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The role of health behaviors in links between stress and epigenetic aging☆

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ABSTRACT

High levels of stress are associated with accelerated epigenetic aging and health risks. However, less is known about the independent effects of different types of stress and whether these effects are explained by unhealthy behaviors. This study involved 1308 adults from the Midlife in the United States (MIDUS) study (53% female; 69% White, 22% Black; mean age: 54 years) to examine the independent effects of stressful life events and perceived stress on three DNA methylation-based measures of epigenetic aging (DNAm measures: GrimAge, DunedinPACE, PhenoAge). We also tested whether tobacco use, alcohol use, sleep problems, low diet quality, and low physical exercise mediate these effects. The results indicated that both perceived stress and stressful life events independently predict accelerated epigenetic aging indirectly through unhealthy behaviors across multiple DNAm measures of aging. Specifically, perceived stress predicted accelerated epigenetic aging through more sleep problems, lower diet quality, and lower physical exercise, whereas stressful life events predicted accelerated epigenetic aging through more tobacco and alcohol use as well as sleep problems. In addition, stressful life events directly predicted accelerated GrimAge above and beyond health behaviors. In summary, these findings underscore the critical role of health behaviors in linking different types of stress with biological aging and associated health issues.

1. Introduction

Exposure to stressful life events as well as subjective perceptions of stress are associated with greater risk for poor health, including the onset of chronic disease, such as coronary heart disease, diabetes, and cancer (Renzaho et al., 2014; Richardson et al., 2012). One mechanism through which stress can affect physical health is by increasing the rate at which an individual is aging biologically, which can be assessed using patterns of DNA methylation (DNAm; Horvath, 2013). Importantly, these patterns can be modified by environmental and behavioral factors including exposure to stress and health behaviors (Galkin et al., 2023). Therefore, DNA methylation measures of biological aging have played a new role to support the investigation of accelerated aging due to environmental changes (Rutledge et al., 2022). While previous research consistently links stressful life events to accelerated epigenetic aging across various age groups (Palma-Gudiel et al., 2020), findings on the association between perceived stress and epigenetic aging in adulthood have been less consistent (Bourassa et al., 2023; Vetter et al., 2022). The extent to which these two aspects of stress predict epigenetic aging through direct physiological mechanisms and indirectly through differences in health behaviors is not well understood. To address these questions, the present study examined the unique effects of stressful life events and perceived stress on epigenetic aging and examined whether poorer health behaviors (specifically substance use, sleep problems, lower diet quality, and lower physical exercise) explain these effects.

1.1. Stress and epigenetic aging

Over the past century (Selye, 1973), a wealth of empirical research has documented that exposure to chronic stress puts individuals at an elevated risk for physical health issues such as cardiovascular disease (Steptoe and Kivimäki, 2012), diabetes (Gallo et al., 2014), and various

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types of cancer (Dai et al., 2020). One physiological mechanism through which heightened stress can increase chronic disease risk is through accelerated biological aging (O'Connor et al., 2021). While gene transcription is regulated by a variety of epigenetic processes, such as histone modifications and noncoding RNA (Bure et al., 2022), one of the most widely investigated mechanisms is via DNA methylation, which involves the addition of a methyl group to mostly Cytosine-Phosphate-Guanine (CpG) sites that can modify gene transcription (Singal and Ginder, 1999). As such, changes in DNA methylation patterns can index an individual's rate of biological aging, which may respond to the physiological embedding of experienced stress at the cellular level (Palma-Gudiel et al., 2020). Based on the pattern of DNA methylation across different CpG sites, an individual's biological age can be reliably estimated (Simpson and Chandra, 2021). While earlier developed DNA methylation-based epigenetic clocks (i.e., Horvath pan-tissue clock, Hannum blood-tissue clock) were trained using chronological age (Hannum et al., 2013; Horvath, 2013), more recent second-generation clocks (i.e., PhenoAge and GrimAge) were trained and validated using more direct clinical markers of disease risk and also provide an estimate of physical health status (Levine et al., 2018; Lu et al., 2022). Building on these advances, the DunedinPACE is a third-generation DNA methylation-based measure of biological aging, developed from within-subject changes across several organ systems over two decades, and is strongly associated with chronic disease and morbidity risk (Belsky et al., 2022; Bourassa et al., 2024a). When accounting for differences in chronological age, these second- and third-generation DNA methylation-based measures of aging (DNAm measures of aging) provide an estimate of accelerated epigenetic aging and predict future risk for chronic disease over and above self-rated health status (Li et al., 2024). Thus, measures of accelerated epigenetic aging enable researchers to examine the effects of stress on chronic disease risk even before the onset of tangible health issues.

Previous research has linked accelerated epigenetic aging as measured via the GrimAge clock with overall stress based on a combination of stressful life events and perceived psychological stress (Harvanek et al., 2021). However, links between stress and epigenetic aging may vary across different aspects of stress (Bourassa et al., 2023), emphasizing the need to better understand the distinct roles of stressful life events and perceived stress in epigenetic aging and subsequent health outcomes. Stressful life events can be defined as the cumulative experience of adverse events throughout one's lifetime that are emotionally dreadful, shocking, or burdensome and have potentially lasting impacts such as experiences of violence, the loss of a loved one, exposure to a natural disasters or severe occupational/financial hardship (Turner and Wheaton, 1995). Prior research has been relatively consistent in linking stressful life events with accelerated epigenetic aging across various DNAm measures of aging (Lim et al., 2022; Palma-Gudiel et al., 2020). For example, experiencing more stressful life events predicted accelerated epigenetic aging in middle-aged adults on the GrimAge clock (Katrinli et al., 2020; Skinner et al., 2024) and a faster rate of aging on the DunedinPACE indicator (Bourassa et al., 2023; Bourassa et al., 2024b; Suglia et al., 2024). Previous studies have provided less consistent results for the PhenoAge clock. While some studies linked higher exposure to adverse childhood experiences with accelerated epigenetic aging in adulthood across all second and third generation DNAm measures of aging including PhenoAge (Kim et al., 2023), other studies found links between cumulative stressful life events beyond childhood and accelerated epigenetic aging only for GrimAge and DunedinPACE and not the PhenoAge clock (Gonggrijp et al., 2024; Skinner et al., 2024).

Chronic stress can also result from high levels of perceived stress, defined as the subjective experience of feeling overwhelmed, angry, nervous, or anxious (Cohen et al., 1983). Due to its focus on subjective experience, perceived stress is not exclusively based on external stress exposure but also captures individual differences in emotionally responding to stress. In comparison to the robust evidence linking stressful life events with accelerated epigenetic aging (Lim et al., 2022), prior research is more mixed on whether perceived stress has a unique effect on epigenetic aging. In a longitudinal study with middle-aged adults from New Zealand, higher perceived stress predicted accelerated epigenetic aging on the DunedinPACE indicator over and above stressful life events, adverse childhood experiences, and posttraumatic stress disorder (Bourassa et al., 2023). In contrast, two studies involving older adult samples found no links between perceived stress and epigenetic aging on any of the second- and third-generation DNAm measures of aging when adjusting for stressful life events (Nwanaji-Enwerem et al., 2023; Vetter et al., 2022). These two studies also adjusted for health behaviors, especially tobacco and alcohol consumption, when predicting epigenetic aging from perceived stress. Unhealthy behaviors including substance use may present behavioral mechanisms underlying effects of stress on accelerated epigenetic aging (Bourassa and Sbarra, 2024) and subsequent health issues (O'Connor et al., 2021). Indeed, a recent study showed that greater engagement in health-compromising behaviors partially mediated links between psychosocial stress and DNA methylation on stress-related CpG loci (Opsasnick et al., 2024). Thus, it is important to consider health behaviors as potential mechanisms when evaluating stressful life events and perceived stress as risk factors for accelerated epigenetic aging and associated health problems.

1.2. Health behaviors as mechanisms

One unhealthy behavior many individuals engage in when they are unable to cope with stress is alcohol use (Böke et al., 2019). Experiencing more stressful life events predicted increased alcohol use and dependence in young and middle-aged persons (Boden et al., 2014) as well as older adults (Sacco et al., 2014). Higher levels of perceived stress were associated with greater alcohol consumption in adults from Korea (Yoon et al., 2016) and the US (Grigsby et al., 2023). Similarly, higher tobacco use has been associated with both greater exposure to stressful life events (Feldner et al., 2007) as well as higher levels of perceived stress (Stubbs et al., 2017). Heavy alcohol and tobacco use in turn have been linked with accelerated epigenetic aging on multiple DNAm measures of aging (Lei et al., 2022).

Chronic stress may also interfere with one's ability to maintain healthy sleep practices. Indeed, both the experience of many stressful life events (Li et al., 2019) as well as high levels of perceived stress (Kashani et al., 2012) have been linked with poorer sleep quality in adulthood. Sleep quality plays a critical role in biological aging as well (Carroll and Prather, 2021) with insufficient and disrupted sleep predicting accelerated epigenetic aging in adulthood (Kusters et al., 2024). There is also robust empirical evidence for links between chronic stress exposure and poorer diet quality. According to a comprehensive meta-analysis, experiencing more stressful life events is associated with lower diet quality including both greater consumption of heavily processed and high-fat foods and lower consumption of healthy nutrient-rich foods such as fruits and vegetables (Hill et al., 2022). Similarly, heightened perceived stress has been linked with lower diet quality, specifically greater consumption of high-fat and sugary foods and soda beverages among college students (Errisuriz et al., 2016; Vidal et al., 2018). Keeping an unhealthy diet characterized by high amounts of heavily processed, sugary, and sodium-rich foods and low amounts of whole grains, fruits, and vegetables has been associated with accelerated epigenetic aging across multiple second-generation DNAm measures of aging (Kresovich et al., 2022).

Finally, stress exposure may interfere with one's physical activity. A recent study has reported bidirectional links between higher perceived stress and lower physical exercise in adulthood (Yoon et al., 2023). While the link between the exposure to stressful life events and subsequent physical exercise is incompletely understood, a recent study reported that experiencing more stressful life events increased the likelihood of adopting a health-damaging lifestyle, which included low physical exercise, substance use, sleep problems, and low diet quality (Siew et al., 2025). Low physical exercise has been linked with accelerated epigenetic aging on the GrimAge and PhenoAge DNAm measure of aging (Fox et al., 2023) as well as the DunedinPACE indicator (Ammous et al., 2025). Based on the research reviewed in this section, substance use, sleep problems, low diet quality, and low physical exercise may present key behavioral mechanisms through which exposure to stress predicts epigenetic aging.

1.3. The current study

This study examines the independent effects of perceived stress and stressful life events on epigenetic aging in middle aged and older adults using three second and third generation DNA methylation-based measures of epigenetic aging (i.e., GrimAge, DunedinPACE, PhenoAge). Second, we examine whether health behaviors (tobacco use, alcohol use, sleep problems, diet quality, and physical exercise) account for links between stress and epigenetic aging. A conceptual model is displayed in Fig. 1. We hypothesized that perceived stress and stressful life events predict accelerated epigenetic aging and that these effects are partially mediated by higher tobacco use, higher alcohol use, more sleep problems, lower diet quality, and lower physical exercise.

2. Methods

2.1. Procedures and participants

We analyzed data from 1308 participants in the Midlife in the United States (MIDUS) national longitudinal study. Only MIDUS participants with available data on DNA methylation-based epigenetic markers of aging were included in the final sample size of 1308, which included 510 participants from the MIDUS core sample and 798 participants from the MIDUS Refresher cohort. Survey data were derived from the "Project 1" and "Biomarker assessments" of the MIDUS-2 assessment in the core sample and of the MIDUS-Refresher 1 assessment in the refresher sample. The survey data were collected between 2004 and 2009 for the core sample and between 2011 and 2016 for the Refresher sample. Blood draws were collected as part of the biomarker assessments between 2004 and 2009 for the core sample and between 2012 and 2016 for the refresher sample. Participants had both a median and mean age of 54 years when the blood samples were collected as part of the Biomarker assessments. A majority of participants identified as White (69%) or Black (22%) and 53% reported their biological sex as female. The median annual household income was $75,000.

2.2. Measures

2.2.1. Accelerated epigenetic aging

Three DNA methylation-based epigenetic clocks from the second and third generations (i.e., GrimAge, PhenoAge, and DunedinPACE) were estimated from blood samples that were collected using BD vacutainer tubes with EDTA anticoagulant. Blood draws were frozen after collection and the DNA methylation analyses were performed in 2019 using the Illumina Methylation EPIC microarrays. The degrees of methylation on the specific CpG sites were quantified as beta values, which express the ratio of methylated fluorescent to overall intensity. The beta-values were normalized, and standard quality controls were conducted. Estimates for GrimAge version 2, DunedinPACE, and PhenoAge were computed in 2022 using published algorithms (Belsky et al., 2022; Levine et al., 2018; Lu et al., 2022). Unlike the first generation pan-tissue clock (DNAm Horvath; Horvath et al., 2013) and blood-tissue clock (DNAm Hannum; Hannum et al., 2013), these second- and third-generation clocks not only predict chronological age but were specifically developed to provide an estimate of chronic disease and mortality risk across the lifespan (Belsky et al., 2022; Palma-Gudiel et al., 2020). The raw scores for GrimAge version 2 (M = 62.68) and PhenoAge (M = 43.64) were similar to those obtained in the National Health and Nutrition Examination Survey (NHANES) for adults aged 54 years (Zou et al., 2025).

To adjust for differences in chronological age, the epigenetic age scores were regressed on participants' chronological age and the resulting residuals were saved and used as indicators of accelerated epigenetic aging. ANOVA tests indicated significant differences in the residual scores across the individual 96 sample well-plates that were used to store the sample. Therefore, the epigenetic scores were also regressed on well-plate prior to the analysis to adjust for differences in storage time. The same approach has been used in a prior study with MIDUS data (Freilich et al., 2024). DunedinPACE scores were standardized prior to the analyses to facilitate model convergence. For more information on the sample collection and estimation of the epigenetic age variables, please reference the data documentation on the MIDUS Portal (https://midus.colectica.org/).

2.2.2. Stressful life events

Stressful life events were assessed from participant self-reports on the MIDUS checklist for stressful life events (Turner and Wheaton, 1995), which includes stressful life events that occurred during childhood as well as adulthood and has been used in previous research with MIDUS data (Hansen et al., 2024). The checklist included 27 individual items covering a range of stressful life events including loss of family

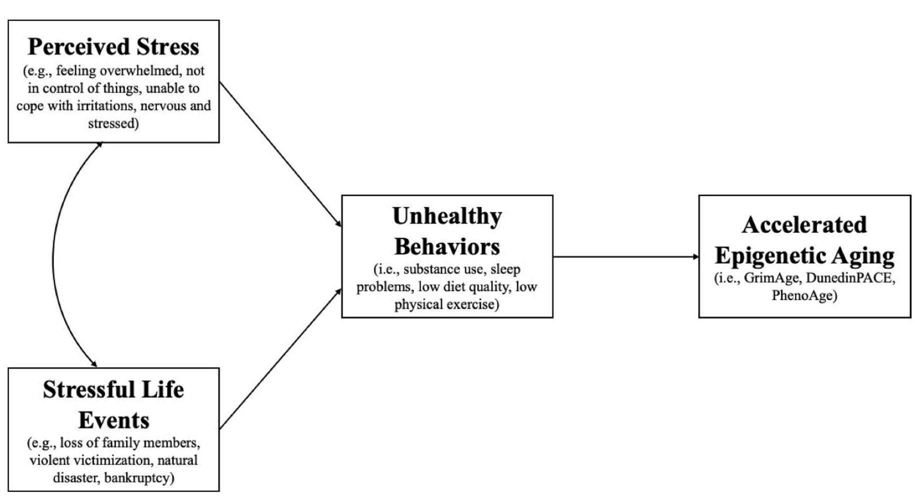

Fig. 1. Conceptual mediation model.

members, experiences of victimization, being affected by environmental disasters, major financial and occupational difficulties, divorce or separation, serious legal difficulties, parental drug issues during childhood, and serious issues at school during childhood. Participants indicated for each event whether they experienced it (1) or did not experience it (0). An overall index of stressful life events was computed by summing the responses to each event with higher scores indicating more stressful life events.

2.2.3. Perceived stress

Participants reported their perceived level of stress by responding to the 10-item Perceived Stress Scale (Cohen et al., 1983). The individual items covered a range of emotions including feeling overwhelmed, angry and nervous. Participants reported on a 5-point Likert-scale how often these occurred over the past month. Items were reverse coded as needed so that higher values indicate more perceived stress, and the individual items were then averaged to create an overall index indicating perceived stress (Cronbach's $\alpha = .87$). The mean and standard deviation of the perceived stress scale in the present sample ($M = 2.25$, $SD = 0.63$) was similar to those obtained in the initial validation study with US adults aged 45 to 54 ($M = 2.26$, $SD = 0.61$) and 55 to 64 ($M = 2.19$, $SD = 0.69$) (Cohen et al., 1983).

2.2.4. Tobacco use

Participants reported their tobacco use by responding to two items; how many years they smoked cigarettes as well as how many cigarettes they smoked on a typical day during that time. Responses to both items were standardized and then averaged to indicate the overall level of lifetime tobacco smoking.

2.2.5. Alcohol use

Participants reported their alcohol use over the past month by responding to two items during the Biomarker assessments of the MIDUS-2 and MIDUS-Refresher studies: First, they reported the frequency of their alcohol use by indicating on how many days they consumed alcohol over the past month using a six-point ordinal scale with response options ranging from '1 – Never' to '6 – Everyday'. Second, participants reported the average number of drinks they had on days they drank alcohol. Responses from both items were standardized and averaged to provide an estimate of overall quantity of alcohol use.

2.2.6. Sleep problems

Participants reported their sleep quality by responding to the 19 items of the Pittsburgh Sleep Quality Index (Buysse et al., 1989). The individual items map onto seven dimensions including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances range, use of sleep medication, and daytime dysfunction. Based on responses to the individual items, participants were scored for each component using a four-point scale with higher values indicating more sleep problems. Responses from the seven components were then summed to create an overall index of sleep problems on a continuous scale with higher values indicating more sleep problems (Chung, 2017).

2.2.7. Diet quality

Participants reported their dietary behavior by indicating how many sugared beverages, servings of fruits and vegetables, and servings of whole grain they consume on an average day as well as how often they consume fish, beef or high fat meat, and fast food in an average week. Participants responded to each item on a five-point scale with varying response options and higher values indicating more consumption of the food/drink item. After reverse coding reports for sugared beverages, beef or high fat meat, and fast food, the individual items were averaged to create an overall index with higher values indicating better diet quality (Levine et al., 2016).

2.2.8. Physical exercise

Participants reported on their physical exercise per week as part of the Biomarker Assessments of the MIDUS-2 and MIDUS-Refresher studies. Participants were first given the following definitions for three types of physical exercise. First, vigorous exercise was defined as activities that cause a rapid heartbeat, substantial sweat, and heavy breathing such as competitive sports, running, swimming, or lifting heavy objects. Second, moderate exercise was defined as activities that lead to a slight increase in heart rate and light sweat such as leisurely sports, brisk walking, and light yard work. Third, light exercise was defined as activities that require little physical effort such as light housekeeping and easy walking. Participants then reported whether they performed regular physical activity of any type and if yes, whether they rate this activity as light, moderate, or vigorous, how often they perform each activity per week. From these answers, the total number of Metabolic Equivalent of Task (MET) minutes per week were computed. Specifically, minutes per week were multiplied by intensity level (1.1 for light, 3.0 for moderate, 6.0 for vigorous) and summed across each activity reported following the same method used in prior MIDUS studies (Strohacker et al., 2013). Since the United States Department of Health and Human Services (USDHHS) utilizes intervals of 500 MET minutes intervals to classify weekly exercise as below recommended (<500 MET minutes), recommended (500-1000 MET minutes) and with additional benefits (>1000) (Strohacker et al., 2013), total values were truncated to a maximum of 1500 MET minutes per week.

2.2.9. Covariates

During the Project 1 assessments of the MIDUS-2 and MIDUS Refresher samples, participants reported their biological sex, which was coded as a dichotomous variable (0 = male, 1 = female) as well as their annual household income in dollars including wage, pension, social security, and other sources. 18 high outliers were truncated to 300,000 dollars per year (3.5 standard deviations above the mean) and the variable was standardized prior to the analyses. Participants also reported on their race and ethnicity. Since most participants in the sample identified as Non-Hispanic White, race/ethnicity was coded as a dichotomous variable (Non-Hispanic White vs. other race/ethnicity). BMI scores were calculated from participants' self-reported height and weight measures during the Project 1 assessments. Finally, a dichotomous variable indicating which sample participants were part of (Core = 0, Refresher = 1) was included to control for potential cohort effects.

2.3. Data analyses

Prior to the main analysis, the normality assumption was tested for all endogenous variables (i.e., DNAm measures of accelerated aging and health behaviors) by examining skewness and kurtosis statistics. As part of preliminary analyses, the amount of missing data was determined, and independent samples t-tests and chi-square tests of independence were used to examine whether participants with missing data differed from participants with complete data on any variables included in the analyses. Additionally, descriptive statistics and bivariate correlations were examined. All preliminary analyses were performed in SPSS version 29.

As part of the main analyses, multiple regressions were conducted to examine the independent main effects of perceived stress and stressful life events on the three DNAm measures of aging adjusting for the covariates (i.e., biological sex, household income, BMI, and MIDUS cohort). The main analyses then constructed three mediation path models with perceived stress and stressful life events predicting each of the five health behaviors (i.e., tobacco use, alcohol use, sleep problems, diet quality, and physical exercise) and the five health behaviors predicting an indicator of accelerated epigenetic aging (i.e., GrimAge in Model 1, DunedinPACE in Model 2, and PhenoAge in Model 3). Each model also included direct paths from perceived stress and stressful life events to epigenetic aging. The models also included covariances between the two stress variables and covariances between the five health behaviors. A conceptual diagram of the mediation model is depicted in Fig. 1. Each individual path adjusted for the covariates (i.e., biological sex, household income, race/ethnicity, BMI, and cohort). Since skewness and kurtosis statistics were within the acceptable range for all endogenous variables, regular maximum likelihood estimation was used in all models. The indirect effects of perceived stress and stressful life events on epigenetic aging through health behaviors were tested with bias-corrected bootstrapping using 5000 bootstrap samples and significance was determined by whether the 95% confidence interval included zero (Preacher and Hayes, 2008). Missing data were handled with full information maximum likelihood estimation, which preserves the overall sample size and minimizes bias when data are missing at random (Cham et al., 2017). All main analyses were performed in R using the Lavaan package for structural equation modeling (Rosseel, 2012).

Three sets of sensitivity analyses were conducted to better understand the robustness of the results if alternative analytic decisions were made. The first set of sensitivity analyses re-examined the mediation models adding chronological age as a covariate to each path. While raw DNAm epigenetic age scores were regressed on chronological age prior to the main analyses, some methodological studies recommend also including age as a covariate on the regression paths in addition to age-adjusting the epigenetic scores prior to the analyses (Krieger et al., 2023). A second set of sensitivity analyses examined separate mediation models for perceived stress and stressful life events to better understand their independent effects on health behaviors and epigenetic aging. The third set of sensitivity analyses re-examined the mediation models with a truncated version of stressful life events. Since over 95% of participants reported eight or fewer stressful life events, reports of more than eight events were truncated to “8” so that all data points were within three standard deviations from the mean.

2.4. Transparency and openness

Some parts of the present study were preregistered. The statistical analyses for the mediation models with perceived stress and stressful life events predicting epigenetic aging through four of the health behaviors (i.e., tobacco use, alcohol use, sleep problems, and diet quality) were pre-registered in the Open Science Framework (OSF). Physical exercise was added as another mediator at the revision stage. The sensitivity analyses were not pre-registered and conducted after the main analyses to examine the robustness of the results. The MIDUS data are available from the Inter-university Consortium for Political and Social Research (https://www.icpsr.umich.edu/icpsrweb/ICPSR/studies/2760; https://www.icpsr.umich.edu/icpsrweb/ICPSR/studies/36532). The dataset and analysis code for the main analyses are available in the Open Science Framework (https://osf.io/p93ew/?view_only=4c2513b208b94a62a37375672ed02ac9).

3. Results

3.1. Preliminary analyses

Owing to missing data on one or more variables, 29% of the participants had incomplete data and 7% of total data points were missing. Compared with participants with complete data, participants with missing data reported on average more sleep problems (7.46 vs. 5.89, $t_{(1280)} = 6.76$, $p < .001$), lower diet quality (3.10 vs. 3.28, $t_{(1301)} = -5.68$, $p < .001$), higher accelerated epigenetic aging on the GrimAge (1.94 vs. −0.78, $t_{(1305)} = 7.99$, $p < .001$) and DunedinPACE indicator (0.06 vs. −0.02, $t_{(1306)} = 10.18$, $p < .001$), more perceived stress (2.40 vs. 2.20, $t_{(1287)} = 4.84$, $p < .001$), more tobacco use (0.12 vs. −0.05, $t_{(1272)} = 2.83$, $p = .005$), and lower physical exercise (638.97 vs. 836.40, $t_{(1302)} = -5.23$, $p < .001$). Moreover, participants in the core sample were more likely to have missing data compared with participants in the refresher cohort ($\chi^2_{(1)} = 19.46$, $p < .001$). Participants with missing data did not differ from participants with complete data in PhenoAge acceleration, alcohol use, stressful life events, household income, BMI, biological sex and race/ethnicity.

Descriptive statistics and results from the bivariate correlation analyses are shown in Table 1.

3.2. Main analyses

Results from the multiple regression analyses are shown in Table 2. Higher perceived stress and more stressful life events independently predicted accelerated GrimAge even when adjusting for the covariates (i.e., biological sex, race, income, BMI, cohort). However, only more stressful life events predicted accelerated DunedinPACE and PhenoAge. The effect of perceived stress on accelerated DunedinPACE did not reach statistical significance at the 0.05 level ($p = .052$) and perceived stress had no effect on the PhenoAge DNAm measure of aging.

Results from the first mediation path model predicting epigenetic aging on the GrimAge clock are shown in Fig. 2. Higher perceived stress predicted more sleep problems, lower diet quality and lower physical exercise, but perceived stress had no effect on alcohol or tobacco use. More stressful life events predicted more tobacco use, more alcohol use, and more sleep problems, but stressful life events had no effect on diet quality or physical exercise. All health behaviors predicted GrimAge acceleration in expected directions with higher alcohol use, higher tobacco use, more sleep problems, lower diet quality and lower physical exercise predicting accelerated GrimAge. While perceived stress had no direct effect on GrimAge acceleration, the results showed mediation effects of higher perceived stress on accelerated GrimAge through more sleep problems (B = 0.31, $CI_{95\%}$ [0.180, 0.465]) as well as lower diet quality (B = 0.15, $CI_{95\%}$ [0.077, 0.241]) and lower physical exercise (B = 0.03, $CI_{95\%}$ [0.004, 0.081]). In contrast, more stressful life events had an independent direct effect on accelerated GrimAge even when adjusting for health behaviors, perceived stress, and the covariates. Additionally, the effect of more stressful life events on accelerated GrimAge was partially mediated by higher tobacco use (B = 0.23, $CI_{95\%}$ [0.168, 0.300]), higher alcohol use (B = 0.025, $CI_{95\%}$ [0.009, 0.050]), and more sleep problems (B = 0.05, $CI_{95\%}$ [0.030, 0.087]). The model explained 43% of variance in GrimAge acceleration.

Results from the second mediation path model predicting epigenetic aging on the DunedinPACE indicator are shown in Fig. 3. As in the first model, higher perceived stress predicted more sleep problems, lower diet quality, and lower physical exercise, whereas more stressful life events predicted higher tobacco use, higher alcohol use, and more sleep problems. Higher tobacco use, more sleep problems, lower diet quality, and lower physical exercise predicted accelerated epigenetic aging on the DunedinPACE indicator. Alcohol use had no effect on DunedinPACE acceleration. Neither perceived stress nor stressful life events had direct effects on the DunedinPACE indicator of epigenetic aging. However, the results showed mediation effects of higher perceived stress on accelerated DunedinPACE through more sleep problems (B = 0.03, $CI_{95\%}$ [0.009, 0.060]), lower diet quality (B = 0.02, $CI_{95\%}$ [0.008, 0.040]), and lower physical exercise (B = 0.01, $CI_{95\%}$ [0.002, 0.021]). Finally, the results showed mediation effects of more stressful life events on accelerated DunedinPACE through higher tobacco use (B = 0.03, $CI_{95\%}$ [0.017, 0.034]) and more sleep problems (B = 0.01, $CI_{95\%}$ [0.002, 0.011]). The model explained 31% of variance in DunedinPACE acceleration.

Results from the third mediation path model predicting epigenetic aging on the PhenoAge clock are shown in Fig. 4. As in the previous models, perceived stress predicted more sleep problems, lower diet quality, and lower physical exercise, while experiencing more stressful life events predicted higher tobacco and alcohol use as well as more sleep problems. Higher tobacco use and more sleep problems predicted accelerated PhenoAge, whereas alcohol use, diet quality, and physical exercise did not predict PhenoAge acceleration. Neither perceived stress

Table 1
Descriptive statistics and correlations among the main variables of interest.

	M (SD)/%	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. Perceived Stress	2.25 (0.63)	1.00								
2. Stressful Life Events	3.31 (2.47)	0.16**	1.00							
3. Tobacco Use	0.00 (0.90)	0.05	0.27**	1.00						
4. Alcohol Use	0.00 (0.86)	−0.01	0.05	0.14**	1.00					
5. Sleep Problems	6.32 (3.52)	0.35**	0.25**	0.13**	0.02	1.00				
6. Diet Quality	3.23 (0.51)	−0.18**	−0.04	−0.10**	0.02	−0.17**	1.00			
7. Physical Exercise	780.4 (621.2)	−0.11**	−0.05	−0.09**	0.07*	−0.13**	0.18**	1.00		
8. Accel. GrimAge	0.00 (5.24)	0.14**	0.28**	0.55**	0.17**	0.27**	−0.23**	−0.14**	1.00	
9. Accel. DunedinPACE	0.00 (0.13)	0.13**	0.21**	0.34**	0.04	0.22**	−0.22**	−0.18**	0.75**	1.00
10. Accel. PhenoAge	0.00 (5.90)	0.03	0.11**	0.18**	0.07*	0.12**	−0.05	−0.07*	0.47**	0.44**

Note: Accel. – Accelerated.
*$p < .05$, **$p < .01$.

Table 2
Multiple regressions predicting accelerated epigenetic aging from perceived stress, stressful life events, and covariates.

Variable	GrimAge (T2) β (*p*)	DunedinPACE (T2) β (*p*)	PhenoAge (T2) β (*p*)
Step 1	$R^2 = 0.19$	$R^2 = 0.23$	$R^2 = 0.04$
Perceived Stress	**.07 (.007)**	0.05 (0.052)	0.00 (0.938)
Stressful Life Events	**.23 (< .001)**	**.12 (< .001)**	**.10 (.003)**
Female	**−.19 (< .001)**	**−.11 (< .001)**	−0.05 (0.136)
Non-Hispanic White	**−.10 (.001)**	**−.15 (< .001)**	−0.05 (0.134)
Income	**−.10 (.001)**	**−.12 (< .001)**	0.04 (0.198)
BMI	**.16 (< .001)**	**.33 (< .001)**	**.15 (< .001)**
Cohort	−0.05 (0.085)	**−.06 (.026)**	−0.03 (0.240)

Note: Epigenetic variables are adjusted for chronological age and well-plate. Standardized coefficients are shown. Significant effects (α = .05) in **bold** font.

nor stressful life events had direct effects on epigenetic aging on the PhenoAge clock. However, the results showed a mediation effect of higher perceived stress on accelerated PhenoAge through more sleep problems (B = 0.23, $CI_{95\%}$ [0.075, 0.399]). The results also showed mediation effects of more stressful life events on accelerated PhenoAge through higher tobacco use (B = 0.08, $CI_{95\%}$ [0.046, 0.126]) and more sleep problems (B = 0.039, $CI_{95\%}$ [0.011, 0.071]). The model explained 7% of variance in PhenoAge acceleration.

3.3. Sensitivity analyses

Results from the first set of sensitivity analyses, which included chronological age as an additional covariate on each regression path completely replicated the results from the main analyses. Model figures from these analyses are shown in the supplementary material (Figs. S1–S3). Results from the second set of sensitivity analyses, which examined perceived stress and stressful life events in separate mediation models also fully replicated the main results. Specifically, all previously identified effects of perceived stress and stressful life events on health behaviors and epigenetic outcomes replicated and there were no additional effects for either of the stress variables. Model figures from these analyses are shown in the supplementary material (Figs. S4–S9). Finally, we conducted a third set of sensitivity analyses using a truncated version of the stressful life events variable (i.e., truncated to eight or more). The models using this approach replicated all previously identified indirect and direct effects and no additional effects emerged in any models.

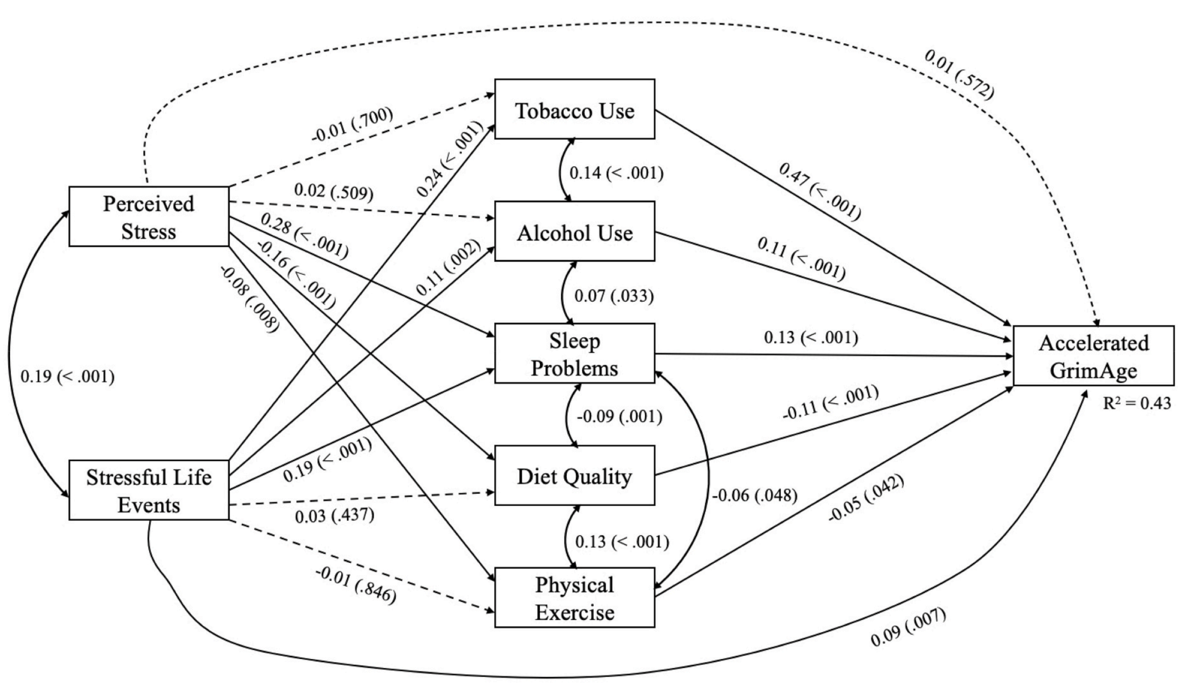

Fig. 2. Mediation path model predicting accelerated GrimAge
Note: Standardized path coefficients (and *p*-values) are shown. All paths adjusted for the covariates (i.e., biological sex, household income, race/ethnicity, BMI, and cohort). Only significant covariances are shown. Non-significant paths are depicted by dashed lines.

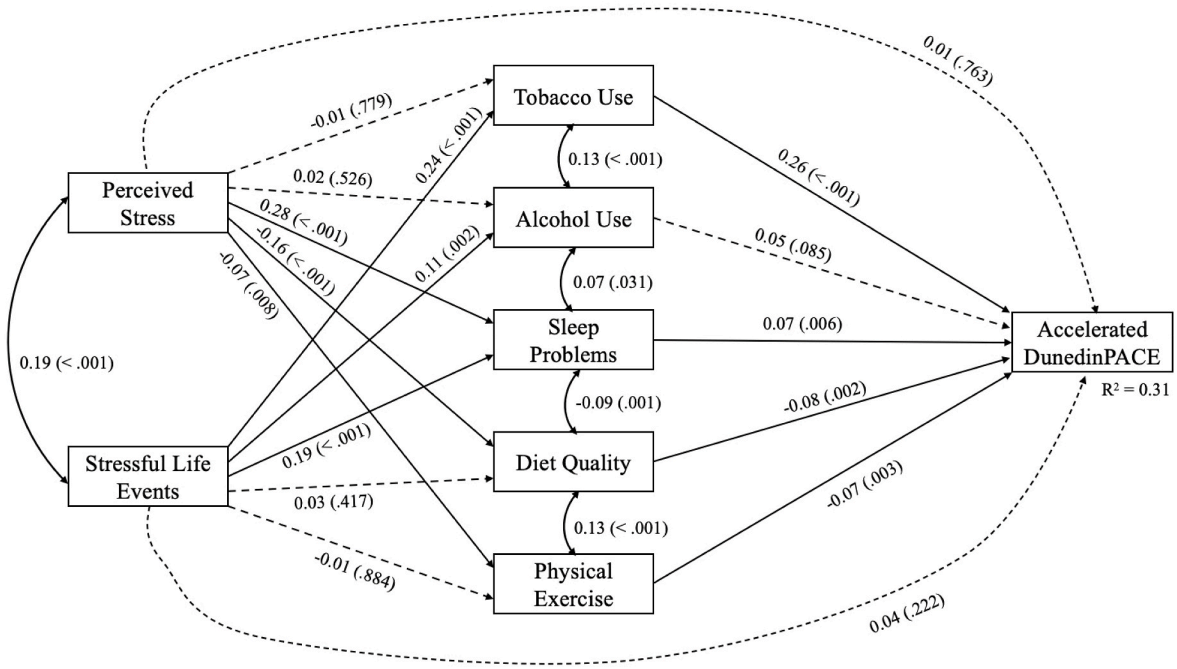

Fig. 3. Mediation path model predicting accelerated DunedinPACE
Note: Standardized path coefficients (and *p*-values) are shown. All paths adjusted for the covariates (i.e., biological sex, household income, race/ethnicity, BMI, and cohort). Only significant covariances are shown. Non-significant paths are depicted by dashed lines.

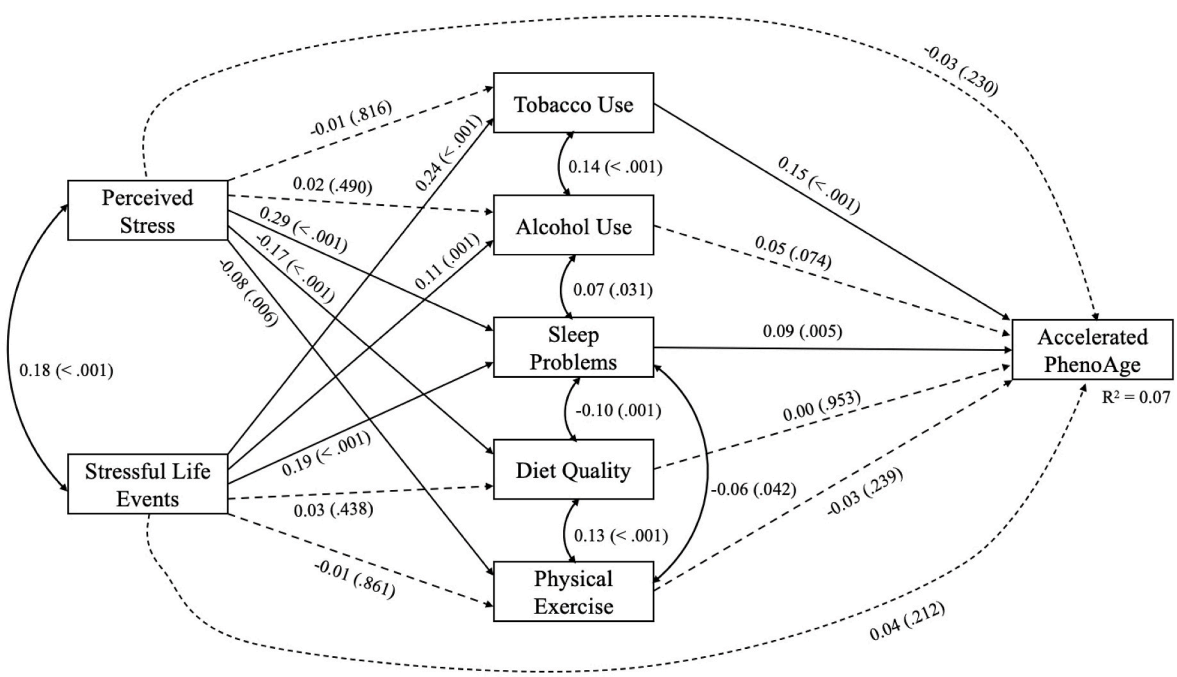

Fig. 4. Mediation path model predicting accelerated PhenoAge
Note: Standardized path coefficients (and *p*-values) are shown. All paths adjusted for the covariates (i.e., biological sex, household income, race/ethnicity, BMI, and cohort). Only significant covariances are shown. Non-significant paths are depicted by dashed lines.

Model figures from these analyses are shown in the supplementary material (Figs. S10–S12).

4. Discussion

This study used data from 1308 adults enrolled in the MIDUS study to examine the independent effects of stressful life events and perceived stress on epigenetic aging, whether unhealthy behaviors are mechanisms of these effects. The results revealed that both stressful life events and perceived stress predicted accelerated epigenetic aging indirectly through unhealthy behaviors. Specifically, stressful life events predicted greater tobacco use, alcohol use, and sleep problems, which in turn predicted accelerated epigenetic aging. Perceived stress predicted more sleep problems, lower diet quality, and lower physical exercise, which in turn predicted accelerated epigenetic aging. Stressful life events remained associated with accelerated GrimAge when accounting for health behaviors, whereas health behaviors fully accounted for the associations between perceived stress and every DNAm measure of aging.

4.1. Types of stress and epigenetic aging

The present findings align with prior research showing that experiencing high levels of stress is associated with accelerated epigenetic aging (Harvanek et al., 2021). Results from the unadjusted correlation analyses showed that stressful life events and perceived stress were only modestly associated ($r = 0.16$) supporting their treatment as distinct constructs. Multiple regression analyses suggested that both stressful life events and perceived stress were independently associated with accelerated epigenetic aging on the GrimAge and marginally the DunedinPACE measure of aging. Experiencing many stressful life events was also associated with accelerated PhenoAge. Accelerated epigenetic aging across these second- and third-generation DNAm measures indicates an elevated risk for chronic disease (Li et al., 2024). Thus, these findings align with previous evidence that individuals who experience many stressful life events or perceive high levels of stress are at greater risk for chronic disease later in life (Renzaho et al., 2014; Richardson et al., 2012) and that accelerated aging may be a biological mechanism underlying these links (Palma-Gudiel et al., 2020). Noteworthy, the effect size for stressful life events was more than twice as large as that of perceived stress in the DunedinPACE model and more than three times as large in the GrimAge model. These findings in the present sample of middle-aged adults (mean age = 54) contrast with a previous study on young to middle-aged adults that found perceived stress to have stronger associations with accelerated epigenetic aging (Bourassa et al., 2023). Prior research with older adults (mean age: 73 years) found no association of perceived stress and accelerated epigenetic aging (Nwanaji-Enwerem et al., 2023). Taken together, these studies may suggest that the effect of perceived stress on epigenetic aging could decline with increasing chronological age.

4.2. Stress, health behaviors and epigenetic aging

The central finding of the present study is that unhealthy behaviors serve as critical mechanisms linking stress to accelerated epigenetic aging. The particular health behaviors underlying links between stress and epigenetic aging differed for stressful life events and perceived stress. Consistent with the hypotheses, the results revealed indirect effects of stressful life events on accelerated epigenetic aging through higher tobacco use across all DNAm measures of aging, whereas indirect effects through higher alcohol were limited to accelerated GrimAge. The finding that individuals who experience more stressful life events are at a greater risk for tobacco and alcohol use is consistent with previous evidence (Boden et al., 2014; Feldner et al., 2007) and may be explained by heightened sensitivity to dopamine and vulnerability to addiction (Sinha, 2008). Tobacco use in turn predicted accelerated epigenetic aging across all three indicators, which is consistent with prior research linking substance use to epigenetic aging across GrimAge, DunedinPACE, and PhenoAge DNAm measures of biological aging (Klopack and Crimmins, 2024). Importantly, when accounting for stress and the other health behaviors, alcohol use predicted accelerated aging only on the GrimAge DNAm measure of aging. Similar to this present finding, a recent study reported links between heavy drinking and accelerated epigenetic aging only for the GrimAge but not the DunedinPACE and PhenoAge DNAm measures of aging (Klopack et al., 2025). This may suggest that tobacco use is more robustly associated with accelerated epigenetic aging than alcohol use.

Contrary to expectations, neither tobacco use nor alcohol use mediated links between perceived stress and epigenetic aging. Specifically, perceived stress was not associated with either tobacco or alcohol use, which is inconsistent with previous studies (Grigsby et al., 2023; Stubbs et al., 2017). A possible explanation may be that the measure utilized for perceived stress focused specifically on the past month. Longitudinal studies have shown that trajectories of problematic substance use often emerge during the adolescent years (Chen and Jacobson, 2012) and due to their addictive characteristics increase the risk for persistent substance use later in life (Meier et al., 2016). If problematic substance use behaviors began earlier in life, past stressful events may exert a stronger influence on adult substance use than current subjective perceptions of stress measured over the past month.

In line with the hypotheses, the results revealed independent indirect effects of both stressful life events and perceived stress on accelerated epigenetic aging through more sleep problems. Experiencing many stressful life events may interfere with sleep quality through cognitive and emotional arousal from reappraising past events before falling asleep (Li et al., 2019). Similarly, the observed effect of perceived stress on lower sleep quality aligns with prior research (Kashani et al., 2012) and has been explained by greater physiological arousal before and during sleep (Hall et al., 2007). Experiencing more sleep problems in turn predicted accelerated epigenetic aging, which is consistent with previous reports linking sleep to epigenetic aging (Kusters et al., 2024).

While diet quality did not mediate links between stressful life events and epigenetic aging, lower diet quality emerged as a key mechanism linking higher perceived stress with accelerated epigenetic aging for the GrimAge and DunedinPACE indicators. The present finding of perceived stress predicting lower diet quality extends prior research with college students (Errisuriz et al., 2016) by replicating this link in adults. Maintaining a high-quality diet requires both time and dedication (Traill et al., 2012) for which individuals who feel highly stressed and overwhelmed may have limited capacity. Contrary to the hypotheses, the results showed no links between stressful life events and lower diet quality, which is inconsistent with previous meta-analytic findings (Hill et al., 2022). This inconsistency may reflect age-related differences in how individuals respond to stress, with low diet quality potentially being a more salient coping mechanism in younger age groups (Tao et al., 2024).

Mirroring the results for diet quality, physical exercise mediated the link between perceived stress and accelerated epigenetic aging on the GrimAge and DunedinPACE DNAm measures of aging. These results complement prior research indicating a bidirectional relationship between higher perceived stress and lower physical exercise (Yoon et al., 2023) and a link between lower physical exercise and accelerated epigenetic aging (Fox et al., 2023). Contrary to the hypotheses and paralleling the findings for diet quality, stressful life events had no effect on physical exercise patterns. In sum, these findings suggest that subjective perceptions of ongoing stress may interfere more with middle-aged adults diet exercise habits than specific stressful experiences from the past.

In addition to these indirect effects, the results showed an independent direct effect of stressful life events on accelerated GrimAge over and above perceived stress, health behaviors, and relevant covariates. Links between stressful life events and accelerated aging may therefore not be fully explained by behavioral mechanisms but also occur through direct physiological mechanisms. Previous research identified increased cortisol reactivity as one mechanism linking stressful life events with accelerated epigenetic aging (Steptoe et al., 2017). Another physiological mechanism may be elevated blood pressure levels, which have been associated with both stressful life events (Gasperin et al., 2009) and accelerated epigenetic aging (Xiao et al., 2022). Since perceived stress had no direct effect on any DNAm measures, the present results suggest that unhealthy behaviors in the form of sleep problems, lower diet quality, and lower physical exercise fully explain links between perceived stress and epigenetic aging.

4.3. Differences across DNAm measures of aging

While many of the observed effects replicated across the three DNAm measures of aging, individual paths showed some differences across the three epigenetic outcomes. Most notably, the direct effects of stressful life events and alcohol use were limited to the GrimAge clock, whereas the effect of diet quality and physical exercise on epigenetic aging was only evident for GrimAge and DunedinPACE. While the three DNAm measures of aging are conceptually similar in estimating biological aging and physical health, their associations with risk factors vary in magnitude (Yusipov et al., 2024). The GrimAge clock explicitly focuses on lifestyle factors, and includes an epigenetic measure of smoking in its algorithm (Lu et al., 2022), which may explain why links between substance use and epigenetic aging are strongest for the GrimAge clock. However, smoking is only one component of the composite, and GrimAge captures additional biomarkers that are relevant for health and longevity (Lu et al., 2019). Consistent with this, the correlation between tobacco use and accelerated GrimAge in the present sample was only moderate ($r = 0.55$) indicating that the two constructs are related but not redundant. Notably, all other health behaviors showed the hypothesized effects on accelerated GrimAge even after adjusting for tobacco use and tobacco use itself predicted accelerated epigenetic aging across all three DNAm measures. Thus, the link between tobacco use and accelerated epigenetic aging is not entirely due to conceptual overlap in the measurement of tobacco use and GrimAge. Including GrimAge while accounting for tobacco use is consistent with prior research examining links between stress and epigenetic aging (Vetter et al., 2022). In general, most observed effect sizes were similar for GrimAge and DunedinPACE and substantially weaker for PhenoAge. This may be due to PhenoAge being a less reliable marker for health status, and therefore less accurate in capturing stress effects on aging-related chronic disease risk, as compared to GrimAge and DunedinPACE (Bourassa et al., 2024; McCrory et al., 2021).

4.4. Implications

The present findings have both theoretical and practical implications. Theoretically, the present findings underscore the importance of examining different types of stress as predictors of epigenetic aging and associated health outcomes. Since tobacco use, alcohol use, sleep problems, diet quality, and physical exercise emerged as mechanisms, future research should continue examining health behaviors as mediators rather than mere covariates to comprehensively capture both direct and indirect effects of stress on aging and physical health. Additionally, the present findings have practical implications by highlighting the potential importance of maintaining or adopting positive health behaviors to slow down epigenetic aging and reduce the health-risks associated with chronic stress exposure. Specifically, monitoring substance use and strategies to improve sleep quality may be effective lifestyle interventions to reduce health risks in individuals with a history of many stressful life events. Individuals with high levels of perceived concurrent stress may benefit from strategies to maintain healthy sleep, dietary, and exercise patterns, and future research should explore these possibilities.

4.5. Limitations

Several limitations need to be considered when interpreting the present findings. First, the mediation models were cross-sectional and could therefore not statistically determine the order of temporal precedence from the stress variables through the health behaviors to epigenetic aging. To draw more directional conclusions, future studies should utilize longitudinal designs with observations at a minimum of three time points and adjust for previous assessments of health behaviors and epigenetic aging. Second, the measures of stressful life events, perceived stress, and health behaviors were based on participants' self-reports, which may have inflated associations between these variables due to common method variance (Spector et al., 2019). A third limitation may be the inconsistent reporting periods for tobacco use and alcohol use. While tobacco use was measured as lifetime use, alcohol use was measured within the last month. Thus, any differences in effect sizes between these two measures cannot be directly interpreted as a difference between the substances. Additionally, the assessment of cumulative lifetime tobacco use did not capture whether, when, and for how long participants stopped smoking. Prior work has shown that smoking cessation can reverse smoking-related alterations in the DNA methylation pattern and decelerate epigenetic aging (Wu et al., 2019). Thus, the present analysis may be limited by not differentiating current-smokers from those who stopped using tobacco many years ago if the cumulative amounts were similar.

5. Conclusion

The present study investigated whether and how different forms of stress predict epigenetic aging in midlife. The findings showed that both the experience of many stressful life events as well as perceptions of high levels of stress put individuals at risk for accelerated biological aging and associated health issues. Unhealthy behaviors were identified as key mechanisms underlying these links. Specifically, individuals who experienced more stressful life events reported greater tobacco and alcohol use as well as more sleep problems, whereas individuals with higher levels of perceived stress experienced more sleep problems, had lower diet qualities, and engaged in fewer physical exercise. Higher tobacco and alcohol use, more sleep problems, lower diet quality, and lower physical exercise were in turn associated with accelerated epigenetic aging on one or more DNAm measures of aging. Thus, the present findings underscore the importance of trying to maintain a healthy lifestyle, especially when experiencing high levels of stress to slow down epigenetic aging and reduce the risk for chronic health issues in the long-term.

CRediT authorship contribution statement

Marlon Goering: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Kyle J. Bourassa:** Writing – review & editing, Methodology, Conceptualization. **Maxine Weinstein:** Writing – review & editing, Conceptualization. **Anthony P. Auger:** Writing – review & editing, Methodology. **Casey K. Brown:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2026.101194.

Data availability

The dataset and analysis code for the main analyses are available in the Open Science Framework (OSF)

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