVD, and CKD. The models were adjusted for age, gender, race, education, poverty ratio income, BMI, smoke status, and alcohol use status.

2.11.1. WQS regression

To evaluate the effects of mixed exposure of selected EDCs and biological age acceleration, WQS regression model was applied. WQS was a weighted quartile sum approach in conjunction with either linear (continuous outcomes) or logistic (binary outcomes) regression. (Carrico et al., 2015) By grouping different BA-EDCs into deciles, the WQS regression model calculated a weighted linear index that represented the overall body burden of all BA-EDCs. The weight assigned to each chemical reflected its contribution to the WQS index.

2.11.2. The Bayesian kernel machine regression (BKMR) model

The Bayesian kernel machine regression (BKMR) was performed to evaluate the joint effect of BA-EDCs exposure on biological age acceleration. (Bobb et al., 2015) The cumulative effects of the mixtures were assessed by estimating the expected change in biological age acceleration associated with concurrent changes in all of the components of the mixture from their median levels. We fitted separate BKMR models for all metrics of biological age acceleration outcomes based on the model below:

$$Y_i = h(PFNA_i, MCPP_i, BPA_i, MEP_i, MMP_i, MeFOSSA_i) + \beta z_i + e_i.$$

where h() was the exposure–response function based on nonlinearity and/or interaction among the mixture components, Zi, and β represented covariates and their coefficients, respectively.

2.11.3. Sensitivity analysis

Since age, race, BMI, and education level are linked to human chemical exposure and adverse health outcomes, propensity score matching (PSM) was applied in our sensitivity analysis to balance differences in these factors between KDM biological aging groups and non-biological aging groups. (Kane et al., 2020) By calculating the propensity score of samples, the method of nearest neighbor matching was used for 1:1 matching using a caliper width of 0.2. A standardized mean difference (SMD) was used to examine the PSM degree, and the association of BA-mixture and KDM biological age acceleration was evaluated using the WQS regression model.

The likelihood ratio test was used to assess the interaction effect of dietary and supplementary vitamins. The level of statistical significance was set at $p < 0.05$. For GO and KEGG analyses, a q value < 0.05 was considered statistically significant. Statistical analysis was performed using R software, EmpowerStats software (https://www.empowerstats.com), and FreeStatistic software. (Yang et al., 2021) WQS regression was performed using the R package gWQS, BKMR was performed using the R package bkmr, and PSM was performed using the R package Matchit.

2.12. Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

3. Results

3.1. Population characteristics

Among all recruited participants, only 1,439 participants successfully passed all the filters. The characteristics of participants categorized by tertile of KDM biological age acceleration are presented in Table 1. It is shown that individuals with higher KDM biological age acceleration tended to be males, while participants with higher KDM biological age acceleration were more likely to be younger and well-educated. Participants in tertile 3 of KDM biological age acceleration had higher levels of BMI, alkaline phosphatase, total cholesterol, Hba1c, creatinine, SBP, BUN, and WBC, compared with tertile 1, as opposed to their lymphocyte percentages and poverty income ratio which were lower. Participants

Table 1
Characteristics of the study population by categories of KDM biological age acceleration tertile.

Variables	Total (n = 1439)	Tertile 1 (n = 480)	Tertile 2 (n = 479)	Tertile 3 (480)	P-value
Gender, n (%)					0.003
Male	742 (51.6)	220 (45.8)	272 (56.8)	250 (52.1)	
Female	697 (48.4)	260 (54.2)	207 (43.2)	230 (47.9)	
Age (Years), Mean ± SD	47.8 ± 17.6	56.3 ± 15.7	44.2 ± 16.0	42.8 ± 18.0	< 0.001
Race, n (%)					< 0.001
Mexican American	134 (9.3)	33 (6.9)	53 (11.1)	48 (10)	
Other Hispanic	159 (11.0)	56 (11.7)	50 (10.4)	53 (11)	
Non-Hispanic White	546 (37.9)	194 (40.4)	193 (40.3)	159 (33.1)	
Non-Hispanic Black	346 (24.0)	98 (20.4)	94 (19.6)	154 (32.1)	
Other Race	254 (17.7)	99 (20.6)	89 (18.6)	66 (13.8)	
Education, n (%)					0.016
Less than 9th grade	140 (9.7)	50 (10.4)	44 (9.2)	46 (9.6)	
9-11th grade	183 (12.7)	63 (13.1)	60 (12.5)	60 (12.5)	
High school graduate/GED or equivalent	285 (19.8)	89 (18.5)	90 (18.8)	106 (22.1)	
Some college or AA degree	434 (30.2)	125 (26)	144 (30.1)	165 (34.4)	
College graduate or above	396 (27.5)	153 (31.9)	141 (29.4)	102 (21.3)	
Poverty income ratio, Mean ± SD	2.5 ± 1.7	2.7 ± 1.6	2.5 ± 1.7	2.4 ± 1.7	0.012
BMI, Mean ± SD	28.8 ± 6.9	27.2 ± 5.6	28.7 ± 6.8	30.5 ± 7.8	< 0.001
Alcohol use status, n (%)					< 0.001
Never	194 (14.8)	82 (18.9)	51 (11.8)	61 (13.7)	
Former	219 (16.7)	78 (18)	75 (17.4)	66 (14.9)	
Current mild user	441 (33.7)	157 (36.3)	150 (34.7)	134 (30.2)	
Current moderate user	194 (14.8)	59 (13.6)	65 (15)	70 (15.8)	
Current heavy user	261 (19.9)	57 (13.2)	91 (21.1)	113 (25.5)	
Smoke status, n (%)					0.915
No	836 (58.1)	279 (58.1)	275 (57.4)	282 (58.8)	
Yes	603 (41.9)	201 (41.9)	204 (42.6)	198 (41.2)	
Albumin (g/dL), Mean ± SD	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.3	4.2 ± 0.3	< 0.001
Alkaline phosphatase (u/L), Mean ± SD	65.9 ± 20.7	62.4 ± 18.4	64.6 ± 18.8	70.7 ± 23.7	< 0.001
Total cholesterol (mg/dL), Mean ± SD	193.2 ± 40.1	186.4 ± 35.8	191.2 ± 37.9	202.1 ± 44.6	< 0.001
HbA1c (%), Mean ± SD	5.7 ± 0.9	5.6 ± 0.6	5.6 ± 0.7	5.9 ± 1.2	< 0.001
Creatinine (mg/dL), Mean ± SD	0.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.3	< 0.001
SBP (mmHg), Mean ± SD	122.5 ± 17.4	117.3 ± 14.4	120.2 ± 14.5	130.1 ± 20.1	< 0.001
BUN (mg/dL), Mean ± SD	12.7 ± 4.8	11.6 ± 3.7	12.4 ± 4.3	14.1 ± 5.9	< 0.001
Lymphocyte percent (%), Mean ± SD	30.9 ± 8.7	31.8 ± 9.1	31.1 ± 8.3	29.8 ± 8.7	0.002
MCV (fl), Mean ± SD	89.5 ± 5.7	89.4 ± 6.3	89.7 ± 5.5	89.5 ± 5.2	0.742
WBC (10^3/uL), Mean ± SD	6.9 ± 2.0	6.3 ± 1.6	7.0 ± 2.0	7.3 ± 2.1	< 0.001
Diabetes mellitus, n (%)					0.02
No	1159 (81.4)	390 (81.8)	400 (84.7)	369 (77.7)	
Yes	265 (18.6)	87 (18.2)	72 (15.3)	106 (22.3)	
Hypertension, n (%)					< 0.001
No	860 (59.8)	298 (62.1)	311 (64.9)	251 (52.3)	
Yes	579 (40.2)	182 (37.9)	168 (35.1)	229 (47.7)	
CKD, n (%)					< 0.001
No	1210 (84.1)	422 (87.9)	430 (89.8)	358 (74.7)	
Yes	228 (15.9)	58 (12.1)	49 (10.2)	121 (25.3)	

Abbreviation: ASCVD: Atherosclerotic cardiovascular disease; Hba1c:Glycosylated hemoglobin; SBP: Systolic blood pressure; BUN: Blood urea nitrogen; MCV: Mean cell volume; WBC: White blood cell; CKD: Chronic kidney disease.

suffering from diabetes, hypertension, and CKD were more likely to have KDM biological age acceleration.

Biological aging participants in tertile 3 of KDM biological age acceleration had higher levels of BPA, MnBP, MEP, MHHP, MiNP, MBzP, MMP, MCPP, MEHHP, MEOHP, MiBP, MECPP, MCNP, and MCOP compared to tertile 1 (Table 2).

Table S1 demonstrates the characteristics of the study sample. Our results revealed that smokers and alcohol drinkers were more likely to have ASCVD. Besides, people with ASCVD were more likely to be older, males, and have lower family PIR and poorer education levels. People with diabetes, hypertension, CKD, or hyperlipidemia were more likely to have ASCVD. Albumin, total cholesterol, and lymphocyte percentage were lower in participants with ASCVD, as opposed to Hba1c, SBP, BUN, and MCV which were higher. Table S2 exhibits that participants with ASCVD had higher levels of MnBP, PFOA, PFHxS, PFOS, MeFOSAA, PFDA, PFNA, and PFUA.

3.2. *Validation analysis of KDM biological aging measures*

In this study, certain biomarkers were found to differ from the original set of biomarkers used by Levine (Levine, 2013) for calculating KDM biological age. These differences included the exclusion of C-reactive protein, forced expiratory volume, FEV1, and cytomegalovirus optical density, which were not measured in the NHANES 2011–12 cycle. Additionally, the present study introduced additional parameters such as the inclusion of lymphocyte percentage, mean cell volume, and WBC count, which were previously utilized for measuring KDM biological age. (Kwon and Belsky, 2021; Mian et al., 2022) Since the biomarkers used to calculate KDM biological age in our study were not completely consistent with those stated in the Levine's study (Levine, 2013); a validation analysis was conducted to establish that the calculated KDM biological age was comparable to the original version published by Levine *et al.*

Consequently, 36,562 participants with available blood test data in all NHANES cycles were used to perform this validation analysis. Figure S1 exhibits that our modified versions of KDM biological age measures showed similar associations with respect to functional test performance, subjective ratings of health, mortality, and socioeconomic status as the original version published by Levine. Therefore, our findings suggest that our KDM biological aging measures were reasonable and comparable to the ones presented in the Levine's version.

Table 2
Concentration of endocrine disrupting chemicals by categories of tertile of KDM biological age acceleration.

Chemicals	Total (n = 1439)	Tertile 1 (n = 480)	Tertile 2 (n = 479)	Tertile 3 (n = 480)	P
Phenols					
BPA (ng/mL), Median (IQR)	1.4 (0.7, 2.8)	1.2 (0.6, 2.4)	1.5 (0.7, 2.8)	1.6 (0.9,3.1)	< 0.001
BP3 (ng/mL), Median (IQR)	14.1 (3.8, 67.8)	12.1 (3.5, 56.8)	16.3 (4.4, 71.6)	14.1 (3.6,64.8)	0.298
TCS (ng/mL), Median (IQR)	7.4 (1.6, 45.0)	7.3 (1.6, 58.7)	7.3 (1.6, 33.5)	7.5 (1.6,51.8)	0.980
Parabens					
EP (ng/mL), Median (IQR)	0.7 (0.7, 6.4)	0.7 (0.7, 7.0)	0.7 (0.7, 5.8)	0.7 (0.7,6.1)	0.592
MP (ng/mL), Median (IQR)	58.1 (11.9, 216.5)	62.5 (13.4, 229.5)	42.2 (9.9, 179.0)	69.2 (13.4,24.1)	0.008
PrP (ng/mL), Median (IQR)	7.7 (1.1, 41.7)	9.9 (1.4, 48.2)	4.5 (0.8, 32.8)	9.1 (1.3,43.5)	0.006
Phalates					
MnBP (ng/mL), Median (IQR)	9.7 (3.9, 20.5)	8.5 (2.9, 19.4)	9.2 (3.9, 19.7)	11.1 (4.8,23.4)	0.005
MEP (ng/mL), Median (IQR)	42.9 (15.2, 132.8)	38.7 (14.7, 109.6)	42.5 (13.9, 134.3)	51.2 (17.9,162.2)	0.042
MHHP (ng/mL), Median (IQR)	1.4 (0.4, 3.0)	1.2 (0.3, 2.4)	1.4 (0.6, 3.0)	1.7 (0.5,3.8)	<0.001
MiNP (ng/mL), Median (IQR)	0.7 (0.4, 2.5)	0.3 (0.3, 1.5)	0.7 (0.3, 2.3)	1.0 (0.3,3.4)	< 0.001
MBzP (ng/mL), Median (IQR)	4.1 (1.8, 9.5)	3.4 (1.4, 7.5)	4.4 (1.9, 9.6)	4.7 (2.2,10.8)	< 0.001
MMP (ng/mL), Median (IQR)	1.0 (0.4, 2.9)	0.8 (0.3, 2.6)	1.1 (0.3, 2.7)	1.2 (0.3,3.6)	0.024
MCPP (ng/mL), Median (IQR)	2.5 (1.2, 6.0)	2.1 (1.0, 4.7)	2.5 (1.2, 6.1)	3.0 (1.6,7.1)	< 0.001
MEHHP (ng/mL), Median (IQR)	8.1 (4.1, 15.7)	7.2 (3.6, 13.6)	7.3 (4.1, 15.6)	10.0 (5.2,17.6)	< 0.001
MEOHP (ng/mL), Median (IQR)	5.2 (2.8, 10.1)	4.7 (2.4, 8.8)	4.8 (2.8, 10.1)	6.2 (3.3,11.3)	< 0.001
MiBP (ng/mL), Median (IQR)	6.8 (3.2, 13.4)	6.1 (2.6, 12.5)	6.6 (3.3, 13.3)	7.5 (4.0,14.1)	0.003
MECPP (ng/mL), Median (IQR)	13.1 (6.8, 24.4)	12.4 (6.5, 22.4)	12.8 (6.8, 24.2)	14.1 (7.5,27.4)	0.032
MCNP (ng/mL), Median (IQR)	2.1 (1.1, 4.5)	2.0 (1.0, 4.2)	2.1 (1.1, 4.2)	2.5 (1.3,5.1)	0.006
MCOP (ng/mL), Median (IQR)	16.8 (6.8, 46.7)	13.6 (5.8, 39.2)	16.0 (7.0, 45.8)	21.9 (9.0,57.6)	< 0.001
Per- and polyfluoroalkyl substances					
PFOA (ng/mL), Median (IQR)	2.2 (1.5, 3.1)	2.2 (1.6, 3.3)	2.1 (1.4, 2.9)	2.2 (1.5,3.0)	0.007
PFOS (ng/mL), Median (IQR)	7.2 (4.3, 11.4)	8.1 (4.7, 12.9)	7.2 (4.1, 11.1)	6.7 (4.1,10.7)	0.006
PFHxS (ng/mL), Median (IQR)	1.3 (0.7, 2.2)	1.4 (0.7, 2.3)	1.3 (0.7, 2.1)	1.2 (0.7,2.0)	0.248
MeFOSAA (ng/mL), Median (IQR)	0.1 (0.1, 0.2)	0.1 (0.1, 0.3)	0.1 (0.1, 0.2)	0.1 (0.1,0.2)	0.300
PFDA (ng/mL), Median (IQR)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)	0.2 (0.1, 0.3)	0.2 (0.1,0.3)	0.006
PFNA (ng/mL), Median (IQR)	0.9 (0.6, 1.4)	0.9 (0.6, 1.5)	0.9 (0.6, 1.4)	0.9 (0.6,1.4)	0.187
PFUA (ng/mL), Median (IQR)	0.1 (0.1, 0.3)	0.2 (0.1, 0.3)	0.1 (0.1, 0.2)	0.1 (0.1,0.2)	<0.001

Abbreviation: BPA:Bisphenol A; BP3:Benzophenone-3; TCS:Triclosan; EP:Ethyl paraben; MP:Methyl paraben; PrP:Propyl paraben; MnBP:Mono-n-butyl phthalate; MEP:Mono-ethyl phthalate; MHHP:Mono-(2-ethyl)-hexyl phthalate; MiNP:Mono-isononyl phthalate; MBzP:Mono-benzyl phthalate; MMP:Mono-n-methyl phthalate; MCPP:Mono-(3-carboxypropyl) phthalate; MEHHP:Mono(2ethyl5-hydroxyhexyl) phthalate; MEOHP:Mono-(2-ethyl-5-oxohexyl) phthalate; MiBP:Mono-isobutyl phthalate; MECPP: Mono-2-ethyl-5-carboxypentyl phthalate; MCNP:Mono(carboxynonyl) Phthalate; MCOP:Mono(carboxyoctyl) Phthalate; PFOA:Perfluorooctanoic acid; PFOS:Perfluorooctane sulfonic acid; PFHxS:Perfluorohexane sulfonic acid; MeFOSAA:2-(N-Methyl-perfluorooctane sulfonamido) acetic acid; PFDA:Perfluorodecanoic acid; PFNA:Perfluorononanoic acid; PFUA:Perfluoroundecanoic acid.

3.3. Selection of BA-EDCs

WQS regression was used to identify EDCs with a non-negligible contribution to KDM biological age acceleration. Fig. 2 shows the mean weight of each EDCs contributing to KDM biological age acceleration. EDCs with a mean weight over the threshold parameter (0.038) were selected as BA-EDCs for further analysis. The detailed weight of each EDCs is summarized in Table S3. The highest weighted BA-EDCs was PFNA, followed by MCPP, BPA, MMP, MEP, and MeFOSAA.

3.4. Individual and mixed effects of BA-EDCs on biological age acceleration

As shown in Fig. 3A, our multivariable linear regression analysis revealed that only MCPP was significantly associated with KDM biological age acceleration. Specifically, each unit increased in MCPP was associated with 0.287 years of KDM biological age acceleration (β: 0.287 95 %CI: 0.045 ~ 0.529, $p = 0.020$). To assess the mixed effect of BA-EDCs on KDM biological age acceleration, WQS regression was performed. After adjusting for confounders, the WQS indices were significantly associated with KDM biological age acceleration (Fig. 3B). A decile increase in the WQS index, was found to correspond to a KDM biological age acceleration of 0.31 years (β: 0.31, 95 % CI: 0.08 ~ 0.53, $p = 0.008$), which is attributable to the contribution of PFNA (35.9 %), MCPP (29.1 %), BPA (20.9 %), MMP (8.1 %), MEP (5.7 %), and MeFOSAA (0.3 %), as shown in Fig. 3B.

Furthermore, Fig. 3D. demonstrates that BPA was positively associated with phenotypic age acceleration (β: 0.310, 95 %CI: 0.012 ~ 0.608, $p = 0.042$), while PFNA was negatively associated (β: −0.452, 95 % CI: −0.850 ~ -0.053, $p = 0.026$). In WQS regression model, co-exposure of BA-EDCs associated with phenotypic age acceleration (β:0.17, 95 CI%: 0.01 ~ 0.34, $p = 0.038$) (Fig. 3E). Either individual and mixed effect of BA-EDCs on HD was not significant (Fig. 3G, Fig. 3H, and Fig. 3I).

In BKMR analysis, the latent continuous outcome of KDM biological age acceleration showed significant increase when all the chemicals were at their 55th percentile or above, compared to their 50th percentile, indicating a positive, significant association between the overall effect of BA-EDCs and KDM biological age acceleration (Fig. 3C). Although no statistically significant association was found in the phenotypic age acceleration model, there was an increasing trend

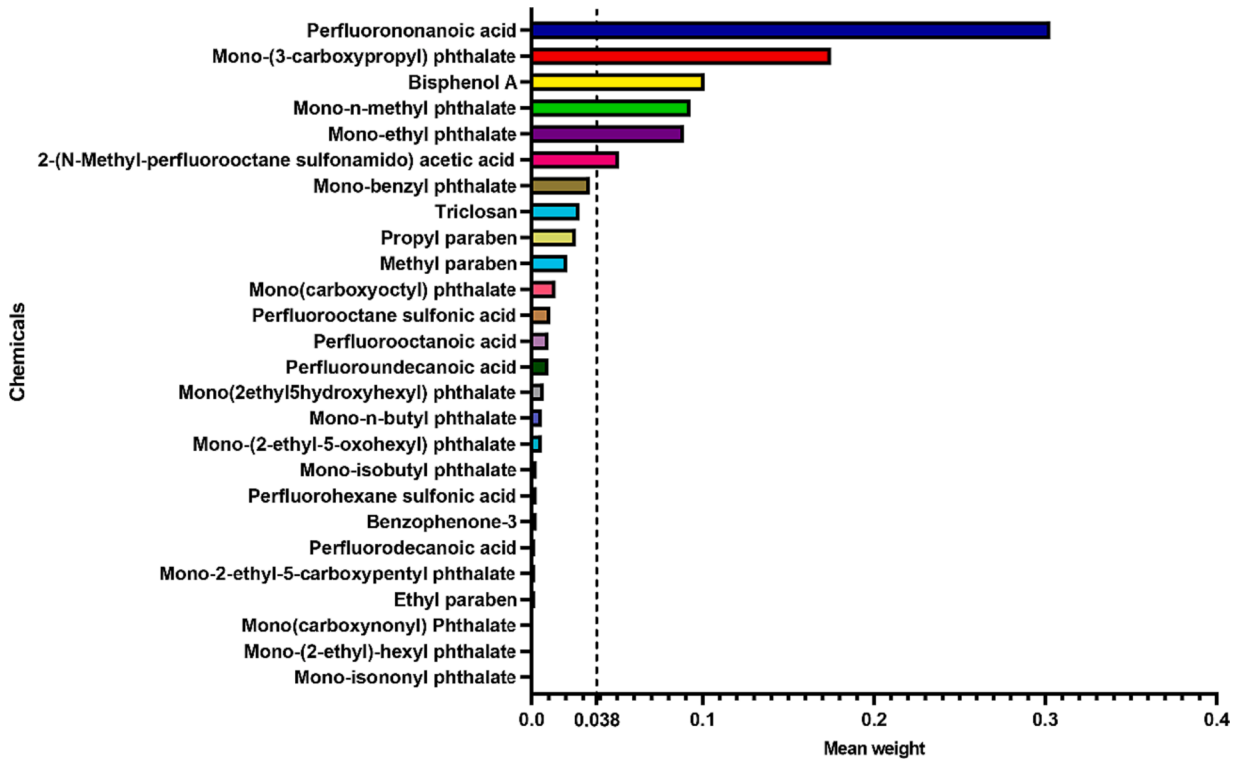

Fig. 2. Mean weight of endocrine-disrupting chemicals for biological age acceleration.

(Fig. 3F). However, in the HD model, the increasing trend was not found (Fig. 3I).

3.5. The interaction effect of dietary vitamins C and E on the association of BA-mixture and biological aging

To identify the potential actionable target for KDM biological aging prevention, we established an interaction effect test for dietary and supplementary vitamin intake. Among individuals who had a dietary vitamin C intake of more than 100 mg/day, the co-exposure effect of BA-EDCs on KDM biological age acceleration was insignificant. However, among individuals whose dietary vitamin C intake levels were less than 100 mg/d, a decile increase in the WQS index of BA-EDCs was significantly associated with 0.476 years of KDM biological age acceleration (β: 0.476, 95 % CI: 0.151 ~ 0.800, $p = 0.004$). The interaction effect of vitamin C was significant in the fully adjusted model, indicating that vitamin C intake may reduce the risk of KDM biological age acceleration (Fig. 4). Similarly, the interaction effect of vitamin E was also observed. Among individuals with low dietary vitamin E intake, the mixed effect of BA-EDCs on KDM biological age acceleration were significant. A decile increase in the WQS index was associated with a KDM biological age acceleration of 0.632 years (β: 0.632, 95 %CI: 0.215 ~ 1.048, $p = 0.003$). For individuals with high dietary vitamin E intake, this relationship was insignificant. Furthermore, the interaction effect of vitamin E was significant in the adjusted model (Fig. 4). The interaction effect of the dietary intake of other vitamins is shown in Fig. S2; however, we did not find any other actionable preventive targets. We also tried to explore the interaction effect of supplementary vitamin C intake (Data on Supplementary vitamin E intake were not collected in the NHANES 2011–12 cycle) on the relationship of BA-EDCs co-exposure and KDM biological age acceleration; although the positive association between BA-EDCs co-exposure and KDM biological age acceleration was significant among participant with low supplementary vitamins C and B1 intake, however, no significant interaction effect was observed.

3.6. Individual and mixed effect on clinical aging-related diseases

To explore the impact of BA-EDCs on clinical aging-related diseases. we assessed the both individual and mixed effects on ASCVD, diabetes, CKD, and hypertension. In the generalized linear regression analysis, individual exposure of PFNA and MeFOSSA was significantly positively associated with ASCVD. In the fully adjusted model, each unit increase in PFNA and MeFOSSA was associated with 73 % (OR: 1.73, 95 % CI: 1.16 ~ 2.58, $p = 0.007$) and 28 % (OR:1.28, 95 % CI:1.02 ~ 1.62, $p = 0.036$) prevalence of ASCVD, respectively (Fig. 5A). Besides, no individual effect or mixed effect of BA-EDCs was significantly related to diabetes, CKD, and hypertension (Fig. S3).

WQS regression was performed to evaluate the mixed effect of BA-EDCs on the aging-related diseases. Co-exposure of BA-EDCs was positively associated with ASCVD (Fig. 5B). For each decile increase in BA-EDCs co-exposure, the prevalence of ASCVD was 25 % higher (OR: 1.25, 95 % CI: 1.01 ~ 1.54), primarily due to PFNA (40.9 %), MeFOSSA (24.4 %), MCPP (23.1 %), and MMP (9.7 %) (Fig. 5B).

3.7. Sensitivity analysis

After PSM, 750 participants were included in a subsequent sensitivity analysis. Compared to unmatched data, the matched covariates between biological aging and non-biological aging groups demonstrated smaller values of SMD (Fig. S4A). Moreover, the propensity score between these two groups tended to be consistent (Fig. S4B). The association of the mixed effect of BA-EDCs and KDM biological age acceleration remained stable after PSM. A decile increase in the WQS index was significantly associated with 0.48 years of KDM biological age acceleration (β: 0.48, 95 % CI: 0.10 ~ 0.86, $p = 0.015$) (Fig. S4C).

3.8. Information on assayed target

To identify the underlying mechanism responsible for the adverse cardiovascular effect of BA-EDCs, we built an interaction network of BA-EDCs targets and aging-related genes expressed in the cardiac tissue. As

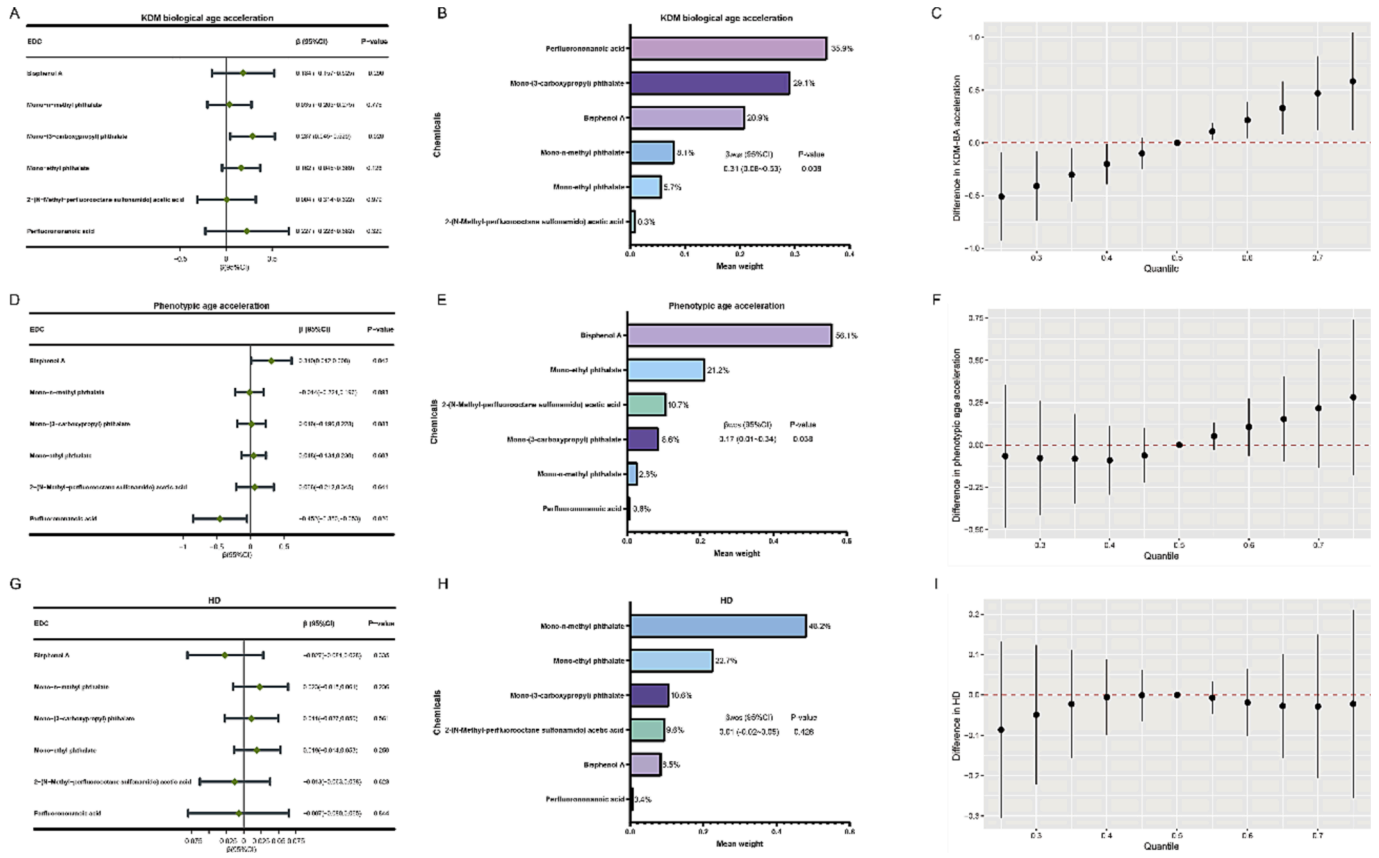

Fig. 3. The association of BA-EDCs with biological age acceleration. A) Individual effects of each BA-EDCs on KDM biological age acceleration. B) Mixed effect of BA-EDCs on biological age acceleration in WQS model. C) Mixed effect of BA-EDCs on KDM biological age acceleration in BKMR model. D) Individual effects of each BA-EDCs on phenotypic age acceleration. E) Mixed effect of BA-EDCs on phenotypic age acceleration in WQS model. F) Mixed effect of BA-EDCs on phenotypic age acceleration in BKMR model. G) Individual effects of each BA-EDCs on HD. H) Mixed effect of BA-EDCs on HD in WQS model. I) Mixed effect of BA-EDCs on HD in BKMR model. All models were adjusted for age, gender, race, education, poverty income ratio, BMI, smoke status, and alcohol use status.

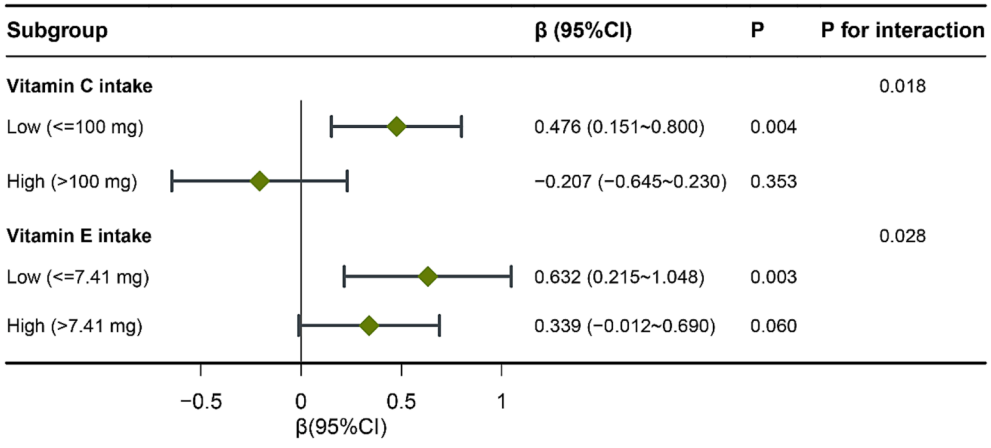

Fig. 4. Subgroup analysis of the association of BA-EDCs co-exposure and KDM biological age acceleration stratified by dietary vitamin C and E intake.

a result, 449 genes were collected from the Aging Anno database. Furthermore, 25,833 BA-EDCs target genes were obtained from the CTD database, 273 genes from SwissTargetPrediction, and 485 genes from TargetNet. Consequently, 140 intersect BA-EDCs target genes from these three databases were considered potential target genes of BA-EDCs. Finally, 583 BA-EDCs targets and heart aging-related genes were collected by merging heart aging-related genes and BA-EDCs target genes, as shown in Fig. 6A and 6B.

3.9. *PPI network and clustering analysis*

We used the STRING database to generate data on the PPI network for 583 combined genes of BA-EDCs targets and heart aging-related genes. As shown in Fig. 6C, the PPI network had 582 nodes, which were interconnected and associated with 6,748 edges. Each node represented a protein, each edge represented the correlation confidence between two targets, and the edge thickness indicated the strength of data support. The PPI enrichment *p*-value was smaller than 1.0e-16,

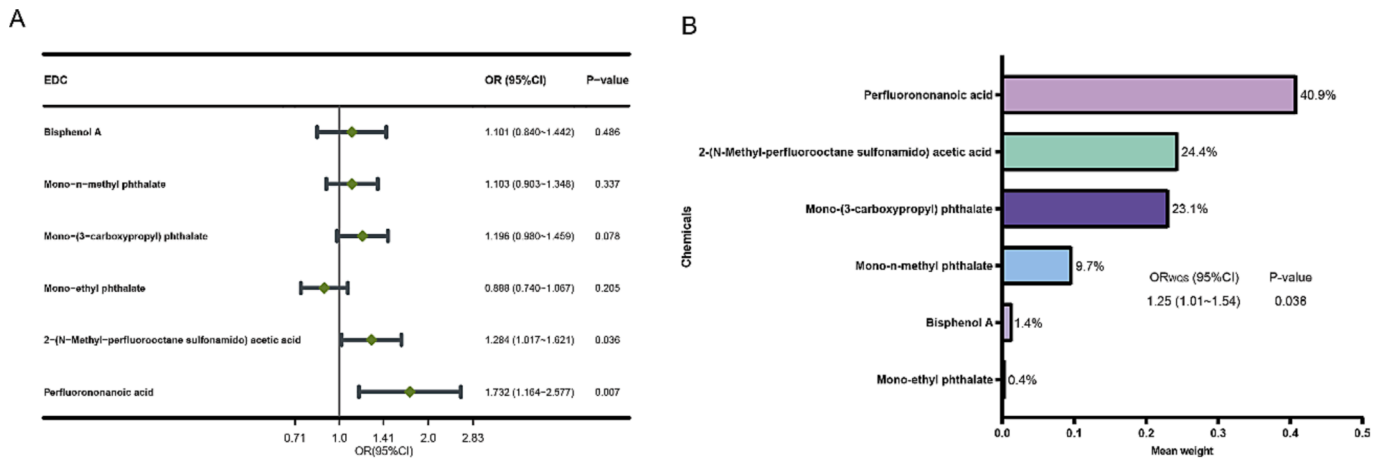

Fig. 5. Individual and mixed effects of BA-EDCs on ASCVD. A) Individual effect of each BA-EDCs on ASCVD assessed by multivariable logistic regressions. B) Mixed effect of BA-EDCs on biological age acceleration assessed by WQS regression. All models were adjusted for age, gender, race, education, poverty income ratio, BMI, smoke status, and alcohol use status.

suggesting that proteins in the PPI network significantly interacted with each other. We further used the MCODE algorithm to produce cluster networks, and a clustering analysis was then performed. Fig. 6D shows the modules with the highest average scores, which may play more important roles in PPI networks. The highest score module (32.91) contained 45 nodes and 1448 edges, indicating that this module was more important in the PPI network.

*3.10. **Functional** enrichment **analyses of intersecting genes of BA-mixture target genes and aging-related genes in heart tissue***

As highlighted in Fig. S5A, the biological processes of the BA-EDCs targets and heart aging-related genes were mainly involved in response to peptides, steroid hormones, muscle system processes, regulation of inflammatory responses, and regulation of lipid metabolic processes. The cellular component combined various genes involving collagen-containing extracellular matrix, vesicle lumen, cytoplasmic vesicle lumen, secretory granule lumen, and contractile fiber. Furthermore, the molecular function of the intersecting genes comprised extracellular matrix structural constituents, carbonate dehydratase activity, amide binding, catecholamine binding, and protease binding.

In the KEGG enrichment analysis, the combined genes were closely associated with pathways, including the AGE-RAGE signaling pathway in diabetic complications, IL-17 signaling pathway, and TNF signaling pathway, but also lipids, atherosclerosis, nitrogen metabolism, focal adhesion, prostate cancer, fluid shear stress and atherosclerosis, viral myocarditis, longevity regulating pathway-multiple species, osteoclast differentiation, toxoplasmosis, diabetic cardiomyopathy, and legionellosis. The results are shown in Fig. S5B.

The enrichment analysis of Module 1 is presented in Fig. 6E and 6F. The GO analysis for module 1 indicated that the highest scoring module was highly correlated with neuron death, smooth muscle cell proliferation, regulation of smooth muscle cell proliferation, regulation of neuron death, and muscle cell proliferation. With respect to cellular components, it was associated with the membrane microdomain, membrane raft, caveola, plasma membrane raft, and collagen-containing extracellular matrix. The related molecular functions of module 1 consisted of ubiquitin-like protein ligase binding, ubiquitin protein ligase binding, R-SMAD binding, cytokine receptor, binding, SMAD binding. As shown in Fig. 3, or findings suggest that the top 4 most related pathways of the highest score module were lipids and atherosclerosis, prostate cancer, the AGE-RAGE signaling pathway in diabetic complications, IL-17 signaling pathway, Hepatitis B, colorectal cancer, endocrine resistance, fluid shear stress and atherosclerosis, TNF signaling pathway, EGFR tyrosine kinase inhibitor resistance, Kaposi sarcoma-associated herpesvirus infection, diabetic cardiomyopathy, small cell lung cancer, FoxO signaling pathway, and apoptosis. Therefore, it can be inferred that the enriched AGE-RAGE signaling pathway could be a possible underlying mechanism for the association of BA-EDCs and ASCVD.

4. Discussion

Our findings clearly suggested that co-exposure of BA-EDCs was positively correlated with biological aging, and BA-EDCs were significantly associated with the increasing prevalence of ASCVD. Network and functional enrichment analyses indicated the significant interaction between BA-EDCs targets and heart aging-related genes, thereby suggesting that BA-EDCs may also be associated with heart aging-related disease via a potential biological mechanism. The most important module in this network was significantly associated with the AGE-RAGE signaling pathway. The interaction effect test revealed that dietary vitamin C may be an actionable target for preventing EDC-induced biological aging.

In accordance with the definition of EDC, exposure to EDC was found to impair glucose homeostasis and insulin resistance, which are considered to be distinct characteristics of aging. (Gore et al., 2015) However, limited studies have laid emphasis on exploring the association of EDC exposure with biological age acceleration. Some research studies investigated the exposure of either a single EDC or a mixture of one category of EDCs. (Khodasevich et al., 2023; Chaney and Wiley, 2023; Curtis et al., 2019) The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study found that prenatal exposure to phthalate accelerated epigenetic age throughout childhood. (Khodasevich et al., 2023) Furthermore, Sarah *et al.* found that polybrominated biphenyl was associated with epigenetic age and phenotypic age acceleration. (Curtis et al., 2019) In contrast, NHANES 1999–2018 revealed that exposure to PFAS was negatively associated with biological age deceleration in several biological age measures, including phenotypic age, and KDM biological age. (Chaney and Wiley, 2023) In our study, we also found that individuals exposed to PFNA were negatively associated with phenotypic age acceleration. Since this study focused on a single category of EDC exposure, the effect of multiple categories exposure of EDCs may be ignored. (Chaney and Wiley, 2023; Wen et al., 2022).

Previous studies indicated that EDCs exposure may contribute to atherosclerosis heart disease. (Melzer et al., 2012; Posnack, 2014; Lind and Lind, 2011; Wen et al., 2022) In particular, it was suggested that Di (2-ethylhexyl) phthalate and its metabolites pose adverse cardiovascular effects, including the development of atherosclerosis and coronary heart

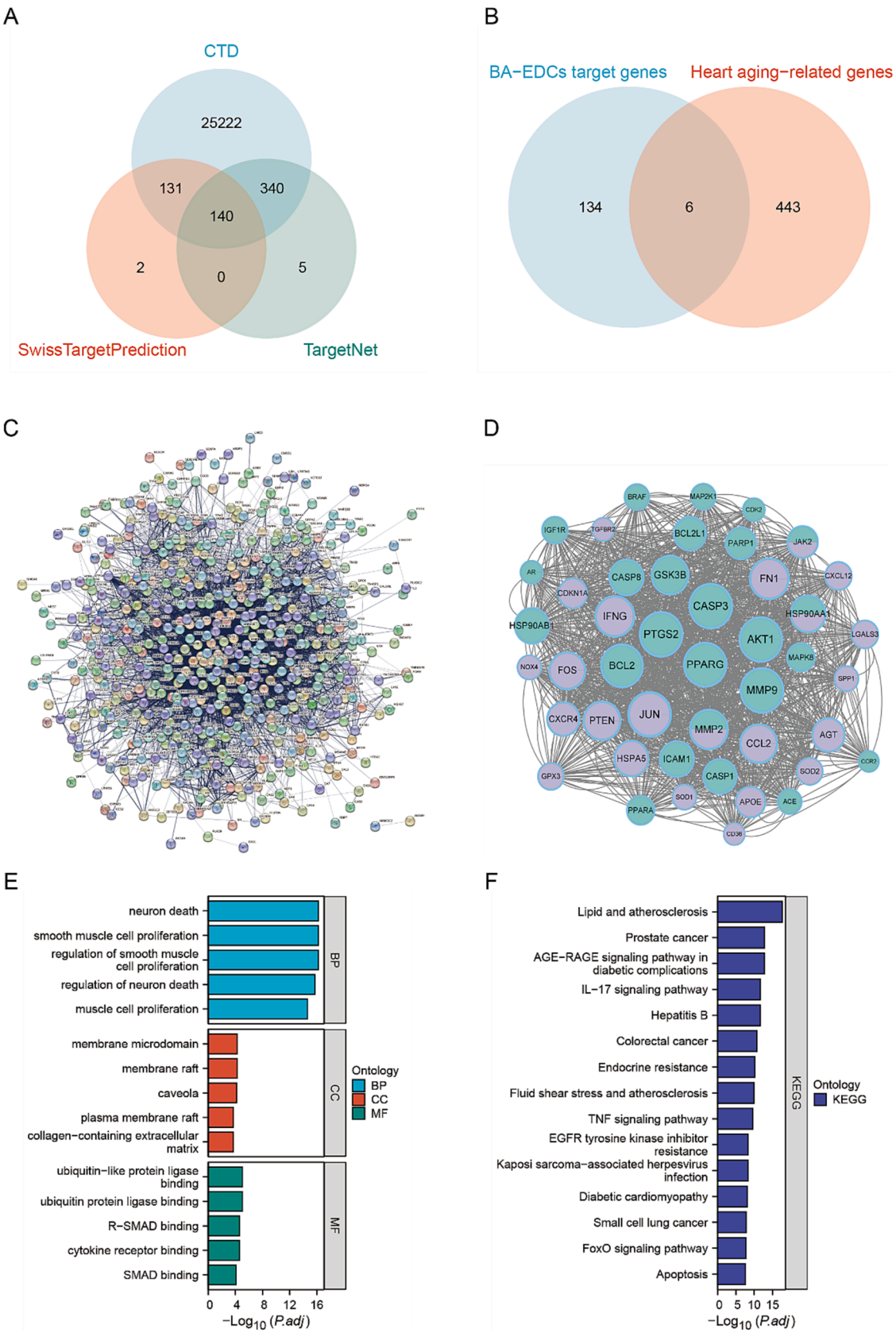

Fig. 6. A. The intersected potential targets of BA-EDCs from three databases used in the present study. B) The potential targets of BA-EDCs and heart aging-related genes from the Aging Anno database. C) The protein–protein interaction network. D) Module 1, the module with the highest average score in the PPI network. Green and purple circles represent BA-mixture targets and heart aging-related genes, respectively. E) Bar plot of GO enrichment analysis. F) Bar plot of KEGG enrichment analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

disease. (Wen et al., 2022) In a prospective cohort study, urinary bisphenol A exposure was found to increase the risk of future coronary heart disease in a healthy population. (Melzer et al., 2012) Phthalate was also found to have an inverted U shape association with the development of atherosclerotic plaques. (Lind and Lind, 2011) At the same time, several phthalates and bisphenol A were significantly associated with the echogenicity of atherosclerotic plaques. (Lind and Lind, 2011) A population-based cross-sectional study identified that co-exposure to paraben and phenol increased cardiovascular disease risk. (Yin et al., 2023) Wen *et al.* demonstrated that PFAS mixture exposure could increase heart disease mortality rates. (Wen et al., 2022) These studies clearly support our findings in that EDCs exposure is indeed related to an increased risk of developing atherosclerosis and heart disease, thereby paving the way to associating co-exposure to more types of EDCs with ASCVD. Notably, we examined the mixed effect of four types of biological aging-related EDCs, namely phthalate, paraben, phenol, and PFAS, since they may be the uncontrolled aging-related risk factors for ASCVD. Therefore, we believe that our findings may improve our understanding on how adverse cardiovascular effects are associated with environmental EDCs exposure.

In the PPI network analysis, we identified that the enrichment *P-value* of the network was smaller than 1.0e-16, suggesting that BA-EDCs targets were significantly interacting with heart aging-related genes. Since ASCVD is an aging-related disease, EDCs may contribute to ASCVD via the corresponding aging-related process. To gain a further understanding of the underlying mechanism associating BA-EDCs and ASCVD, we performed GO and KEGG enrichment analysis. Our results demonstrated that the AGE-RAGE signaling pathway was significantly enriched.

The AGE-RAGE signaling pathway in diabetic complications refers to the signaling cascade which involves Advanced Glycation End-products (AGEs) and their receptor, i.e., the Receptor for Advanced Glycation End-products (RAGE). AGEs are a group of complex compounds produced by nonenzymatic glycosylation and oxidation of proteins, lipids, and nucleic acids, mainly due to aging and certain pathological conditions such as hyperglycemia. (Chaudhuri et al., 2018) These AGEs can then interact with RAGE, triggering a series of cellular responses that contribute to the development and progression of diabetic complications, including cardiovascular disease. In diabetes, persistent high blood sugar levels can lead to the formation and accumulation of AGEs, and thus further activate the AGE-RAGE signaling pathway. Although this pathway specifies “diabetic complication,” it may also be activated in people without diabetes, because AGEs can be accumulated as a result of the aging process or oxidative stress. As mentioned before, EDCs were found to disrupt glucose homeostasis, but also increase the risk of diabetes; (Gore et al., 2015) therefore, EDCs may trigger the AGE-RAGE signaling pathway by inducing chronic hyperglycemia. There is significant evidence of the effect of AGEs in the aging process and their causal influence on organismal aging. (Chaudhuri et al., 2018) A previous study presented that a decrease in the accumulation of AGEs by Rifampicin could enhance the lifespan of worms via DAF-16/FOXO activation. (Golegaonkar et al., 2015) In a previous proteomics research, enrichment analysis of 232 age-associated proteins revealed that the insulin-like growth factor signaling pathway and AGE and RAGE metabolic pathways were two of the most remarkable metabolic pathways connected with biological age. (Moaddel et al., 2021).

To date, a growing number of evidence supports that AGEs play an important role in the pathogenesis of cardiovascular disease. AGE-RAGE activation may lead to arterial stiffness, atherosclerosis, endothelial dysfunction, oxidative stress, mitochondrial dysfunction, etc. (Lee et al., 2019) AGEs were found to impair endothelial dysfunction by means of suppressing endothelial nitric oxide production. (Xu et al., 2003) Upon ligand binding, RAGE could activate nuclear factor (NF)-κB, oxidative stress, and inflammation through Janus kinase/signal transducers and activators of transcription, nicotinamide adenine dinucleotide phosphate hydrogen oxidase, and mitogen-activated protein kinases pathway. (Chaudhuri et al., 2018) As a result, proinflammatory and proatherogenic factors, such as vascular cell adhesion molecule-1 (VCAM-1), are increasingly produced, possibly further enhancing RAGE expression. (Chaudhuri et al., 2018).

Notably, a recent review showed that the metabolic disorder caused by EDC exposure, oxidative stress, inflammatory activation, endothelial dysfunction, arterial stiffness, and other pathological processes related to the AGE-RAGE pathway was associated with the aging and longevity of the cardiovascular system. (Pietri and Stefanadis, 2021).

Our results also revealed the interaction effect of dietary vitamins C and E on the association of BA-EDCs and biological aging, which may provide an actionable target for BA-EDCs-induced biological aging prevention. Oluwayiose *et al.* reported that phthalate metabolites and their mixture were associated with advanced sperm epigenetic aging. (Oluwayiose et al., 2022) Interestingly, vitamins A and C supplementation was found to attenuate the toxic effects of DEHP on testicular functions, morphology, and semen characterization in adult male Wistar rats. (Ogunlade et al., 2022) These results support the hypothesis that vitamin C may be a potential and actionable target to attenuate BA-EDCs-induced biological aging. Considering that biological aging is a long-term process, it is necessary to conduct larger-scale prospective studies to thoroughly validate the potential therapeutic role of vitamins C and E.

Our study has several advantages. First, to the best of our knowledge, the present study provided an innovative assessment of the mixed effect of biologically-related EDCs on ASCVD and a novel insight into the role of biological aging as a result of EDC exposure and ASCVD. Second, we investigated the mixing effect of four categories of EDCs, which better reflects the effect of EDCs exposure on biological age acceleration and aging-related diseases. Third, we made use of the Aging Anno database to further explore the underlying mechanism of the adverse effect of BA-EDCs on ASCVD, and finally identify a possible explanation, i.e., BA-EDCs may lead to the accumulation of AGEs or activation of the AGE-RAGE signaling pathway, thereby promoting the manifestation of ASCVD. Most importantly, the present study revealed that dietary vitamins C and E may comprise an actionable approach to prevent BA-EDCs-induced biological aging. Lastly, our study also serves as a proof-of-concept for integrating single-cell RNA sequencing and environmental molecular epidemiology, which will be increasingly important as public health and translational science advance toward a precision medicine model.

Our study also has some limitations which must be seriously considered. As a cross-sectional study, we could not provide casual evidence on the association between BA-EDCs and ASCVD. Although covariates were adjusted, the results may be confounded by some unmeasured confounders. Besides, to assess the mixed exposure of as many EDCs as possible, we only included data from the NHANES 2011–12 cycle. Consequently, our study population was relatively small. In addition, the results presented do not account for the oversampling or under-sampling of certain groups in the NAHNES design, since it is unlikely to consider survey weights and strata in WQS regression. Hence, prospective studies with larger samples and repeated analysis in other sub-populations are required to confirm our findings. In addition, the use of NHANES 2011–12 exposure information may be relatively outdated and may not be aligned with the current pattern of EDCs exposure. Furthermore, BA-EDCs are mainly non-persistent chemicals, such as BPA and phthalates, thus a single urinary measurement may not reflect a representative estimate of internal exposure, and the impact of more persistent chemicals that bio-accumulate during human lifespan may have been ignored. Finally, long-term follow-up research is essential to assess the potential effects of vitamins C and E in preventing and delaying the adverse effects of EDCs. Moreover, cellular experiments and animal model studies are bound to provide a deeper understanding of how vitamins C and E intervenes in the effects of EDCs on cells and organisms. This will subsequently help uncover the potential signaling pathways, regulatory factors, or molecular mechanisms through which

vitamins C and E exerts their protective effects.

Taken together, our results demonstrate the positive correlation between EDC and a major aging-related disease, ASCVD. However, these detrimental effects may be ameliorated by the intake of vitamin C, thereby providing a distinct strategy to mitigate aging-related cardiac disease. Finally, this could pave the way to the development of novel therapeutic approaches based on vitamins or natural compounds.

5. Conclusion

In this study, we constructed biological age-related EDCs (BA-EDCs) and found a positive association between them and biological age acceleration, including KDM biological age acceleration and phenotypic age acceleration. Besides, BA-EDCs were also found to be positively associated with ASCVD. Dietary vitamins C and E may be actionable targets to prevent BA-EDCs-induced biological aging. Finally, based on our PPI and functional enrichment analysis, we can suggest that the AGE-RAGE signaling pathway may be the underlying mechanism.

6. Contributors

Weichao Huang contributed to conceptualization, methodology, formal analysis, and writing the original draft, reviewing and editing the manuscript. Zilong Zhang and Manuel Colucci contributed to conceptualization, software methodology, reviewing and editing the manuscript. Linghui Deng, Mi Yang, Xinyi Huang, Xianghong Zhou, Yumin Jin, Edoardo Lazzarini, Carolina Balbi, Oriol Juanola, Aurora Valdata, Silvia Bressan, Yu Zhan, and Fang Qi contributed to methodology, reviewing and editing the manuscript. Qiang Wei, Lu Yang, Xiaoli Zou, and Shi Qiu contribute to supervision, conceptualization, methodology, reviewing the manuscript.

7. Data sharing

The data that support the findings of this study are available from the National Health and Nutrition Examination Survey (https://www.cdc.gov/nchs/nhanes/about_nhanes.htm), Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/), Swiss Target Prediction (https://swisstargetprediction.ch/), TargetNet (https://targetnet.scbdd.com/home/index/), AgeAnno (https://relab.xidian.edu.cn/AgeAnno/#/).

CRediT authorship contribution statement

Weichao Huang: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Zilong Zhang:** Conceptualization, Methodology, Writing – review & editing. **Manuel Colucci:** Conceptualization, Methodology, Writing – review & editing. **Linghui Deng:** Methodology, Writing – review & editing. **Mi Yang:** Methodology, Writing – review & editing. **Xinyi Huang:** Methodology, Writing – review & editing. **Xianghong Zhou:** Methodology, Writing – review & editing. **Yumin Jin:** Methodology, Writing – review & editing. **Edoardo Lazzarini:** . **Carolina Balbi:** . **Oriol Juanola:** . **Aurora Valdata:** . **Silvia Bressan:** Methodology, Writing – review & editing. **Yu Zhan:** . **Fang Qi:** . **Qiang Wei:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Lu Yang:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Xiaoli Zou:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Shi Qiu:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by the National key research and development program of China (2022YFC3602901, 2022YFC3602902) , China Postdoctoral Science Foundation (2020M670057ZX), Programs from Science and Technology Department of Sichuan Province (2023NSFSC1906, 2021YJ0462), Post-doctoral Science Research Foundation of Sichuan University (2020SCU12041), National Natural Science Foundation of China (82372831). Thanks to Jing Zhang (Shanghai Tongren Hospital) for his work on the NHANES database. His outstanding work, nhanesR package and webpage, makes it easier for us to explore the NHANES database.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108447.

References

Altaf-Ul-Amin, M., Shinbo, Y., Mihara, K., Kurokawa, K., Kanaya, S., 2006. Development and implementation of an algorithm for detection of protein complexes in large interaction networks. BMC Bioinf. 7, 207.

Bloom, M.S., Whitcomb, B.W., Chen, Z., Ye, A., Kannan, K., Buck Louis, G.M., 2015. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum. Reprod. 30 (11), 2645–2657.

Bobb, J.F., Valeri, L., Claus Henn, B., et al., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16 (3), 493–508.

Campisi, M., Mastrangelo, G., Mielzynska-Svach, D., et al., 2023. The effect of high polycyclic aromatic hydrocarbon exposure on biological aging indicators. Environ. Health 22 (1), 27.

Caporale, N., Leemans, M., Birgersson, L., et al., 2022. From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures. Science 375 (6582), eabe8244.

Carrico, C., Gennings, C., Wheeler, D.C., Factor-Litvak, P., 2015. Characterization of Weighted Quantile Sum Regression for Highly Correlated Data in a Risk Analysis Setting. J. Agric. Biol. Environ. Stat. 20 (1), 100–120.

Chaney, C., Wiley, K.S., 2023. The variable associations between PFASs and biological aging by sex and reproductive stage in NHANES 1999–2018. Environ. Res. 227, 115714.

Chang, W.H., Herianto, S., Lee, C.C., Hung, H., Chen, H.L., 2021. The effects of phthalate ester exposure on human health: A review. Sci. Total Environ. 786, 147371.

Chaudhuri, J., Bains, Y., Guha, S., et al., 2018. The Role of Advanced Glycation End Products in Aging and Metabolic Diseases: Bridging Association and Causality. Cell Metab. 28 (3), 337–352.

Chen, F., Du, M., Blumberg, J.B., et al., 2019. Association Among Dietary Supplement Use, Nutrient Intake, and Mortality Among U.S. Adults: A Cohort Study. Ann. Intern. Med. 170 (9), 604–613.

Chen, L., Zhang, Y.H., Wang, S., Zhang, Y., Huang, T., Cai, Y.D., 2017. Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. PLoS One 12 (9), e0184129.

Clarity, C., Trowbridge, J., Gerona, R., et al., 2021. Associations between polyfluoroalkyl substance and organophosphate flame retardant exposures and telomere length in a cohort of women firefighters and office workers in San Francisco. Environ. Health 20 (1), 97.

Curtis, S.W., Cobb, D.O., Kilaru, V., et al., 2019. Environmental exposure to polybrominated biphenyl (PBB) associates with an increased rate of biological aging. Aging (Albany NY) 11 (15), 5498–5517.

Ferrucci, L., Levine, M.E., Kuo, P.L., Simonsick, E.M., 2018. Time and the Metrics of Aging. Circ. Res. 123 (7), 740–744.

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat. Rev. Endocrinol. 14 (10), 576–590.

[16] Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015; **43**(Database issue): D1049–D1056.

Golegaonkar, S., Tabrez, S.S., Pandit, A., et al., 2015. Rifampicin reduces advanced glycation end products and activates DAF-16 to increase lifespan in Caenorhabditis elegans. Aging Cell 14 (3), 463–473.

Gore, A.C., 2016. Endocrine-Disrupting Chemicals. JAMA Intern. Med. 176 (11), 1705–1706.

Gore, A.C., Chappell, V.A., Fenton, S.E., et al., 2015. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr. Rev. 36 (6), E1–e150.

Hu, W.Y., Shi, G.B., Hu, D.P., Nelles, J.L., Prins, G.S., 2012. Actions of estrogens and endocrine disrupting chemicals on human prostate stem/progenitor cells and prostate cancer risk. Mol. Cell. Endocrinol. 354 (1–2), 63–73.
Kane, L.T., Fang, T., Galetta, M.S., et al., 2020. Propensity Score Matching: A Statistical Method. Clin. Spine Surg. 33 (3), 120–122.
Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M., 2016. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 44 (D1), D457–D462.
Kang, H., Lee, J.P., Choi, K., 2021. Exposure to phthalates and environmental phenols in association with chronic kidney disease (CKD) among the general US population participating in multi-cycle NHANES (2005–2016). Sci. Total Environ. 791, 148343.
Karle, V.A., Short, B.L., Martin, G.R., et al., 1997. Extracorporeal membrane oxygenation exposes infants to the plasticizer, di(2-ethylhexyl)phthalate. Crit. Care Med. 25 (4), 696–703.
KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int* 2021; **100**(4s): S1-s276.
Kennedy, B.K., Berger, S.L., Brunet, A., et al., 2014. Geroscience: linking aging to chronic disease. Cell 159 (4), 709–713.
Khodasevich, D., Holland, N., Hubbard, A., et al., 2023. Associations between prenatal phthalate exposure and childhood epigenetic age acceleration. Environ. Res. 231 (Pt 1), 116067.
Klemera, P., Doubal, S., 2006. A new approach to the concept and computation of biological age. Mech. Ageing Dev. 127 (3), 240–248.
Kresovich, J.K., Martinez Lopez, A.M., Garval, E.L., et al., 2021. Alcohol Consumption and Methylation-Based Measures of Biological Age. J. Gerontol. A Biol. Sci. Med. Sci. 76 (12), 2107–2111.
Kwon, D., Belsky, D.W., 2021. A toolkit for quantification of biological age from blood chemistry and organ function test data: BioAge. Geroscience 43 (6), 2795–2808.
Lang, I.A., Galloway, T.S., Scarlett, A., et al., 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. J. Am. Med. Assoc. 300 (11), 1303–1310.
Lee, T.W., Kao, Y.H., Chen, Y.J., Chao, T.F., Lee, T.I., 2019. Therapeutic potential of vitamin D in AGE/RAGE-related cardiovascular diseases. Cell. Mol. Life Sci. 76 (20), 4103–4115.
Levey, A.S., Stevens, L.A., Schmid, C.H., et al., 2009. A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150 (9), 604–612.
Levine, M.E., 2013. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? J. Gerontol. A Biol. Sci. Med. Sci. 68 (6), 667–674.
Li, A., Koch, Z., Ideker, T., 2022. Epigenetic aging: Biological age prediction and informing a mechanistic theory of aging. J. Intern. Med. 292 (5), 733–744.
Libby, P., Bornfeldt, K.E., Tall, A.R., 2016. Atherosclerosis: Successes, Surprises, and Future Challenges. Circ. Res. 118 (4), 531–534.
Lin, Y., Wei, J., Li, Y., et al., 2011. Developmental exposure to di(2-ethylhexyl) phthalate impairs endocrine pancreas and leads to long-term adverse effects on glucose homeostasis in the rat. Am. J. Phys. Endocrinol. Metab. 301 (3), E527–E538.
Lind, P.M., Lind, L., 2011. Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. Atherosclerosis 218 (1), 207–213.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153 (6), 1194–1217.
Melzer, D., Rice, N.E., Lewis, C., Henley, W.E., Galloway, T.S., 2010. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PLoS One 5 (1), e8673.
Melzer, D., Osborne, N.J., Henley, W.E., et al., 2012. Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. Circulation 125 (12), 1482–1490.
Mian, O., Belsky, D.W., Cohen, A.A., et al., 2022. Associations between exposure to adverse childhood experiences and biological aging: Evidence from the Canadian Longitudinal Study on Aging. Psychoneuroendocrinology 142, 105821.
Michels, K.B., De Vivo, I., Calafat, A.M., Binder, A.M., 2020. In utero exposure to endocrine-disrupting chemicals and telomere length at birth. Environ. Res. 182, 109053.
Moaddel, R., Ubaida-Mohien, C., Tanaka, T., et al., 2021. Proteomics in aging research: A roadmap to clinical, translational research. Aging Cell 20 (4), e13325.
Monacelli, F., Acquarone, E., Giannotti, C., Borghi, R., Nencioni, A., Vitamin, C., 2017. Aging and Alzheimer's Disease. Nutrients 9, 7.
Moreno-Gomez-Toledano, R., 2022. Relationship between emergent BPA-substitutes and renal and cardiovascular diseases in adult population. Environ. Pollut. 313, 120106.
Moreno-Gómez-Toledano, R., Sánchez-Esteban, S., Cook, A., et al., 2021. Bisphenol a induces accelerated cell aging in murine endothelium. Biomolecules 11 (10).
Naidu, K.A., 2003. Vitamin C in human health and disease is still a mystery? An Overview. Nutr. J. 2, 7.
Napolitano, G., Fasciolo, G., Di Meo, S., Venditti, P., 2019. Vitamin E Supplementation and Mitochondria in Experimental and Functional Hyperthyroidism: A Mini-Review. Nutrients 11 (12).
Niemiec, S.S., Kechris, K., Pattee, J., et al., 2023. Prenatal exposures to per- and polyfluoroalkyl substances and epigenetic aging in umbilical cord blood: The Healthy Start study. Environ. Res. 231 (Pt 2), 116215.
Ogunlade, B., Gbotolorun, S.C., Adedotun, O.A., Itiere, K.A., Adejayi, J.A., 2022. Vanillic Acid and Vitamin C Attenuated Dehp-Induced Testicular Toxicity in Male Rats. Reprod Fertil 3 (3), 220–230.
Oluwayiose, O.A., Houle, E., Wu, H., et al., 2022. Urinary phthalate metabolites and their mixtures are associated with advanced sperm epigenetic aging in a general population. Environ. Res. 214 (Pt 4), 114115.
Padayatty, S.J., Katz, A., Wang, Y., et al., 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr. 22 (1), 18–35.
Pietri, P., Stefanadis, C., 2021. Cardiovascular Aging and Longevity: JACC State-of-the-Art Review. J. Am. Coll. Cardiol. 77 (2), 189–204.
Posnack, N.G., 2014. The adverse cardiac effects of Di(2-ethylhexyl)phthalate and Bisphenol A. Cardiovasc. Toxicol. 14 (4), 339–357.
Priego, A.R., Parra, E.G., Mas, S., Morgado-Pascual, J.L., Ruiz-Ortega, M., Rayego-Mateos, S., 2021. Bisphenol A Modulates Autophagy and Exacerbates Chronic Kidney Damage in Mice. Int. J. Mol. Sci. 22 (13).
Pupo, M., Pisano, A., Lappano, R., et al., 2012. Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. Environ. Health Perspect. 120 (8), 1177–1182.
Rattan, P., Penrice, D.D., Ahn, J.C., et al., 2022. Inverse Association of Telomere Length With Liver Disease and Mortality in the US Population. Hepatol Commun 6 (2), 399–410.
Ribeiro-Varandas, E., Pereira, H.S., Monteiro, S., et al., 2014. Bisphenol A disrupts transcription and decreases viability in aging vascular endothelial cells. Int. J. Mol. Sci. 15 (9), 15791–15805.
Shankar, A., Teppala, S., 2011. Relationship between urinary bisphenol A levels and diabetes mellitus. J. Clin. Endocrinol. Metab. 96 (12), 3822–3826.
Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014; **63**(25 Pt B): 2889-934.
Szklarczyk, D., Gable, A.L., Lyon, D., et al., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47 (D1), D607–D613.
Trasande, L., Attina, T.M., 2015. Association of exposure to di-2-ethylhexylphthalate replacements with increased blood pressure in children and adolescents. Hypertension 66 (2), 301–308.
Tsao, C.W., Aday, A.W., Almarzooq, Z.I., et al., 2022. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation 145 (8), e153–e639.
Upson, K., Sathyanarayana, S., De Roos, A.J., et al., 2013. Phthalates and risk of endometriosis. Environ. Res. 126, 91–97.
Vaiserman, A., Krasnienkov, D., 2020. Telomere Length as a Marker of Biological Age: State-of-the-Art, Open Issues, and Future Perspectives. Front. Genet. 11, 630186.
von Mering, C., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P., Snel, B., 2003. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res. 31 (1), 258–261.
Wang, T., Li, M., Chen, B., et al., 2012. Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. J. Clin. Endocrinol. Metab. 97 (2), E223–E227.
Wen, X., Wang, M., Xu, X., Li, T., 2022. Exposure to Per- and Polyfluoroalkyl Substances and Mortality in U.S. Adults: A Population-Based Cohort Study. Environ. Health Perspect. 130 (6), 67007.
Wen, Z.J., Wang, Z.Y., Zhang, Y.F., 2022. Adverse cardiovascular effects and potential molecular mechanisms of DEHP and its metabolites-A review. Sci. Total Environ. 847, 157443.
Wu, D., Meydani, S.N., 2014. Age-associated changes in immune function: impact of vitamin E intervention and the underlying mechanisms. Endocr Metab Immune Disord Drug Targets 14 (4), 283–289.
Xu, B., Chibber, R., Ruggiero, D., Kohner, E., Ritter, J., Ferro, A., 2003. Impairment of vascular endothelial nitric oxide synthase activity by advanced glycation end products. FASEB J. 17 (10), 1289–1291.
Yang, Q., Zheng, J., Chen, W., et al., 2021. Association Between Preadmission Metformin Use and Outcomes in Intensive Care Unit Patients With Sepsis and Type 2 Diabetes: A Cohort Study. Front Med (lausanne) 8, 640785.
Yin, T., Zhu, X., Cheang, I., et al., 2023. Urinary phenols and parabens metabolites associated with cardiovascular disease among adults in the United States. Environ. Sci. Pollut. Res. Int. 30 (10), 25093–25102.
Zhang, Y., Dong, T., Hu, W., et al., 2019. Association between exposure to a mixture of phenols, pesticides, and phthalates and obesity: Comparison of three statistical models. Environ. Int. 123, 325–336.
Zhang, S.H., Shen, Y.X., Li, L., Fan, T.T., Wang, Y., Wei, N., 2018. Phthalate exposure and high blood pressure in adults: a cross-sectional study in China. Environ. Sci. Pollut. Res. Int. 25 (16), 15934–15942.
Zhang, X., Wolff, M.S., Shen, J., et al., 2022. Phthalates and Phenols, Leukocyte Telomere Length, and Breast Cancer Risk and Mortality in the Long Island Breast Cancer Study Project. Cancer Epidemiol.. Biomarkers Prev. 31 (1), 117–123.
Zingg, J.M., Stamatiou, C., Montalto, G., Daunert, S., 2022. Modulation of CD36-mediated lipid accumulation and senescence by vitamin E analogs in monocytes and macrophages. Biofactors 48 (3), 665–682.