Brain, Behavior, and Immunity 127 (2025) 72–80

Contents lists available at ScienceDirect

Brain Behavior and Immunity

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

Age and Inflammation: Insights on "Age Three Ways" from Midlife in the United States Study

Dakota D. Witzel [a,b,*], Aarti C. Bhat [b,c,d], Jennifer E. Graham-Engeland [b,e], David M. Almeida [b,c,d]

[a] College of Education, Counseling, and Human Development, South Dakota State University, United States
[b] Center for Healthy Aging, Penn State University, United States
[c] Human Development and Family Studies, Penn State University, United States
[d] Population Research Institute, Penn State University, United States
[e] Biobehavioral Health, Penn State University, United States

ARTICLE INFO

Keywords:
Chronological Age
Subjective Age
Epigenetic Age
MIDUS
Inflammation

ABSTRACT

Introduction: Chronological age is a particularly well-known indicator of variability in systemic inflammation. Other pertinent aspects of age (or "age proxies") – subjective or epigenetic age – may offer nuanced information about age and inflammation associations. Using the Midlife in the United States Study, we explored how chronological, subjective, and epigenetic age were associated with inflammation. Further, we tested whether chronological age remained a unique predictor of inflammation after accounting for the variance of subjective and epigenetic age. Using an intersectionality framework, we also tested whether associations differed by race and gender. **Method:** 1,307 (85.39% White, 52.99% men) participants reported on their chronological and subjective age and provided blood from which epigenetic DNA and inflammatory biomarkers (IL-6, IL-8, fibrinogen, TNF-α, and E-selectin) were determined. **Results:** Linear regressions showed that being chronologically older was related to higher levels of inflammation. Being biologically older (higher epigenetic age or pace of aging) was also related to higher levels of all but IL-8. Subjective age was related to inflammatory biomarkers but only for people who identified their racial identity as White. Gender differences emerged, primarily with biological and chronological age. With all age indicators in one model, chronological age remained a unique indicator of inflammation in the sample, as similar to or a better predictor than biological age. **Conclusion:** The current study provides a better scientific understanding of the relative association of chronological age versus subjective and epigenetic age on inflammation with evidence suggesting that chronological age provides novel information above and beyond other proxies of age.

1. Introduction

Aging – often operationalized as *chronological aging*, or the number of years since birth – is related to reduced efficiency in innate and adaptive immune functioning (e.g., Chung et al., 2019; Graham et al., 2006; Kiecolt-Glaser et al., 2003; Xia et al., 2016), resulting in a higher risk for impairment or immune diseases in older age. The term *inflammaging* was coined to reflect this chronic elevation of inflammation related to aging processes and is highly relevant to morbidity and mortality from a range of conditions (Franceschi et al., 2018). However, chronological age differences in immune functioning show within-age variability, such that not every same-aged adult will experience similar levels of inflammatory markers or uniform declines in immune functioning across older ages (Kiecolt-Glaser et al., 2003). A potential source of this age heterogeneity is that chronological age is often used as a proxy for other possible age-related factors. Although researchers sometimes use chronological age as a proxy for status or experiences, chronological age is often interpreted as signifying differences across a host of phenomena such as emotional maturity, life experiences, biological age, or functional age (Settersten & Godlewski, 2015).

Other indicators of age may provide a more nuanced understanding of what chronological age represents. *Subjective age* – defined as how old a person feels – often represents a more personal experience or psychosocial construct of age (Settersten & Godlewski, 2015; Levy, 2009).

* Corresponding author at: 206 Wenona Hall, Brookings, SD 57006, United States.
E-mail address: Dakota.witzel@sdstate.edu (D.D. Witzel).

https://doi.org/10.1016/j.bbi.2025.03.018
Received 22 May 2024; Received in revised form 1 January 2025; Accepted 8 March 2025
Available online 9 March 2025

In recent years, DNA methylation biomarkers have also been developed as a representation of *biological age* or aging (Salameh et al., 2020). The overarching goal of the current research is to provide an exploration of the potential varying importance of these three age phenomena using three example indicators: (1) year of birth representing chronological age, (2) a discrepancy score between felt age and chronological age representing subjective age, and (3) epigenetic age representing biological age. To characterize how aspects of aging may inform health, the current research aims to provide a preliminary assessment of the unique and independent associations of chronological, subjective, and biological age with an outcome well-known to be associated with chronological age: inflammatory biomarkers. Given that inflammation differs across racial groups (e.g., (Ferguson et al., 2013) and by gender/sex (Knight et al., 2022), we also explore whether these associations vary across racial identities and gender.

1.1. Subjective age and inflammation

Internalizing aging stereotypes has been related to poorer physical health symptoms (Witzel et al., 2022), cardiovascular health (Levy et al., 2009), and broad health conditions (Levy et al., 2020). Stereotype embodiment theory (Levy, 2009) suggests that internalizing aging – sometimes presented as feeling older than you are – can affect health. Across health levels (e.g., poor to good health), people often feel about 20 % younger than their actual age (Rubin & Bernsten, 2006), and there is evidence that feeling younger than one's chronological age is related to better physical and cognitive health (Sargent-Cox et al., 2012). Conversely, feeling older and having poorer perceptions of age have been related to higher cortisol levels (Levy et al., 2016), increased blood pressure and skin conductance (Levy et al., 2000), and increased cardiovascular stress (Levy et al., 2000). Similar theoretical perspectives (Diehl et al., 2015) suggest that subjective age may, in some cases, reflect a person's knowledge of their general health and, as such, may be associated (albeit non-causally) with inflammation.

Subjective age is related to physical health (e.g., Wettstein et al., 2021; Witzel et al., 2022), but only a handful of empirical studies tie aspects of subjective age to inflammation. Two studies found that greater subjective age was related to higher systemic inflammation via C-reactive protein levels (CRP; Stephan et al., 2015) and other circulating inflammatory markers (Stephan et al., 2023). Specifically, Stephan and colleagues (2015) found that in an older adult sample (*Mage* = 69), feeling younger than one's chronological age was associated with lower levels of CRP, after adjusting for chronological age. In the same sample, they found that feeling younger than one's chronological age was related to lower pro- and anti-inflammatory cytokines up to eight years later (Stephan et al., 2023). Another study using a sample of middle-aged adults observed higher CRP and fibrinogen (but not IL-6, E-selectin, and ICAM-1) among people who felt older than they chronologically were; however, in the same study a discrepancy score between chronological age and subjective age was not significantly related to a composite of five inflammatory biomarkers (interleukin-6 [IL-6], CRP, Fibrinogen, E-selectin, ICAM-1; Hartanto et al., 2021) after accounting for health behaviors. Importantly, associations observed with one inflammatory marker may not be seen with others and, as such, examining associations across a range of inflammatory biomarkers may provide nuanced information compared to a composite.

1.2. Epigenetic age and inflammation

Although chronological aging seems to be related to strengths in emotion regulation and well-being, at least in certain contexts, aging also confers some biological vulnerability, including reduced physiological flexibility (Charles, 2010). The strength and vulnerability integration (SAVI) model highlights that middle-aged and older adults are more able to avoid or down-regulate emotional reactions than younger adults. This model also posits, however, that older adults have a physiological vulnerability especially when encountering chronic stress (Charles, 2010). Therefore, biological age indicators, such as the biological pace of aging, may inform inflammation in addition to subjective and chronological age.

Some researchers have attempted to estimate biological age based on *epigenetic clocks*, which are operationalized as the methylation of DNA at sites related to aging markers (Belsky et al, 2020; Horvath & Raj, 2018). The creation of these clocks utilizes regions within a DNA sequence comprising of cytosine and guanine base pairs in a particular pattern (i.e., CpG sites) and is one of many ways (e.g., RNA modification, chromatin remodeling, and histone modification) to characterize epigenetic differences related to aging. Although epigenetic clocks have been acknowledged for a decade, the "first generation" clocks estimated chronological age. Only more recently – in the second and third "generations" of epigenetic clock creation – were epigenetic clocks utilized to estimate biological age. These new iterations of epigenetic clocks often predict two things: health span/mortality (e.g., PhenoAge and GrimAge), and the pace of aging (e.g., DunedinPACE or DPACE for short; Belsky et al, 2020). Notably, GrimAge and PhenoAge were developed to predict morbidity and functional decline, offering a broader view of an individual's biological age compared to first generation clocks. Further, DPACE was developed to predict the rate of change in various biological and physiological parameters over time, offering different information than first generation clocks. As the "first generation" clocks predict chronological age, we utilize three second and third epigenetic clocks as a preliminary test of biological age associations with inflammation – two predicting lifespan/mortality (GrimAge and PhenoAge) and one predicting the pace of aging (DPACE) – for the current study.

Chronological age (e.g., age in years) is one of the biggest risk factors for aging-related death and disease and is distinct from biological aging (Marioni et al., 2015). Although epigenetic clocks have been used to predict disease and mortality (see review by Fransquet et al., 2019), and research has addressed whether inflammation informs epigenetic aging two years later (Cribb et al., 2022), no research to our knowledge has tested the reverse pathway that epigenetic aging may relate to inflammatory biomarkers. When DNAm occurs, particularly at CpG sites in gene promoters or enhancers, gene expression can be impacted by being dampened, or sometimes potentially amplified, contributing to increased levels of physiological inflammation (Aristizabal et al., 2020). Given that chronological age may often be considered a proxy for biological aging phenomena like epigenetic aging, a goal of the present work is to examine the possibility that epigenetic age may be related to inflammatory biomarkers.

1.3. Intersectionality in inflammation

The health disparities framework developed by the National Institute on Aging includes a noted priority to shift aging research into a more intersectional focus (Hill et al., 2015). Indeed, age is not a mutually exclusive demographic characteristic that informs inflammation. Intersectional approaches highlight that race or gender are critical characteristics that inform health with other aspects of a person (Etherington et al., 2015). Moreover, intersectional theories illuminate the importance of considering multiple aspects of identity when testing hypotheses (Alvidrez et al., 2021; Bauer, 2014; Crenshaw, 2013). As noted in a National Institute of Health perspective piece (Alvidrez et al., 2021), intersectionality frameworks attempt to understand how multiple disadvantaged social statuses interact with lived experiences to reflect systems of oppression or privilege. For example, research (e.g., (Mitchell and Aneshensel, 2016), has tested such intersectional theories regarding chronological age, race, and one indicator of inflammation (C-reactive protein; CRP), finding that racial differences in inflammation did not vary by chronological age. Further, gender and age interact to inform health outcomes (e.g., blood pressure, Robles et al. (accepted, in press)). As such, the intersection of race or gender and age may be a pertinent avenue of research for the current examination. No research to date that

we are aware of has explored these findings with (1) other indicators of inflammation, or (2) other indicators of age; as such, it becomes important to clarify whether and how chronological age, subjective age, and epigenetic age are associated with inflammation and how associations differ by race and gender/sex.

1.4. Current study

Using the second wave of the Midlife in the United States (MIDUS) study and the Refresher subsample of the MIDUS study, the current research proposes two aims to test whether chronological, subjective, and epigenetic age *uniquely* inform inflammatory biomarkers. First, we aim to determine how three indicators of age are linked with inflammatory biomarkers (interleukin-6 [IL-6], interleukin-8 [IL-8], fibrinogen, TNF-α, E-selectin). We anticipate that lower chronological age, feeling younger than one's chronological age, and lower epigenetic age will be uniquely related to lower levels of inflammatory biomarkers. Further, as chronological age and subjective age may be proxies for biological age (Settersten & Godlewski, 2015), we anticipate that when chronological age, subjective age, and epigenetic age are included as simultaneous predictors of inflammation, epigenetic age will remain a significant predictor of inflammation, whereas associations with subjective and chronological age will be reduced or nullified. Second, from an intersectionality perspective, we explore whether these associations differ across individual differences – particularly race and gender. We will explore if the associations between age and inflammation will be different for people who (1) are racialized as non-White compared to people who are racialized as White and (2) identify as men or women.

1.5. Method

Participants and Procedure.

The current study utilized data from MIDUS, a national study of health and well-being in midlife (https://midus.wisc.edu). Although three waves of data are publicly available for the MIDUS study, only the second wave and the refresher components offer measures of epigenetic aging. The second wave of MIDUS began in 2004/2005; in 2011, an additional sample was recruited to refresh and expand on the MIDUS study and to allow for testing of cohort effects on pertinent phenomena. The protocol for MIDUS Wave 2 and the MIDUS Refresher were the same. Participants were recruited through random digit dialing and completed telephone interviews that took approximately 20 min. A subsample of both MIDUS Wave 2 and MIDUS Refresher were additionally recruited and completed a biomarker assessment including an overnight stay at a clinical research unit, during which participants completed biological and health assessments, including blood sample collections. Notably, these clinical stays occurred six months to two years following the initial MIDUS telephone interviews. See Table 1 for more information about the sample descriptives.

Table 1
Demographic information of pertinent variables.

	N	M (SD)	Range / %
Self-rated health	1053	2.31 (0.99)	1—5
# of chronic conditions	1307	4.20 (3.22)	0—28
Woman	1053	–	47.01 %
White	1047	–	85.39 %
Education	1219	8.20 (2.38)	1 – 12
No school	1	–	0.08 %
Junior high	9	–	0.74 %
Some high school	30	–	2.46 %
GED	7	–	0.57 %
High school diploma	207	–	16.98 %
1–2 years of college	206	–	16.90 %
3 + years of college	54	–	4.43 %
Graduated 2-year program	106	–	8.70 %
Bachelor's degree	288	–	23.63 %
Some graduate school	47	–	3.86 %
Master's degree	207	–	16.98 %
PhD or equivalent	57	–	4.68 %
Smoking status	417	–	75.30 %
Chronological age	1053	52.06 (12.76)	25—81
Felt age	1031	43.29 (12.54)	3—100
Felt age discrepancy	1031	−0.04 (0.96)	−4.52 – 4.13
GrimAge residual	1053	0.01 (5.01)	−37.41 – 17.81
PhenoAge residual	1053	0.001 (5.76)	−19.15 – 19.95
DPACE	1307	0.99 (0.14)	0.53 – 1.45
IL-6	1307	1.12 (1.33)	0.06 – 21.03
IL-8	1301	12.73 (6.40)	2.81 – 69.11
TNF-A	1300	2.06 (0.72)	0.41 – 6.14
Fibrinogen	1297	349.60 (74.03)	101.00 – 590.00
E-selectin	1286	42.74 (19.42)	2.10 – 111.09

1.6. Measures

1.6.1. Inflammation

We utilize the following inflammatory biomarkers in the current study analyses: interleukin 6 (IL-6), interleukin 8 (IL-8), fibrinogen, E-selectin, and TNF-α. Although CRP was assayed in the MIDUS study, CRP was utilized as in the creation of the epigenetic clocks (see below); as such CRP was not included as an outcome. Blood draws for these biomarkers were collected before breakfast on the second day of the clinical stay. IL-6 and IL-8 were measured using the Quantikine High-sensitivity ELISA kit (#HS600B and #DR600; R&D Systems, Minneapolis, MN). IL-6 and IL-8 were assayed in the MIDUS Biocore Laboratory at the University of Wisconsin, Madison, WI. Fibrinogen antigen was measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Siemens, Malvern, PA). Fibrinogen was assayed at the Laboratory for Clinical Biochemistry Research at the University of Vermont, Burlington, VT. TNF-α was measured using a V-plex Custom Human Cytokine Kit (catalog #K151A0H-2) manufactured by Meso Scale Diagnostics, Rockville, MD. Finally, E-selectin was measured in serum by sandwich ELISA using Quantikine® kit #SSLE00 (R&D Systems, Minneapolis, MN). More information pertaining to levels assay ranges and procedure surrounding inflammation can be found at midus.colectica.org/.

1.6.2. Chronological age

Participants provided their date of birth in the initial MIDUS questionnaire. This was subtracted from the current year of data collection to obtain chronological age.

1.6.3. Subjective age

Subjective age was measured with one question, "What age do you feel?". Participants responded with the number of years old they felt. In line with previous research (Hartanto et al., 2021; Wahl et al., 2022), we created a discrepancy score between the age a participant felt and their chronological age. Higher scores represented feeling younger than their chronological age, and lower scores represented feeling older than chronological age.

1.6.4. Epigenetic age

Three epigenetic clocks (GrimAge, PhenoAge, and DPACE) were used in the present work characterize epigenetic age; although first generation clocks were available, because they were modeled for chronological age, we did not include them. To create these clocks, DNA was extracted from blood samples during the second clinical visit and tested for suitable DNA yield and DNA integrity and subjected to genome-wide DNAm profiling using the Illumina Methylation EPIC v1 microarray. The GrimAge epigenetic clock was created with surrogate DNAm biomarkers including plasma proteins (adrenomedullin, CRP, PAI-1, and GDF15) and years of smoking packs. PhenoAge was created with a host of 20,169 CpGs sites (Levine et al., 2018). The creation of the DunedinPACE (DPACE) clock included the use of 18 unique biomarkers and 46 specific CpG sites. Training models for these clocks used previously published algorithms to determine the clocks (Belsky et al., 2022; Levine et al.,

2018). PhenoAge and Grim Age clocks were regressed on chronological age to represent biological aging. Residuals were outputted from this model to create a unique indicator that represents the epigenetic pace of aging. For GrimAge, PhenoAge, and DPACE, higher scores represent older epigenetic age. More information about quality control and assaying procedures, as well as training models for epigenetic clock creation can be found at https://midus.colectica.org/.

1.6.5. Covariates

All models covaried for gender (0 = *women,* 1 = *men*), education (1 = *no school*, 2 = *junior high*, 3 = *some high school*, 4 = *GED*, 5 = *high school diploma*, 6 = *1–2 years college*, 7 = *3 + years of college, no degree*, 8 = *graduate 2-year program*, 9 = *Bachelors*, 10 = *some graduate school*, 11 = *Master's degree*, 12 = *PhD or equivalent*), number of chronic conditions, and subjective health status (0 = *poor*, 4 = *excellent*). Gender (Gubbels Bupp, 2015; Knight et al., 2022) and education (Muscatell et al., 2018) have previously been associated with differences in levels of some inflammatory markers. Moreover, number of chronic conditions and subjective health status) were utilized to account for conditions that may have links to inflammation (e.g., arthritis).

1.7. Analyses

Inflammatory variables were log-transformed and winsorized given the positive skew. All other variables were z-scored for more direct comparisons across the age indicators. We ran regression analyses in SAS (SAS Institute, 2013), using PROC REG to test for variance inflation factors and tolerance levels for the variables. All models, including sensitivity analysis, covaried for education, and subjective health status (poor-excellent) and gender and race (where appropriate). Moreover, we ran additional sensitivity analyses covarying for smoking ("do you smoke cigarettes regularly"). First, to test how subjective, chronological, and epigenetic ages were uniquely linked with inflammatory biomarkers, we ran linear regressions for each inflammatory biomarker regressed on each age indicator. Then, we tested whether accounting for the variance of each age indicator (subjective, chronological, epigenetic) influenced the effect of age on inflammatory biomarkers by running one model for each outcome. Next, we explored whether these associations were similar across race and gender. First, models were stratified by racialization of White or non-White racial identification to better understand whether associations were similar across people who are racialized as a minority (e.g., non-White Black, Hispanic) or White. Importantly, because being White is usually the comparator for race related analyses (Baker & Gamaldo, 2022), and we did not want to assume underlying processes were the same, we stratified by race and examined the size, significance, and direction of effects within group, rather than statistically comparing these groups. Then, we explored differences in gender by stratifying samples by men and women.

2. Results

2.1. Descriptive Results

Of the entire MIDUS wave 2 and MIDUS Refresher participants, 1,307 had both inflammatory and epigenetic data available. Most participants were White (85.39 %), women (52.99 %), and were in fair to excellent health (88.22 %). Participants were on average approximately 52 years old (Range = 25 – 81) and the average difference between chronological and felt age was approximately 9 years (*SD* = 9.99) suggesting that subjective age was approximately 9 years younger than chronological age. GrimAge predicted the average biological age of the sample to be similar to chronological age (*M* = 52.63, Range = 22.50 – 104.59). PhenoAge predicted the average biological age to be about 10 years younger than the average chronological age (*M* = 43.64, Range = 10.27 – 85.01). The DPACE showed that people had on average, a slower pace of aging (M = 0.99, Range = 0.53 – 1.45) compared to their chronological age. Feeling younger (OR = 1.04, %95CI: 1.01, 1.07) but being chronologically older (OR = 1.06, %95CI: 1.04, 1.09) was related to a significantly higher likelihood of smoking. Moreover, feeling younger (OR = 0.98, %95CI: 0.96, 0.99), but being chronologically older (OR = 0.96, %95CI: 0.95, 0.98), was related to a decreased likelihood of being characterized as a racial minority.

T-tests revealed that being a woman was associated with a *higher* level of chronic health conditions t(1051) = 2.78, $p = 0.01$, E-selectin t(1040) = -2.90, $p = 0.0004$, fibrinogen t(1045) = 4.02, $p < 0.001$, IL-8 t(1050) = 3.96, $p < 0.001$, and biological GrimAge t(1051) = 2.51, $p = 0.01$, and *lower* education t(1049) = -2.38, $p = 0.02$, TNF-α t(1046) = -3.25, $p = 0.001$, biological DPACE t(1051) = -2.91$p = 0.004$, subjective age t(1029) = -2.33, $p = 0.02$, and chronological age t(1051) = -2.59, $p = 0.01$. Further, being a racialized minority was related to *higher* levels of fibrinogen t(1039) = -2.12, $p = 0.04$, IL-6 t(1045) = -2.53, $p = 0.01$, biological DPACE t(1045) = -4.16, $p < 0.001$, and biological PhenoAge t(1045) = 2.42, $p = 0.02$, and *lower* levels of general health t(1045) = -3.32, $p = 0.001$, and chronological age t(1045) = 3.54, $p < 0.001$. Finally, smoking was significantly associated with a *higher* number of chronic conditions t(415) = -2.30, $p = 0.02$, education t(415) = -4.48, $p < 0.001$, TNF-α t(413) = -2.26, $p = 0.03$, and chronological age t(415) = -6.40, $p < 0.0001$, and *lower* general health t(415) = 4.60, $p < 0.001$, biological DPACE t(415) = 4.37, $p < 0.001$, biological PhenoAge t(415) = -3.89, $p < 0.001$. As shown in Table 2, the inflammatory biomarkers were significantly and positively correlated. Similarly, higher chronological age was significantly related to higher epigenetic age and subjective age.

2.2. Main effects of Chronological, Subjective, and epigenetic age on inflammation

2.2.1. Independent models

Standardized beta coefficients and variances for independent models of chronological, subjective, and epigenetic age predicting inflammation can be found in Table 3. Being chronologically older was associated with higher levels IL-6 (b = 0.05, β = 0.18, 95 %CI: [0.03, 0.07]), IL-8 (b = 0.08, β = 0.22, 95 %CI: [0.06, 0.11]), fibrinogen, (b = 19.60, β = 0.26, 95 %CI: [14.71, 24.48]), and TNF-α (b = 0.10, β = 0.13, 95 %CI: [0.05, 0.15]).

As higher scores in subjective age indicate feeling younger than a person is, younger subjective age was related to both higher IL-8 (b = 0.03, β = 0.09, 95 %CI: [0.01, 0.06]) and higher fibrinogen (b = 4.68, β = 0.06, 95 %CI: [0.08, 9.29]). IL-6, TNF-α, and E-selectin were not statistically significantly associated with subjective age.

The relation between epigenetic age and inflammation varied by the epigenetic age indicator used. DPACE was significantly associated with higher IL-6 (b = 0.12, β = 0.41, 95 %CI: [0.10, 0.14]), IL-8 (b = 0.03, β = 0.08, 95 %CI: [0.004, 0.06]), fibrinogen (b = 24.998, β = 0.32, 95 % CI: [20.17, 29.82]), TNF-α (b = 0.11, β = 0.14, 95 %CI: [0.06, 0.16]), and E-selectin (b = 4.72, β = 0.23, 95 %CI: [3.42, 6.01]). Although PhenoAge was not significantly associated with fibrinogen or TNF-α PhenoAge was associated with IL-6 (b = -0.004, β = -0.09, 95 %CI: [-0.007, −0.001]), IL-8 (b = 0.004, β = 0.07, 95 %CI: [0.001, 0.008]), and E-selectin (b = -0.50, β = -0.15, 95 %CI: [-0.69, −0.30]). Moreover, GrimAge was not significantly associated with any inflammatory biomarker.

2.2.2. Combined models

Standardized beta coefficients and the R^2 for combined age indicators predicting inflammation are in the right panel of Table 3. When all age variables were included in the model, significantly more of the variance was accounted for than by one age variable alone. Chronological age remained significantly related to higher IL-6 (b = 0.02, β = 0.09, 95 %CI: [0.004, 0.05]), IL-8 (b = 0.09, β = 0.23, 95 %CI: [0.06, 0.12], fibrinogen (b = 19.68, β = 0.27, 95 %CI: [13.68, 24.75]), TNF-α (b = 0.09, β = 0.12, 95 %CI: [0.03, 0.19]), and became significant for E-

Table 2
Correlations with continuous variables.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
1. Il-6												
2. IL-8	**0.13**											
3. Fibrinogen	**0.42**	**0.11**										
4. TNF-A	**0.33**	**0.19**	**0.09**									
5. E-Selectin	**0.28**	**0.11**	**0.20**	**0.19**								
6. Chronological Age	**0.21**	**0.24**	**0.23**	**0.15**	−0.03							
7. Grim Epigenetic Age	0.06	**0.07**	−0.001	−0.004	−0.05	**0.39**						
8. Pheno Epigenetic Age	**−0.10**	**0.09**	−0.05	−0.03	**−0.17**	**0.45**	**0.61**					
9. Pace of Epigenetic Age	**0.47**	**0.12**	**0.38**	**0.14**	**0.28**	**0.27**	−0.04	**−0.27**				
10. Subjective Age	**0.06**	**0.09**	0.03	−0.01	**−0.08**	**0.41**	**0.15**	**0.17**	0.05			
11. Education	**−0.19**	−0.05	**−0.13**	−0.07	**−0.13**	−0.02	0.02	**0.07**	**−0.24**	**0.08**		
12. Subjective health status	**0.33**	**0.08**	**0.18**	**0.11**	**0.18**	−0.004	−0.02	**−0.12**	**0.31**	**−0.19**	**−0.19**	
13. Chronic health conditions	**0.26**	**0.21**	**0.18**	**0.16**	**0.08**	**0.43**	**0.15**	**0.11**	**0.24**	**0.09**	**−0.07**	**0.34**

Note. All variables are scored such that higher scores reflect higher levels. Higher scores represented higher levels of phenomena. Bolded = $p < 0.05$.

selectin, (b = -2.53, β = -0.13, 95 %CI: [-4.11, −0.95]). Subjective age, however, was no longer a significant predictor of any inflammatory biomarker.

Further, DPACE remained a significant predictor of higher IL-6 (b = 0.12, β = 0.40, 95 %CI: [0.10, 0.13]), fibrinogen (b = 20.49, β = 0.27, 95 %CI: [15.51, 25.47]), TNF-α (b = 0.09, β = 0.12, 95 %CI: [0.04, 0.15]), and E-selectin (b = 5.42, β = 0.27, 95 %CI: [4.06, 6.78]), but was no longer a significant predictor of IL-8. Moreover, GrimAge (b = -1.28, β = -0.09, 95 %CI: [-2.18, −0.39]) and PhenoAge (b = -0.93, β = -0.07, 95 %CI: [-1.81, −0.05]) were significantly associated with fibrinogen such that higher biological age was associated with lower fibrinogen.

2.3. Exploratory analyses

2.3.1. Racial stratification

Independent models. White and non-White groups were similar in the unique effects of chronological, subjective, and epigenetic age. As shown in the first columns of Supplemental Table 1 and Table 2, chronological age was significantly related to higher levels of *all* inflammatory biomarkers for White participants: IL-6 (b = 0.05, β = 0.18, 95 %CI: [0.03, 0.07]), IL-8 (b = 0.09, β = 0.24, 95 %CI: [0.06, 0.12]), fibrinogen (b = 19.01, β = 0.26, 95 %CI: [13.73, 24.29]), TNF-α (b = 0.10, β = 0.14, 95 %CI: [0.05, 0.16]), and E-selectin (b = -1.64, β = -0.09, 95 %CI: [-3.06, −0.21]). For non-White participants, however, chronological age was only statistically significant for IL-6 (b = 0.06, β = 0.20, 95 %CI: [0.01, 0.11]) and fibrinogen (b = 19.01, β = 0.26, 95 %CI: [13.73, 24.29]).

When stratified by minoritization status, only White participants had statistically significant associations between subjective age and IL-8 (b = 0.05, β = 0.13, 95 %CI: [0.02, 0.07]) and fibrinogen (b = 5.86, β = 0.08, 95 %CI: [0.83, 10.89]).

Similar to chronological age, DPACE was significantly related to higher levels of *all* inflammatory biomarkers for White participants: IL-6 (b = 0.12, β = 0.40, 95 %CI: [0.10, 0.14]), IL-8 (b = 0.03, β = 0.07, 95 % CI: [0.001, 0.06]), fibrinogen (b = 25.11, β = 0.32, 95 %CI: [19.85, 30.37]), TNF-α (b = 0.09, β = 0.12, 95 %CI: [0.04, 0.14]), and E-selectin (b = 4.45, β = 0.22, 95 %CI: [3.02, 5.88]). For non-White participants, however, DPACE was only statistically significant for IL-6 (b = 0.12, β = 0.40, 95 %CI: [0.08, 0.17]), fibrinogen (b = 24.55, β = 0.31, 95 %CI: [12.09, 37.02]), and TNF-α (b = 0.16, β = 0.20, 95 %CI: [0.03, 0.30]). Finally, PhenoAge was associated with higher levels of IL-6 (b = -0.004, β = -0.10, 95 %CI: [-0.01, −0.002]), IL-8 (b = 0.004, β = 0.07, 95 %CI: [0.0004, 0.01]), and E-selectin (b = -0.54, β = -0.17, 95 %CI: [-0.73, −0.32]) for White participants but was not significantly related to any inflammation marker for non-White participants. There were no statistically significant associations between GrimAge and inflammation any participants.

Combined models. As shown in Supplemental Table 1 and Table 2, within combined models, chronological age remained significantly associated with IL-6 (b = 0.03, β = 0.09, 95 %CI: [0.005, 0.05]), IL-8 (b = 0.09, β = 0.24, 95 %CI: [0.06, 0.12]), fibrinogen (b = 18.17, β = 0.25, 95 %CI: [11..96, 24.39]), TNF-α (b = 0.10, β = 0.13, 95 %CI: [0.03, 0.16]), and E-selectin (b = -2.48, β = -0.13, 95 %CI: [-4.19, −0.76]) for White participants. The only exception to this was chronological age and E-selection when PhenoAge was included in models. Notably, in combined models for non-White participants, chronological age remained significant for fibrinogen (b = 22.48, β = 0.28, 95 %CI: [5.64, 39.32]), but not IL-6.

DPACE remained a statistically significant predictor of IL-6 (b = 0.12, β = 0.39, 95 %CI: [0.10, 0.13]), fibrinogen (b = 21.07, β = 0.27, 95 %CI: [15.67, 26.47]), TNF-α (b = 0.08, β = 0.10, 95 %CI: [0.02, 0.13]), and E-selectin (b = 5.07, β = 0.25, 95 %CI: [3.58, 6.56]) for White participants. Associations between IL and 8 or TNF-α and DPACE, however, were sensitive to the 2nd generation clock included. For non-White participants, DPACE remained statistically significant for IL-6 (b = 0.12, β = 0.39, 95 %CI: [0.07, 0.17]), fibrinogen (b = 17.54, β = 0.22, 95 %CI: [3.88, 31.20]), and TNF-α (b = 0.16, β = 0.20, 95 %CI: [0.01, 0.31]) and became statistically significant for E-selectin (b = 6.97, β = 0.36, 95 %CI: [3.50, 10.43]). Finally, after combining indicators, PhenoAge remained significantly associated with fibrinogen (b = -1.09, β = -0.09, 95 %CI: [-2.03, −0.16]) for White participants.

2.3.2. Gender stratification

Independent models (Supplemental Table 3 and Table 4) showed that when women had higher levels for PhenoAge (b = 0.01, β = 0.15, 95 %CI: [0.004, 0.02]) and chronological age (b = 0.15, β = 0.19, 95 % CI: [0.08, 0.22]), they also had significantly higher levels of IL-8 and TNF-α respectively, whereas for men, these associations were not statistically significant. Conversely, men had significantly higher levels of (1) IL-6 associated with higher GrimAge (b = 0.004, β = 0.08, 95 %CI: [0.0001, 0.01]) and PhenoAge (b = -0.004, β = -0.09, 95 %CI: [-0.01, −0.0001]), (2) IL-8 associated with higher DPACE (b = 0.05, β = 0.15, 95 %CI: [0.02, 0.09]) and subjective age (b = 0.04, β = 0.12, 95 %CI: [0.01, 0.07]), and (3) E-selectin associated with higher chronological age (b = -2.44, β = -0.13, 95 %CI: [-4.30, −0.57]). These differences were reflected in the combined models, with one exception. In combined models, GrimAge and PhenoAge were no longer significantly associated with IL-6 for men and subjective age was no longer significantly associated with IL-8 for men.

2.4. Sensitivity analyses

Given associations between smoking and inflammation, we ran sensitivity analyses with smoking as a covariate in models seen in Supplemental Table 5–7. Given that smoking status was utilized in creation of the GrimAge clock, we did not test independent or combined models that included GrimAge.

Independent models with chronological age, DPACE, and PhenoAge

Table 3
Main effects of age on inflammatory biomarkers.

	Independent Models		Combined Model GrimAge		Combined Model PhenoAge	
IL-6	**B (SE)**	**R^2**	**B (SE)**	**R^2**	**B (SE)**	**R^2**
GrimAge	0.05 (0.002)	0.15	0.04 (0.002)			
PhenoAge	−0.09 (0.001)**	0.15			−0.05 (0.002)	
DPACE	0.41 (0.01)***	0.28	0.40 (0.01)***		0.37 (0.01)***	
Subjective Age	−0.01 (0.01)	0.08	−0.04 (0.01)		−0.04 (0.01)	
Chronological Age	0.18 (0.01)***	0.16	0.09 (0.01)*	0.29	0.14 (0.01)**	0.29
IL-8	**B (SE)**	**R^2**	**B (SE)**	**R^2**	**B (SE)**	**R^2**
GrimAge	0.04 (0.002)	0.06	−0.04 (0.003)			
PhenoAge	0.07 (0.002)*	0.06			−0.02 (0.002)	
DPACE	0.08 (0.01)*	0.06	0.02 (0.01)		0.02 (0.02)	
Subjective Age	0.09 (0.01)**	0.06	0.01 (0.01)		0.01 (0.02)	
Chronological Age	0.22 (0.01)***	0.09	0.23 (0.02)***	0.09	0.22 (0.02)***	0.09
Fibrinogen	**B (SE)**	**R^2**	**B (SE)**	**R^2**	**B (SE)**	**R^2**
GrimAge	−0.02 (0.44)	0.07	−0.09 (0.45)**			
PhenoAge	−0.04 (0.38)	0.07			−0.07 (0.45)*	
DPACE	0.32 (2.46)***	0.15	0.27 (2.54)***		0.25 (2.78)***	
Subjective Age	0.06 (2.35)*	0.07	−0.04 (2.39)		−0.04 (2.40)	
Chronological Age	0.26 (2.49)***	0.12	0.26 (2.95)***	0.18	0.26 (2.78)***	0.18
TNF-A	**B (SE)**	**R^2**	**B (SE)**	**R^2**	**B (SE)**	**R^2**
GrimAge	−0.01 (0.004)	0.04	−0.04 (0.005)			
PhenoAge	−0.03 (0.004)	0.04			−0.06 (0.005)	
DPACE	0.14 (0.03)***	0.05	0.12 (0.03)***		0.10 (0.03)**	
Subjective Age	−0.001 (0.02)	0.04	−0.05 (0.03)		−0.05 (0.03)	
Chronological Age	0.13 (0.03)***	0.05	0.12 (0.03)**	0.06	0.14 (0.03)**	0.06
E-selectin	**B (SE)**	**R^2**	**B (SE)**	**R^2**	**B (SE)**	**R^2**
GrimAge	−0.04 (0.11)	0.05	0.02 (0.12)			
PhenoAge	−0.15 (0.10)***	0.07			−0.05 (0.12)	
DPACE	0.23 (0.66)***	0.10	0.27 (0.69)***		0.24 (0.76)***	
Subjective Age	−0.05 (0.62)	0.05	−0.03 (0.65)		−0.03 (0.65)	
Chronological Age	−0.06 (0.67)	0.06	−0.13 (0.80)**	0.11	−0.09 (0.87)*	0.11

Note. Left panel reports *standardized* Beta coefficients for each independent model for each age indicator. Right panel reports *standardized* Beta coefficients for combined model with all age indicators included. Models covary for gender, race, education, and subjective health status (poor-excellent).
*** $p < 0.001$, ** $p < 0.01$ * $p < 0.05$.

respectively predicting inflammation remained largely the same direction and significance. Notably, however, the following independent associations between for the following were no longer significant after including smoking status: (1) DPACE, PhenoAge, and subjective age uniquely predicting IL-8; (2) subjective age predicting fibrinogen; and (3) chronological age predicting TNF-α. After adding smoking to the combined models, subjective age became a significant predictor of IL-6, such that for every year a person felt younger, there was a.03 pg/ml decrease in IL-6 (b = -0.03, β = -0.11, 95 %CI: [-0.06, −0.004]).

Although stratified independent models for White participants remained largely the same after the inclusion of smoking status, the independent models for non-White participants did differ depending on the inclusion of smoking status. For chronological age, the only association that remained significant for non-White participants was for fibrinogen (b = 37.02, β = 0.44, 95 %CI: [11.84, 62.21]). Further, DPACE was only statistically significantly associated with fibrinogen for non-White participants (b = 32.12, β = 0.37, 95 %CI: [7.27, 56.98]). Combined models remained the same for White participants compared to the entire model with smoking; for non-White participants, there were no longer any significant associations between any age indicator or inflammation, except for DPACE and TNF-α (b = 0.40, β = 0.39, 95 %CI: [0.00, 0.80]).

Similarly, gender stratified models remained largely the same when including smoking status, although direction of associations remained the same, significance level changed for some associations depending on men or women. Although changes are noted in Supplemental Table 7, two notable changes occurred. First, for women, associations between subjective age and IL-6 became statistically significant such that feeling younger was related to lower levels of IL-6 for women but not men. Second, the following associations became non-significant in the combined models for men and women: (1) chronological age associated with TNF-α for women and (2) chronological age associated with E-selectin for men.

3. Discussion

The overarching goal of the current research was to provide a theoretical overview of the potential importance of three age indicators by examining how chronological, subjective, and epigenetic age uniquely informed inflammatory biomarkers in a large sample of middle-aged and older adults. Researchers often construe chronological age as a proxy for status or experiences, emotional maturity, life experiences, biological age, or functional age (Settersten & Godlewski, 2015). The current study provides evidence to suggest that chronological age is a unique indicator of inflammation, above and beyond subjective age and biological/epigenetic age. Specifically, chronological age remained a significant predictor for nearly all inflammatory biomarkers regardless of race or gender and additional indicators. Epigenetic age was more sensitive to other age indicators but did show significant associations for non-White participants in some instances. Further, subjective age seemed to be only related to inflammatory biomarkers (i.e., IL-8, fibrinogen) for people who identified their racial identity as White; however, associations with subjective age were dependent on the inclusion of age proxies and smoking status. Finally, we found that there were gender differences, predominantly with relation to biological age indicators and inflammation; however, many of these associations were void in combined models.

3.1. Age indicators as unique inflammation indicators

We anticipated that lower chronological age, subjectively feeling younger than one's chronological age, and lower epigenetic age would be uniquely related to lower levels of inflammatory biomarkers. This hypothesis was partially supported. Specifically, in line with previous research (Chung et al., 2019; Graham et al., 2006; Kiecolt-Glaser et al., 2003; Xia et al., 2016) being chronologically older was related to higher levels of all three inflammatory biomarkers. Moreover, being biologically older (higher epigenetic age) was related to higher levels of all inflammatory biomarkers. Although past research suggests that epigenetic age and inflammation are uniquely related to poorer health outcomes (e.g., mortality; Cribb et al., 2022) and has examined how first-generation epigenetic age clocks inform IL-6 (e.g., Irvin et al., 2018), no studies to our knowledge have tested whether epigenetic aging indicators based on newer epigenetic age clocks are related to levels of inflammation. As such, the current study provides extended evidence to suggest that chronological age is associated with higher levels of

inflammation and provides new evidence that epigenetic age is associated with higher levels of inflammation.

Contrary to expectations, reporting feeling younger, rather than older, than one's chronological age was associated with higher levels of IL-8 and fibrinogen. Previous studies examining the discrepancy between felt age and chronological age found that feeling older than one's chronological age was related to higher levels of inflammation; however, these studies focused on or included CRP as their main inflammatory biomarker, which is a noted indicator of systemic inflammation (Nater et al., 2013). Subjective age may have unique associations with CRP compared to other inflammatory biomarkers like IL-6, which has numerous functions (Black, 2002; Kishimoto, 2006).

3.2. Chronological age as a proxy for aging

As researchers utilize chronological age as a proxy for biological aging, we hypothesized that when chronological age, subjective age, and epigenetic age were included as simultaneous predictors of inflammation, epigenetic age would remain a significant predictor of inflammation, and chronological and subjective age show lower, or null, associations. This hypothesis was not fully supported. When chronological age, subjective age, and epigenetic aging were included in the same model, chronological age remained a significant predictor of inflammatory markers, but subjective age was no longer significantly associated with inflammatory markers. Although subjective age has been previously associated with inflammation (Hartanto et al., 2021; Stephan et al., 2015, 2023), it may be that chronological and epigenetic age account for the variance that has been attributed to subjective age. Metanalyses have suggested that lower subjective age is also associated with lower depressive symptoms and better cognition (Debreczeni et al., 2021); as such subjective age may simply be a better proxy for well-being outcomes compared to physiological outcomes. Conversely, the age range of the current sample is quite large (25–81); as such, the heterogeneity of chronological age may have introduced statistical noise, potentially masking associations between subjective age and inflammation. Although this is partially addressed by the independent models examining subjective age without adjusting for chronological age, it may be valuable for future work focus on theoretically relevant age groups to address this potential heterogeneity.

Compared to subjective age, chronological and epigenetic age (DPACE specifically) remained statistically significant predictors of almost all inflammatory biomarkers over and above the other age indicators. Being epigenetically older was related to higher levels of IL-6 fibrinogen, and TNF-α even after accounting for chronological and subjective age. Indeed, DPACE was the strongest predictor of IL-6, fibrinogen, and TNF-α. As noted previously, this was expected as literature attributes chronic elevated inflammation (termed inflamaging; Franceschi et al., 2018) to normative aging related changes to physiology; this normative age-related change in inflammation may be captured by epigenetic aging measures. Chronological age also remained a significant indicator of all inflammatory biomarkers, even after accounting for subjective and epigenetic aging. Moreover, chronological age was the strongest and only predictor of IL-8 levels. Although potentially counterintuitive, both subjective and epigenetic age may be influenced by aspects of life that are not accounted for here, such as work experiences, or sickness. Further, it is possible that chronological age may continue to be a proxy for additional phenomena not studied here. For example, previous experiences such as lifetime adversity could be representative of chronological age for some, but not all, people; the lack of inclusion of these phenomena may overestimate associations between chronological age and inflammation. Conversely, although the epigenetic clocks utilized here represent DNA methylation, there could be additional pertinent biological aging processes that account for current associations (e.g., mitochondrial dysfunction). Thus, future research should consider which indicators of age should be utilized for either moderators or covariates.

3.3. Subjective age as a uniquely White indicator of health

When stratifying by race, subjective age was uniquely related to IL-8 and fibrinogen for White participants; associations between subjective age and inflammation were not significant for non-White participants. This was particularly the case for IL-8 and fibrinogen; however, when including smoking status, subjective age was significantly related to IL-6 for White participants.

There are mixed findings related to race and subjective age as some literature suggests that race does not inform levels of subjective age (Henderson et al., 1995), and others suggesting that race was associated with subjective age (Kirkpatrick Johnson et al., 2007). These mixed findings may be related to the internalization of age-related stereotypes across racial identity. Specifically, it is possible that people who are racialized as a racial minority may not equate importance to feeling older or younger than one's age. Menkin and colleagues (2017) note that expectations of aging that differ by both race and ethnicity and cultural differences (e.g., collectivism vs. individualism) may influence the internalization of age stereotypes that connect subjective age and health outcomes (e.g., North & Fiske, 2015). For example, stereotype embodiment theory suggests that the internalization of feeling older or younger may be particularly important for the health of the individual (Levy, 2009). As such, it may be that White individuals tend to internalize aging processes and stereotypes differently compared to racial minorities. It will be important to determine how different racial groups internalize aging to better understand how racial differences in associations between subjective age and inflammation.

Interestingly, subjective age was particularly sensitive to the inclusion of smoking status. Indeed, after including smoking status as a covariate, nearly all associations with subjective age were no longer significant with one exception: subjective age became significantly associated with IL-6 following inclusion of smoking. Stratified models revealed this to be the case for women, but not men. Some (Hartanto et al., 2021; Stephan et al., 2015), but not all (Stephan et al., 2023), research examining subjective age and inflammation has included smoking as a covariate. Moreover, research suggests that aspects of psychological age (e.g., self-perceptions of aging) are associated with smoking status over time (Hooker et al., 2019). Although subjective age was measured a year prior to inflammation, smoking and subjective age were measured in the same point, thus limiting our ability to test smoking as a possible mediator for subjective age and inflammation associations. However, long-term smoking does result in aging-related changes (e.g., wrinkles, (Yazdanparast et al., 2019) that may make people feel older than they are.

3.4. Gender differences associated with chronological and biological age

In combined models, there were gender differences in the significance and direction of associations PhenoAge and DPACE. Moreover, there were noted gender differences with chronological age and E-selectin. This may be the result of the use of inflammation as the outcome of interest. Specifically, men and women have shown different patterning of associations with associations between other phenomena and inflammation (e.g., Knight et al., 2022; Martinez de Toda et al., 2023) and as such, gender may be a particularly important variable to consider regardless of age indicator used. It will be necessary for future work to utilize additional intersectional approaches with gender and other age indicators to clearly understand whether gender, age, or some combination related to differences presented.

3.5. Limitations and future directions

The goal of the present work was to examine the consideration of utilizing additional or different age indicators when examining phenomena of interest; however, this conceptual example was not without limitations. Although we leveraged a large, national dataset, the number

of racial minorities in the subsamples drawn on were still small (n = 190), which impedes our ability to generalize to multiple ethnic and racial groups. Because of the limited sample size, we were unable to disentangle whether associations were similar or different across Black, Hispanic, Asian, or Indigenous peoples. Especially given differences in the significance of subjective age on inflammation by race, future work may need to directly test how subjective age and other aspects subjective age (e.g., awareness of aging related change), may inform biological health indicators for populations racialized as non-White. We were additionally limited in the ability to report chronological age to the day and month. The data acquired only reported birth year, and as such we may have lost minimal specificity in chronological age.

Moreover, we utilized cross-sectional data for these analyses. Although a strength of the present work was the ability to test multiple inflammatory biomarkers, having these measures at just one time point precludes our ability to examine whether there are changes in inflammation or subjective, chronological, or epigenetic age. It may be, for example, that the pace of aging (e.g., DPACE) or changes in subjective age are stronger predictors of how inflammation changes over time compared to chronological age. Future work will be needed to test how different age indicators may underscore longitudinal change. As inflammatory markers were determined at one time point, measurements may capture a participant's typical level of inflammation. For example, if a participant was recovering from a cold, or if it was allergy season, they may have evidenced elevated levels of inflammatory markers compared to their "normal". As such, one valuable direction for future research would be to replicate the current associations with multiple measurement points of inflammation. Additionally, given the cross-sectional nature of the current study, we are unable to disentangle the potential effects of life events or social factors that may inform these age indices. It will be paramount for future work to understand, for example, how events in the life course (e.g., work hazard, pathogen exposure, accidents) may inform subjective and biological age, which then inform health outcomes.

Future work may additionally test whether chronological age, subjective age, and epigenetic age uniquely inform other indicators of health and well-being. Inflammation was utilized for the current research because of the strong associations between chronological age and inflammation (Elisia et al., 2017; Rollandi et al., 2019; Flynn et al., 2019; Wolf et al., 2012). However, it will be pertinent for future research to test whether associations between chronological age and other indicators of well-being hold, after accounting for subjective age or epigenetic age, to better understand what information each age indicator provides unique of another. For example, one potential direction for future research could be to examine whether the presence of acute or chronic diseases further modifies associations between age indicators and inflammation, or to examine whether age indicators additionally predict acute or chronic disease. Finally, given the sensitivity of some analyses to smoking, there may be other health behaviors (e.g., diet, sleep) that may have links with subjective age and inflammation. Future directions may work to disentangle possible associations between smoking and other health behaviors with subjective age and inflammation.

4. Conclusion

Previous research has theorized that chronological age is a proxy for a host of phenomena, including emotional maturity and biological age. Given this, researchers often examine chronological age in relation to health outcomes, rather than examining associations between other indicators of age and health. The current study provides a better scientific understanding of the theoretical idea that chronological age provides important information over and above two example indicators of subjective and biological age using a well-known outcome associated with age: inflammation. Notably, this is a preliminary investigation in understanding *what* chronological age may represent and whether chronological age is a stand-alone phenomenon, or a proxy for other aging indicators or experiences. The current study findings are useful in providing an example of the unique information that chronological age provides, over and above other age indicators for inflammation. It will be crucial for future work to examine associations between previous experiences (e.g., life adversity), and other age indicators (e.g., awareness of age-related changes [AARC], mitochondrial DNA that may represent social and biological age, respectively) and inflammation, and to test these associations with other outcomes.

Author Note: Aarti C. Bhat acknowledges training on epigenetic processes for social scientists funded by the National Institute on Aging (NIA, R25AG053227). Aarti C. Bhat and Dakota D. Witzel were supported in part by the National Institute on Aging Grant T32 AG049676 to The Pennsylvania State University.

CRediT authorship contribution statement

Dakota D. Witzel: Writing – review & editing, Writing – original draft, Visualization, Conceptualization, Software, Methodology, Investigation, Funding acquisition. **Aarti C. Bhat:** Writing – review & editing, Writing – original draft, Conceptualization. **Jennifer E. Graham-Engeland:** Writing – review & editing, Writing – original draft. **David M. Almeida:** Writing – review & editing, Writing – original draft, Data curation.

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.

Acknowledgements

Dakota D. Witzel is supported, in part, by HATCH funds from the USDA National Institute of Food and Agriculture and the National Institute on Aging Grant 1R03AAG088758-01.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.03.018.

Data availability

Data is publicly available and is noted in manuscript.

References

Alvidrez, J., Greenwood, G.L., Johnson, T.L., Parker, K.L., 2021. Intersectionality in public health research: A view from the National Institutes of Health. American Journal of Public Health 111 (1), 95–97. https://doi.org/10.2105/AJPH.2020.305986.

Aristizabal, M.J., Anreiter, I., Halldorsdottir, T., Odgers, C.L., Mcdade, T.W., Goldenberg, A., Mostafavi, S., Kobor, M.S., Binder, E.B., O'Donnell, K.J., 2020. Biological embedding of experience: A primer on epigenetics. *Proc. Natl. Acad. Sci.* 117 (38), 23261–23269. https://doi.org/10.1073/pnas.1820838116.

Baker, T.A., Gamaldo, A.A., 2022. More than just race: Redefining the impact of sociocultural and biologic determinants on aging and well-being of older diverse populations. J. Gerontol.: Series B 77 (11), 1959–1963. https://doi.org/10.1093/geronb/gbac134.

Bauer, G.R., 2014. Incorporating intersectionality theory into population health research methodology: Challenges and the potential to advance health equity. Social Science & Medicine 110, 10–17. https://doi.org/10.1016/j.socscimed.2014.03.022.

Belsky, D.W., Avshalom, C., Corcoran, D.L., Sugden, K., Poulton, R., Arsenault, L., Baccarelli, A., Charmarti, K., Gau, X., Hannon, E., Harrington, H.L., Houts, R., Kothari, M., Kwon, D., Mill, J., Schwartz, J., Vokonas, R., Wang, C., Williams, B.S., Moffit, T.E., 2022. DunedinPACE, a DNA methylation biomarker of the pace of aging. Epidemiology and Global Health. Genetics, and Genomics 1-26. https://doi.org/10.7554/eLife.73420.

Belsky, D.W., Caspi, A., Arseneault, L., Baccarelli, A., Corcoran, D.L., Gao, X., Hannon, E., Harrington, H.L., Rasmussen, L.J.H., Houts, R., Huffman, K., Kraus, W.E., Kwon, D.,

Mill, J., Pieper, C.F., Prinz, J.A., Poulton, R., Sxhwartz, J., Sugden, K., Vokonas, P., Williams, B.S., Moffitt, T.E., 2020. Quantification of the pace of biological aging in humans through a blood test, the Dunedinpoam DNA methylation algorithm. *Elife* 9, 1–25. https://doi.org/10.7554/eLife.54870.

Black, P.H., 2002. Stress and the inflammatory response: A review of neurogenic inflammation. *Brain Behav. Immun.* 16 (6), 622–653. https://doi.org/10.1016/S0889-1591(02)00021-1.

Charles, S.T., 2010. Strength and vulnerability integration: a model of emotional well-being across adulthood. *Psychol. Bull.* 136 (6), 1068–1091. https://doi.org/10.1037/a0021232.

Chung, H.Y., Kim, D.H., Lee, E.K., Chung, K.W., Chung, S., Lee, B., Seo, A.Y., Chung, J.H., Jung, Y.S., Im, E., Lee, J., Deuk Kim, N., Ja Choi, Y., Soon Im, D., Yu, B.P., 2019. Redefining chronic inflammation in aging and age-related diseases: Proposal of the senoinflammation concept. *Aging Dis.* 10 (2), 367–382. https://doi.org/10.14336/AD.2018.0324.

Crenshaw, K., 2013. Demarginalizing the intersection of race and sex: A black feminist critique of antidiscrimination doctrine, feminist theory, and antiracist politics. In: Feminist Legal Theories. Routledge, pp. 23–51.

Cribb, L., Hodge, A.M., Yu, C., Li, S.X., English, D.R., Makalic, E., Southey, M.C., Milne, R.L., Giles, G.G., Dugué, P.A., 2022. Inflammation and epigenetic aging are largely independent markers of biological aging and mortality. *J.Gerontol. Series A* 77 (12), 2378–2386. https://doi.org/10.1093/gerona/glac147.

Diehl, M., Wahl, H.W., Brothers, A., Miche, M., 2015. Subjective aging and awareness of aging: Toward a new understanding of the aging self. In: Diehl, M., Wahl, H. (Eds.), *Annual Review of Gerontology and Geriatrics*. Springer Publishing, pp. 1–35.

Elisia, I., Lam, V., Hofs, E., Li, M.Y., Hay, M., Cho, B., Brooks-Wilson, A., Rosin, M., Bu, L., Jia, W., Krystal, G., 2017. Effect of age on chronic inflammation and responsiveness to bacterial and viral challenges. *PLoS One* 12 (11), 1–22. https://doi.org/10.1371/journal.pone.0188881.

Etherington, C., 2015. Race, gender, and the resources that matter: An investigation of intersectionality and health. *Women Health* 55 (7), 754–777. https://doi.org/10.1080/03630242.2015.1050544.

Ferguson, J.F., Patel, P.N., Shah, R.Y., Mulvey, C.K., Gadi, R., Nijjar, P.S., Usman, H.M., Mehta, N.N., Shah, R., Master, S.R., Propert, K.J., Reilly, M.M., 2013. Race and gender variation in response to evoked inflammation. Journal of Translational Medicine 11 (63), 1–9. https://doi.org/10.1186/1479-5876-11-63.

Flynn, M.G., Markofski, M.M., Carrillo, A.E., 2019. Elevated inflammatory status and increased risk of chronic disease in chronological aging: Inflamm-aging or inflamm-inactivity? *Aging Dis.* 10 (1), 147–156. https://doi.org/10.14336/AD.2018.0326.

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. https://doi.org/10.1038/s41574-018-0059-4.

Fransquet, P.D., Wrigglesworth, J., Woods, R.L., Ernst, M.E., Ryan, J., 2019. The epigenetic clock as a predictor of disease and mortality risk: A systematic review and meta-analysis. *Clin. Epigenetics* 11, 1–17. https://doi.org/10.1186/s13148-019-0656-7.

Graham, J.E., Christian, L.M., Kiecolt-Glaser, J.K., 2006. Stress, age, and immune function: Toward a lifespan approach. *J. Behav. Med.* 29, 389–400. https://doi.org/10.1007/s10865-006-9057-4.

Gubbels Bupp, M.R., 2015. Sex, the aging immune system, and chronic disease. *Cell. Immunol.* 294 (2), 102–110. https://doi.org/10.1016/j.cellimm.2015.02.002.

Hartanto, A., Majeed, N.M., Ng, W.Q., Chai, C.K.N., Lua, V.Y.Q., 2021. Subjective age and inflammation risk in midlife adults: Findings from the Midlife in the United States (MIDUS) studies. *Comprehensive Psychoneuroendocrinology* 7 (100072), 1–7. https://doi.org/10.1016/j.cpnec.2021.100072.

Henderson, K.V., Goldsmith, R.E., Flynnm, L.R., 1995. Demographic characteristics of subjective age. *J. Soc. Psychol.* 135 (4), 447–457. https://doi.org/10.1080/00224545.1995.9712214.

Hill, C.V., Pérez-Stable, E.J., Anderson, N.A., Bernard, M.A., 2015. The National Institute on aging health disparities research framework. *Ethn. Dis.* 25 (3), 245–254. https://doi.org/10.18865/ed.25.3.245.

Hooker, K., Mejía, S.T., Phibbs, S., Tan, E.J., Stevens, J., 2019. Effects of age discrimination on self-perceptions of aging and cancer risk behaviors. *Gerontologist* 59 (Supplement_1), S28–S37. https://doi.org/10.1093/geront/gny183.

Horvath, S., Raj, K., 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* 19 (1), 371–384. https://doi.org/10.1038/s41576-018-0004-3.

Irvin, M.R., Aslibekyan, S., Do, A., Zhi, D., Hidalgo, B., Claas, S.A., Srinivasasainagendra, V., Horvath, S., Tiwari, H.K., Absher, D.M., Arnett, D.K., 2018. Metabolic and inflammatory biomarkers are associated with epigenetic aging acceleration estimates in the GOLDN study. *Clin. Epigenetics* 10, 1–9. https://doi.org/10.1186/s13148-018-0481-4.

Kirkpatrick Johnson, M., Allen Berg, J., Sirotzki, T., 2007. Differentiation in self-perceived adulthood: Extending the confluence model of subjective age identity. *Soc. Psychol. Q.* 70 (3), 243–261. https://doi.org/10.1177/019027250707000030.

Kiecolt-Glaser, J.K., Preacher, K.J., MacCallum, R.C., Atkinson, C., Malarkey, W.B., Glaser, R., 2003. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc. Natl. Acad. Sci.* 100 (15), 9090–9095. https://doi.org/10.1073/pnas.1531903100.

Kishimoto, T., 2006. Interleukin-6: discovery of a pleiotropic cytokine. *Arthritis Res. Ther.* 8, 1–6. https://doi.org/10.1186/ar1916.

Knight, E.L., Majd, M., Graham-Engeland, J.E., Smyth, J.M., Sliwinski, M.J., Engelenad, C.G., 2022. Depressive symptoms and other negative psychological states relate to ex vivo inflammatory responses differently for men and women: Cross-sectional and longitudinal evidence. *Physiological Behavior* 244, 113656–113678. https://doi.org/10.1016/j.physbeh.2021.113656.

Levine, M.E., Lu, A.T., Quach, A., Chen, B.H., Assimes, T.L., Bandinelli, S., Hou, L., Baccarelli, A.A., Stewart, J.D., Li, Y., Whitsel, E.A., Wilson, J.G., Reiner, A.P., Aviv, A., Lohman, K., Liu, Y., Ferrucci, L., Horvath, S., 2018. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)* 10 (4), 573–591. https://doi.org/10.18632/aging.101414.

Levy, B.R., Hausdorff, J.M., Hencke, R., Wei, J.Y., 2000. Reducing cardiovascular stress with positive self-stereotypes of aging. *J. Gerontol. Series b: Psych. Sci. Soc. Sci.* 55 (4), 205–213. https://doi.org/10.1093/geronb/55.4.P205.

Levy, B.R., Slade, M.D., Chang, E., Kannoth, S., Wang, S., 2020. Ageism amplifies cost and prevalence of health conditions. The Gerontologist 60 (1), 174–181. https://doi.org/10.1093/geront/gny131.

Levy, B.R., Zonderman, A.B., Slade, M.D., Ferrucci, L., 2009. Age stereotypes held earlier in life predict cardiovascular events in later life. *Psychol. Sci.* 20 (3), 296–298. https://doi.org/10.1111/j.1467-9280.2009.02298.x.

Levy, B.R., Moffat, S., Resnick, S.M., Slade, M.D., Ferrucci, L., 2016. Buffer against cumulative stress: Positive age self-stereotypes predict lower cortisol over 30 years. GeroPsych 29 (3), 141–146. https://doi.org/10.1024/1662-9647/a000149.

Marioni, R.E., Shah, S., McRae, A.F., Chen, B.H., Colicino, E., Harris, S.E., Deary, I.J., 2015. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.* 16, 1–12. https://doi.org/10.1186/s13059-015-0584-6.

Martinez de Toda, I., Gonzalez-Sanchez, M., Diaz-Del Cerro, E., Valera, G., Carracedo, J., Guerra-Perez, N., 2023. Sex differences in markers of oxidation and inflammation. Implications for ageing. *Mech. Ageing Dev.* 211, 1–13. https://doi.org/10.1016/j.mad.2023.111797.

Mitchell, U.A., Aneshensel, C.S., 2016. Social inequities in inflammation: Age variations in older persons. Journal of Aging and Health 29 (5), 769–787. https://doi.org/10.1177/089826431665546.

Muscatell, K.A., Brosso, S.N., Humphreys, K.L., 2018. Socioeconomic status and inflammation: A meta-analysis. *Mol. Psychiatry* 25 (1), 2189–2199. https://doi.org/10.1038/s41380-018-0259-2.

North, M.S., Fiske, S.T., 2015. Modern attitudes toward older adults in the aging world: A cross-cultural meta-analysis. *Psychol. Bull.* 141 (5), 993–1021. https://doi.org/10.1037/a0039469.

Rollandi, G.A., Chiesa, A., Sacchi, N., Castagnetta, M., Puntoni, M., Amaro, A., Banelli, B., Pfeffer, U., 2019. Biological age versus chronological age in the prevention of age associated diseases. *OBM Geriatrics* 3 (2), 1–11. https://doi.org/10.21926/obm.geriatr.1902051.

Rubin, D.C., Berntsen, D., 2006. People over forty feel 20% younger than their age: Subjective age across the lifespan. *Psychon. Bull. Rev.* 13 (5), 776–780. https://doi.org/10.3758/BF03193996.

Salameh, Y., Bejaoui, Y., El Hajj, N., 2020. DNA methylation biomarkers in aging and age-related diseases. *Front. Genet.* 11 (480672), 1–11. https://doi.org/10.3389/fgene.2020.00171.

Sargent-Cox, K.A., Anstey, K.J., Luszcz, M.A., 2012. The relationship between change in self-perceptions of aging and physical functioning in older adults. *Psychol. Aging* 27 (3), 750–760. https://doi.org/10.1037/a0027578.

Settersten, R.A., Godlewski, B., 2015. Concepts and theories of age and aging. In: Bengston, V.L., Settersten, R. (Eds.), *Handbook of Theories of Aging*. Springer Publishing, pp. 9–28.

Stephan, Y., Sutin, A.R., Terracciano, A., 2015. Younger subjective age is associated with lower C-reactive protein among older adults. *Brain Behav. Immun.* 43, 33–36. https://doi.org/10.1016/j.bbi.2014.07.019.

Stephan, Y., Sutin, A.R., Luchetti, M., Terracciano, A., 2023. The prospective relationship between subjective aging and inflammation: Evidence from the health and retirement study. *Psychophysiology* 60 (2), 1–14. https://doi.org/10.1111/psyp.14177.

Xia, S., Zhang, X., Zheng, S., Khanabdali, R., Kalionis, B., Wu, J., Wan, W., Tai, X., 2016. An update on inflamm-aging: Mechanisms, prevention, and treatment. *J. Immunol. Res.* 2016 (8426874), 1–12. https://doi.org/10.1155/2016/8426874.

Wahl, H.-W., Drewelies, J., Duezel, S., Lachman, M.E., Smith, J., Eibich, P., Steinhagen-Thiessen, E., Demuth, I., Lindenberger, U., Wagner, G.G., Ram, N., Gerstorf, D., 2022. Subjective age and attitudes toward own aging across two decades of historical time. *Psychol. Aging* 37 (3), 413–429. https://doi.org/10.1037/pag0000649.

Wettstein, M., Spuling, S.M., Cengia, A., Nowossadeck, S., 2021. Feeling younger as a stress buffer: Subjective age moderates the effect of perceived stress on change in functional health. *Psychol. Aging* 36 (3), 322–337. https://doi.org/10.1037/pag0000608.

Witzel, D.D., Turner, S.G., Hooker, K., 2022. Self-perceptions of aging moderate associations of within-and between-persons perceived stress and physical health symptoms. *J.Gerontol.: Series B* 77 (4), 641–651. https://doi.org/10.1093/geronb/gbab228.

Wolf, J., Weinberger, B., Arnold, C.R., Maier, A.B., Westendorp, R.G., Grubeck-Loebenstein, B., 2012. The effect of chronological age on the inflammatory response of human fibroblasts. *Exp. Gerontol.* 47 (9), 749–753. https://doi.org/10.1016/j.exger.2012.07.001.

Yazdanparast, T., Hassanzadeh, H., Nasrollahi, S.A., Seyedmehdi, S.M., Jamaati, H., Naimian, A., Karimi, M., Roozbahani, R., Firooz, A., 2019. Cigarettes smoking and skin: A comparison study of the biophysical properties of skin in smokers and non-smokers. *Tanaffos* 18 (2), 163–168. PMID: 32440305.

Robles, P. L., Zawadzki, M. J., Graham-Engeland, J. E., Witzel, D. D., Dial, B., & Brondolo, E. (accepted, in press). How age and gender moderate relationships between negative interpersonal interactions and both blood pressure and mood in daily life.