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Pubertal Timing as a Predictor of Epigenetic Aging and Mortality Risk in Young Adulthood

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Abstract

Early pubertal timing is associated with adverse health in adulthood. These effects may be mediated by DNA methylation changes associated with accelerated cellular aging and mortality risk, but few studies tested associations between pubertal timing and epigenetic markers in adulthood. Additionally, pubertal timing effects often vary by sex and are understudied in diverse youth. Thus, this longitudinal study examined links between pubertal timing and later epigenetic aging and mortality risk together with sex differences in predominantly Black youth. Participants included 350 individuals (58% female, 42% male, 80% Black, 19% Non-Hispanic White). Perceived pubertal timing relative to peers and self-reported phenotypic pubertal timing based on age-adjusted Tanner scores were assessed during early adolescence (mean age 13), whereas epigenetic aging (GrimAge, DunedinPACE, and PhenoAge) and mortality risk were measured during young adulthood (mean age 27). After adjusting for covariates (smoking, BMI, family income, early life stress, race/ethnicity, sex, parenthood), early pubertal timing (both perceived and phenotypic) predicted higher epigenetic mortality risk and early phenotypic pubertal timing predicted accelerated DunedinPACE. Both perceived and phenotypic early pubertal timing were correlated with accelerated GrimAge. Off-time phenotypic pubertal timing (i.e., early and late) was associated with accelerated PhenoAge in males only, whereas perceived off-time pubertal timing was unexpectedly linked with lower PhenoAge acceleration. These findings extend prior research by linking two dimensions of early pubertal timing with epigenetic mortality risk and accelerated aging in racially diverse young adults and showing non-linear effects on PhenoAge acceleration that differ across pubertal timing measures and show some sex differences.

Keywords

Pubertal Timing; Epigenetic Age Acceleration; Mortality Risk; DNA Methylation

The age of pubertal onset has been declining in the western world for the last two centuries (Wyshak & Frisch, 1982), with a temporary cease around 1950 but a continued decline since

1980 (Eckert-Lind et al., 2020). The declining age of puberty has become a public health concern, especially since earlier pubertal timing has been associated with various negative outcomes, including behavioral and emotional problems (Ullsperger & Nikolas, 2017), as well as increased risk for chronic diseases such as cardiovascular disease or cancer (Golub et al., 2008; Gong et al., 2013; Lei et al., 2018). One mechanism that may account for these negative sequelae of early puberty is chronic stress, which is experienced by early maturing youth throughout adolescence (Natsuaki et al., 2009). In turn, higher levels of stress during adolescence contribute to poorer health outcomes in adulthood (Bush et al., 2018).

One pathway through which perceived stress affects health involves accelerated epigenetic aging and other changes in DNA methylation (Demetriou et al., 2015), which serve as biomarkers of subsequent chronic disease and mortality risks (Perna et al., 2016). The present longitudinal study examined associations between pubertal timing and later accelerated epigenetic aging and mortality risk during young adulthood. Given that some of the effects of early pubertal timing vary by sex (Negriff & Susman, 2011) and are relatively understudied in racially diverse youth (Bleil et al., 2017), this study involved a racially diverse sample and examined sex differences in the links between pubertal timing and later epigenetic outcomes.

Theoretical Framework

Early pubertal timing has been linked with increased psychological and interpersonal stress during adolescence (Hamilton et al., 2014; Natsuaki et al., 2009). Several mechanisms have been hypothesized to explain this link through physiological and socio-environmental processes. One physiological explanation states that youth who experience stress-inducing endocrine changes during puberty at a younger age have developed fewer self-regulatory skills in the pre-frontal cortex when experiencing pubertal stress (Dahl, 2004; Ge & Natsuaki, 2009) and are thus less equipped to cope with the elevated stress levels during puberty. Conversely, an environmental explanation has highlighted that adolescents with early pubertal timing appear older to both peers and adults in their social environment and may thus be treated as more mature than their same-age peers (Schelleman-Offermans et al., 2011), which may induce stress. Additionally, youth who perceive their pubertal timing as early compared to peers may seek out affiliations with older peers, leading to environmental influences and behavioral expectations they are not mature enough to adequately cope with (Lynne et al., 2007). Indeed, early pubertal timing has been linked with poorer health behaviors during adolescence and adulthood including substance use and sleep deprivation (Goering et al., 2024; Mendle et al., 2007), which may in turn result in poorer health outcomes in young adulthood.

High levels of stress during childhood and adolescence have been linked with an elevated risk for health problems in adulthood (Dube et al., 2009). One mechanism through which adverse environmental experiences may lead to poorer health outcomes is DNA methylation, which has been investigated as an indicator of the biological embedding of stressful environmental experiences and an epigenetic marker for health (Bush et al., 2018; Demetriou et al., 2015; Lei et al., 2018). As early pubertal timing is associated with higher stress (Hamilton et al., 2014; Natsuaki et al., 2009), these higher stress levels may alter the

epigenome in ways that make early maturing youth more prone to poorer health outcomes during adulthood.

The majority of previous research examined the *early timing* or *stage termination hypothesis*, which postulates that only early pubertal timing is linked with negative outcomes (Petersen & Crockett, 1985). However, two alternative hypotheses have been formulated in the literature. According to the *deviance hypothesis*, any deviation from normative pubertal timing (i.e., early and late) is associated with social stress and negative developmental outcomes (Petersen & Crockett, 1985). The *gender deviation hypothesis* provides an extension to the *deviance hypothesis* that incorporates sex differences. Since female adolescents enter puberty on average 18 months earlier than boys (Mendle et al., 2010; Negri & Susman, 2011), early maturing females and late maturing males diverge the most from the average overall pubertal timing of both sexes. Therefore, the *gender deviation hypothesis* postulates that the negative effects of early pubertal timing are amplified in female youth, while potential negative effects of late maturation are more pronounced in males (Ullsperger & Nikolas, 2017). Thus, the *early timing hypothesis* predicts a linear relationship between early pubertal timing and accelerated epigenetic aging, whereas the *deviance hypothesis* predicts a quadratic (U-shape) relationship of pubertal timing and epigenetic aging. Finally, the *gender deviation hypothesis* predicts linear effects of pubertal timing on epigenetic aging that are in opposite directions for males and females.

A few previous studies have shown differential effects of pubertal timing depending on how pubertal timing is measured, either as subjective youth perceptions of pubertal timing relative to peers or phenotypic maturation based on pubertal status relative to chronological age (Goering & Mrug, 2022; Harden & Mendle, 2012). Moreover, these perceived and phenotypic aspects of pubertal timing affect developmental outcomes in distinct ways (Moore et al., 2014). In the present study, phenotypic pubertal timing is defined as self-reported physical maturation based on Tanner-drawings that were then adjusted for chronological age (Taylor et al., 2001). Using chronological age provides a more objective “phenotypic” criterion for pubertal timing than youth’s subjective peer comparisons. Given these differences in the pubertal timing measures and competing theoretical hypotheses, the present study examined both perceived and phenotypic pubertal timing in relation to epigenetic markers of poorer health, including their linear and quadratic effects and potential sex differences.

Pubertal Timing and Health Outcomes

Pubertal timing has been linked to a variety of adverse health outcomes, with early maturation being associated with more negative outcomes than on-time or late maturation in both males and females (Day et al., 2015). Several studies have reported links between early pubertal timing and risk for chronic diseases and mortality during adulthood. Early pubertal timing has been linked with increased risk for cardiovascular disease and mortality during adulthood in White women from the US (Lakshman et al., 2009), from Iceland (Imai et al., 2013) and the UK (Hardy et al., 2019) as well as male and female young adults from Finland (Widen et al., 2012). While links between early pubertal timing and cardiovascular disease or all-cause mortality risk are understudied in Black individuals (Bleil et al., 2017), one

US study found links between early pubertal timing and increased risk for cardiovascular disease in Black males and females (Lei et al., 2018).

Early pubertal timing has also been linked to a higher risk of breast cancer in predominantly White (Bodicoat et al., 2014) and Black female adults (Bertrand et al., 2017). According to a meta-analysis, early pubertal timing is linked with a higher risk for ovarian cancer in females (Gong et al., 2013). Among males, early pubertal timing has been associated with higher risk for testicular cancer (Golub et al., 2008) and later pubertal timing has been associated with lower risk for prostate cancer (Bonilla et al., 2016; Day et al., 2017). Thus, prior research suggests that specifically early pubertal timing presents a risk factor for chronic disease in both males and females. While some research suggests that both early and late pubertal timing predict an increased risk for mental health problems in males (Benoit et al., 2013), there is no evidence of off-time pubertal timing effects on physical health outcomes in either sex. Thus, prior research is supporting the *early timing hypothesis* over the *deviance hypothesis* or *gender deviation hypothesis* in links between pubertal timing and physical health outcomes.

DNA Methylation as a Biomarker of Health

Several DNA methylation-based biomarkers have been developed to predict biological age as well as risk for chronic disease and mortality. These biomarkers measure DNA methylation across specific CpG sites (cytosine phosphate guanine sites) and the DNA methylation patterns across these CpG sites provide estimates of epigenetic age and mortality risk (Lin et al., 2016; Zhang, Wilson et al., 2017). Three epigenetic age measures from the second and third generation (i.e., GrimAge, DunedinPACE, and PhenoAge) have been validated with clinical biomarkers associated with health problems and mortality in addition to chronological age. These epigenetic clocks are therefore better biomarkers for health and mortality risk than the first-generation pan-tissue epigenetic clock (DNAm age Horvath) and blood tissue clock (DNAm age Hannum), which were only validated with chronological age (Palma-Gudiel et al., 2020). After adjusting for chronological age, higher PhenoAge has been associated with elevated inflammation markers, chronic disease, and all-cause mortality (Levine et al., 2018). Previous studies have also found associations between accelerated PhenoAge and a heightened risk for different types of cancer such as breast cancer (Kresovich et al., 2019), pancreatic cancer (Chung et al., 2021), and lung cancer (Ma et al., 2023). Similarly, accelerated PhenoAge was associated with cardiovascular disease (Cao et al., 2022) and all-cause mortality (Klopach et al., 2022).

Similar associations between epigenetic age acceleration and risk for chronic disease and all-cause mortality have been found for GrimAge (Lu et al., 2019). For example, GrimAge acceleration predicted heart disease and type 2 diabetes (Hillary et al., 2020), lung cancer (Dugue et al., 2021), cardiovascular disease (Ammous et al., 2021; Joyce et al., 2021), and all-cause mortality (Foehr et al., 2021). Compared to previous clocks, the GrimAge clock takes environmental and lifestyle factors into account (e.g., smoking years) and provides a good estimate of longevity (Yusipov et al., 2024). Ultimately, accelerated DunedinPACE indicates not only faster biological aging but is also associated with morbidity and chronic disease risk (Belsky et al., 2022). Specifically, accelerated DunedinPACE has been linked

with breast cancer (Kresovich et al., 2023) and cardiovascular disease (Belsky et al., 2021). While the three clocks differ in their computation and the CpG sites involved, all three epigenetic clocks included in this study are markers for chronic disease risk. Another approach has used DNA methylation on specific CpG sites as a marker for all-cause mortality risk, putting greater emphasis on clinical markers and chronic disease outcomes when validating the index (Zhang, Wilson et al., 2017). This mortality risk index has shown strong associations with cardiovascular and cancer mortality, suggesting that it may be an even more accurate biomarker for negative health outcomes than epigenetic age acceleration (Gao et al., 2019).

Pubertal Timing and Epigenetic Age Acceleration

Several studies have examined the relationship between pubertal timing and epigenetic age acceleration during adolescence. Higher epigenetic age acceleration has been linked to earlier pubertal timing in Chilean female adolescents (Binder et al., 2018) as well as adolescent males and females from Finland (Suarez et al., 2018). A few studies have also examined links between pubertal timing and subsequent epigenetic age acceleration in adulthood. One study found that early pubertal timing in the form of a younger age of menarche predicts accelerated GrimAge but is unrelated to PhenoAge in predominantly White US females during mid-adulthood (Hamlat et al., 2021). Similarly, in a study involving middle-aged males and females from the UK, early pubertal timing assessed as reaching menarche before the age of 12 was associated with accelerated GrimAge but not PhenoAge in females (Maddock et al., 2021). The same study found no links between pubertal timing assessed via physical examination of overt physical changes and epigenetic age acceleration on either the GrimAge or the PhenoAge clock in males.

Black youth in the United States experience more early life adversity in the form of chronic stress such as systemic discrimination and economic insecurity as well as limited access to health care and health promoting lifestyles, which in turn affect health outcomes (Thoits, 2010). Due to a history of race-related oppression and inequities, Black youth are experiencing faster epigenetic aging as compared to White youth in the US (Del Toro et al., 2024) and early pubertal timing is more prevalent among Black youth and youth from lower-income families (Herman-Giddens et al., 2012; James-Todd et al., 2010). Despite these disparities in both pubertal timing and social determinants of health, links between pubertal timing and epigenetic markers of health are understudied in Black youth. One recent study has provided some evidence that early pubertal timing is related to accelerated aging across racial groups in the US, as links between early pubertal timing measured by a younger age of menarche predicted accelerated aging on a latent construct with indicators of GrimAge, telomere length, and C-reactive protein in both Black and White women (Hamlat et al., 2023). However, no prior studies have examined these relationships in racially and economically diverse populations of both males and females. Additionally, no previous studies have examined links between pubertal timing and DunedinPACE or epigenetic markers for mortality risk in adulthood. Moreover, previous studies only assessed pubertal timing in females with age of menarche (Hamlat et al., 2021; Maddock et al., 2021) and physician examinations of pubertal status in males (Maddock et al., 2021). Thus, less understood are the effects of self-perceived pubertal timing relative to peers on

epigenetic age acceleration or mortality risk. Ultimately, no prior research has examined the possibility of off-time pubertal timing effects on epigenetic age acceleration and mortality risk following the *deviance hypothesis* that any deviation from normative pubertal timing puts youth at heightened risk for negative outcomes (Petersen & Crockett, 1985).

Sex Differences

Across racial groups, girls experience puberty earlier than boys (Slyper, 2006). Early pubertal timing may therefore be especially problematic for girls due to experiencing puberty-related stress at an even younger chronological age than early maturing males. While a meta-analysis has shown that early pubertal timing predicts negative developmental outcomes in both males and females and that sex does not moderate risks associated with early puberty (Ullsperger & Nikolas, 2017), some previous studies have shown that the heightened levels of stress associated with early puberty are more problematic for females than for males (Natsuaki et al., 2009). Thus, the effects of early pubertal timing on epigenetic aging and mortality risk that can be explained by increased stress may be more pronounced in female as compared to male adolescents. Only one prior study examined sex differences in these effects and found that early pubertal timing predicts accelerated epigenetic aging in females but not in males (Maddock et al., 2021). However, this study measured pubertal timing as the age of menarche in girls and via physical examination using Tanner criteria in boys. Therefore, these differences in the effects of pubertal timing between males and females may be driven by differences in the measurement method of pubertal timing. Thus, more research is needed on sex differences in links between early pubertal timing and accelerated epigenetic aging using comparable measures for male and female youth.

Current Study

To help elucidate mechanisms through which early pubertal timing contributes to chronic disease and early mortality, the present study examined prospective associations between pubertal timing in early adolescence and epigenetic biomarkers of aging and mortality risk in young adulthood. Extending previous studies that involved predominantly White and female individuals, these questions were examined in a predominantly Black sample of males and females. Since females experience puberty at a younger age than males and some effects of pubertal timing and associated stress differ by sex, the present study also investigated sex differences in the links between pubertal timing and epigenetic age acceleration and mortality risk. Since perception-based and phenotype-based measures of pubertal timing have shown to have independent effects through distinct mechanisms, the present study examined the effects of both perceived and phenotypic pubertal timing. In addition to examining linear links between early pubertal timing and accelerated epigenetic aging, this study also examined a quadratic effect to test if off-time pubertal timing (i.e., early and late) predicts epigenetic aging and mortality risk. It was hypothesized that both perceived and phenotypic early pubertal timing are associated with higher mortality risk and accelerated epigenetic age. Moreover, it was hypothesized that links between early pubertal timing and the epigenetic biomarkers would be stronger in females as compared to males.

Methods

Participants and Procedures

Participants included 350 individuals (58% female, 42% male; 80% Black, 19% Non-Hispanic White, 1% other race/ethnicity) who participated in the Birmingham Youth Violence Study (BYVS) between 2005 and 2022. Initially, adolescents were recruited from 17 public schools from a medium sized city in the Southeastern United States when attending fifth grade. These 17 schools were selected with the objective of recruiting a sample that is representative of the demographic and socioeconomic composition of the local area. This study used primarily data from the Wave 2 (labeled as Time 1) and Wave 4 (Time 2) assessments and only included participants who had epigenetic data at Time 2. The average ages of the youth participants were 13.1 years (Time 1) and 27.7 years (Time 2). At Time 1, adolescents' caregivers were also interviewed. Reflecting the sampled population, there was an overrepresentation of families with lower incomes. At Time 1, annual family income before taxes ranged from less than \$5,000 to more than \$90,000 with a median income of \$25,000 to \$30,000 per year.

The Institutional Review Board of the University of Alabama at Birmingham approved the study. At the Time 1 assessment, caregivers provided informed consent and adolescent participants provided informed assent. At Time 2, the adult participants provided informed consent. At each time point, trained research assistants administered questions using Computer-Assisted-Personal-Interviews. Sensitive questions (e.g., on pubertal development) were administered via Audio-Computer-Assisted-Self-Interview (ACASI) so that participants could complete these questions in private with no interviewer being present in the room. At Time 2, saliva samples were collected from the participants. Participants were financially compensated for their time at each assessment.

DNA Collection, Extraction, and Methylation

At Time 2, the participants provided saliva samples utilizing Oragene DNA OG-500 kits after rinsing their mouths with water and abstaining from food or drink for 30 minutes before collecting the sample. From the samples, DNA was extracted following the PureGene method and all manufacturer's instructions. The saliva samples provided high-quality DNA (> 2.1 µg). The Illumina Infinium MethylationEPIC BeadChip was utilized to perform methylation analyses on the DNA samples (Mrug et al., 2024). Quality control and within-array normalization were carried out in R using the Minfi package. These also encompassed background correction, batch/plate/chip adjustment, and Type I and II chemistry correction. The measurements of methylation were quantified as β -values, which were computed as the ratio of methylated fluorescent intensity and overall intensity (Bibikova et al., 2006). The R-code for the computation of the epigenetic analyses including the quality control and within-array normalization is available in the manuscript's OSF repository [<https://osf.io/h8397>].

Measures

Pubertal Timing—Pubertal timing was measured at Time 1 in two distinct ways, as perceived and phenotypic. Perceived pubertal timing measures youth's subjective

perceptions of their pubertal timing relative to their same-age and same-sex peers. Adolescents were administered the last item from the Pubertal Development Scale (PDS) (Petersen et al., 1988), which asked “Do you think your body has changed any earlier or later than most other girls/boys your age?” Adolescents responded to this question on a five-point scale with response options ranging from “much earlier” to “much later”. Responses were coded so that higher values indicated earlier pubertal timing. Throughout this manuscript, these pubertal timing scores are referred to as “perceived pubertal timing”.

Phenotypic pubertal timing was assessed by administering two sets of five sex-specific line drawings. The drawings showed different stages of pubertal maturation corresponding to the five Tanner stages of pubic hair and breast maturation for girls and pubic hair and genital maturation for boys (Taylor et al., 2001). The images included brief descriptions in the footnote to make it easier for adolescents to detect the differences in the images. From each set of drawings, participants were asked to select the image that most accurately reflected their own pubic hair and breast or genital development. The two scores were moderately correlated ($r = .46$ for males, $r = .39$ for females, both $p < .001$). Therefore, the two sets of Tanner ratings were averaged, so that higher numbers indicated more advanced pubertal status. As expected, older youth had more advanced pubertal status ($r = .38$, $p < .001$). To account for these age differences, the averaged pubertal status scores from the Tanner stage assessments were regressed on adolescents’ age at the time of the Time 1 interview. The resulting unstandardized residuals from this regression indicated adolescents’ pubertal timing, with higher scores indicating earlier pubertal timing (Sumner et al., 2019). Throughout this manuscript, these pubertal timing scores are referred to as “phenotypic pubertal timing”.

Epigenetic Age Acceleration—Three second- and third-generation DNA methylation-based epigenetic age clocks – GrimAge, DunedinPACE and PhenoAge, were used in this study. Unlike the first-generation biomarkers – Horvath DNAm age and Hannum DNAm age, which were validated to only predict chronological age, these more recently developed biomarkers were also validated with health outcomes and therefore serve as better biomarkers of adverse health and mortality (Palma-Gudiel et al., 2020). The scores for GrimAge were computed using the DNAm age calculator (<https://dnamage.genetics.ucla.edu/>) from DNAm on 1030 CpG sites (Lu et al., 2019). GrimAge scores were coded so that higher values indicate higher epigenetic age. Three extreme outliers were identified (z scores above 5) and were coded as missing. To adjust for differences in chronological age, the GrimAge scores were regressed on chronological age and the unstandardized residuals were used as an indicator of epigenetic age acceleration. Higher scores indicated greater epigenetic age acceleration.

The DunedinPACE epigenetic clock was estimated from the pattern of DNA methylation across 173 CpG sites using elastic net regression (Belsky et al., 2022). Scores for the DunedinPACE describe the year increase in biological aging per year-increase in chronological age. The DunedinPACE indicator was initially developed and validated in a longitudinal cohort followed from childhood to age 45 (Belsky et al., 2022). After screening for outliers, the DunedinPACE scores were regressed on chronological age at Time 2 so that

the unstandardized residuals indicate accelerated epigenetic aging on the DunedinPACE with higher positive values indicating more accelerated DunedinPACE.

The scores for PhenoAge were obtained through a linear function (ENmix) in the R package minfi. The scores were computed based on published intercepts and regression coefficients for DNAm levels at 513 CpG sites (Levine et al., 2018). PhenoAge scores were coded so that higher values indicate higher epigenetic age. Two extreme outliers were identified (absolute z-scores above 5) and were coded as missing. As the other clocks, the PhenoAge scores were regressed on chronological age at Time 2 and the unstandardized residuals were used as an indicator of epigenetic age acceleration. Higher scores indicated greater epigenetic age acceleration.

Epigenetic Mortality Risk—An epigenetic mortality risk index was computed from methylation beta values on eight CpG sites (cg14975410, cg05575921, cg01612140, cg25983901, cg10321156, cg19572487, cg24704287, and cg08362785). These CpG sites have been identified in an epigenome wide association study (EWAS) as predictive of all-cause mortality and validated in other studies (Zhang, Hapala et al., 2017). Mortality risk scores based on methylation of these CpG loci have been used in prior studies (Gao et al., 2020; Mrug et al., 2024). All methylation beta-values from the eight CpG sites were standardized, reverse-coded if needed, and averaged to an index so that higher values indicated a higher risk for mortality. The mortality risk scores were age-adjusted in the same way as the epigenetic clocks by regressing the mortality risk scores on chronological age and using the resulting residuals as indicators of mortality risk.

Covariates—Participants' biological sex, race/ethnicity, and family income at Time 1 were included as socio-demographic covariates. Chronological age was not included as a covariate as the epigenetic outcomes were already age-adjusted in their computations. Since almost all participants were either Black or Non-Hispanic White, race/ethnicity was coded as a dichotomous variable indicating whether youth are from a racial/ethnic minority or not. Age at Time 2 was calculated from participants' date of birth and the date of the interview in years. Family income at Time 1 was assessed with caregiver reports of the gross annual family income on a 13-point scale ranging from '1 – Less than \$5,000' to '13 – More than \$90,000'. A few studies have shown that pregnancy and caregiving are associated with shorter telomere length indicating accelerated epigenetic aging in females (Giller et al., 2020; Pollack et al., 2018). Since early pubertal timing is associated with greater odds for early parenthood in females (Hendrick et al., 2016), it is important to adjust for parenthood when examining links between pubertal timing and epigenetic aging. Parenthood was assessed by asking participants if they had any biological children at Time 2.

Previous studies identified early life stress as a risk factor for early pubertal timing (Holdsworth & Appleton, 2019) as well as accelerated epigenetic aging in adulthood (Kim et al., 2023). Thus, this study included early life stress (pre-puberty) as an additional covariate to adjust for its potential confounding effect on links between early pubertal timing and epigenetic aging and mortality risk. Early life stress was assessed via retrospective reports at Time 2 using a shortened 28-item version of the Childhood Trauma Questionnaire, which includes questions on emotional, physical, and sexual abuse as well as emotional and

physical neglect (Bernstein et al., 2003). Participants responded to each item on a three-point scale indicating whether the stressful event was experienced never (1), sometimes (2), or often (3). Responses to ten items on support in the family environment were reverse-coded so that higher values on each item indicate more early life-stress. Each item was followed-up with a question inquiring at what age the stressful event was experienced for the first time. Since pubertal timing was assessed at age 13, only stressful events experienced before the age of 13 were included when computing the early life stress variable. The scores from the 28 items were averaged and the scale had a good internal reliability (Cronbach's $\alpha = .91$).

Since previous research has consistently linked higher epigenetic age with BMI (Foster et al., 2023) and tobacco use (Gao et al., 2016), BMI and cigarette smoking were also included as covariates. BMI was computed from the average of two height and weight measurements at Time 2. A third measurement was taken if the two measurements differed by more than 0.5cm for height and more than 0.2kg for weight, and the two closest values were averaged. Smoking has been assessed at both Wave 3 (age: 17.6 years) and Wave 4 (Time 2; age: 27.7 years). At each time point, participants reported how often they smoked cigarettes in the last 12 months on a seven-point scale with response options ranging from '1 – A few times' to '7 – Every day'. At each time point, participants also reported how many cigarettes they usually smoked per day in the last 12 months on a five-point response scale with response options ranging from '1 – less than 1 cigarette per day' to '5 – More than 20 cigarettes per day'. Responses from the two items were multiplied to compute a frequency-quantity index at each time point. The two frequency-quantity indexes were then averaged to an overall smoking index in late adolescence and early adulthood. If information on smoking was missing at one of the time points, only the score from the available time point was used.

Cell Type Composition—Since cell-type composition has shown large inter-individual variability in saliva samples, which can drive DNA methylation (Middleton et al., 2022), estimated cell counts were obtained using the deconvolution method (Houseman et al., 2012). Specifically, estimated cell counts of CD4T, CD8T, B-cells, Monocytes, and Granular cells were obtained for each saliva sample. Three extreme outliers on B-cells (z scores > 5) and two extreme outliers on CD4T (z scores > 5) were coded as missing.

Data Analysis

Prior to the main analyses, the assumptions of multiple regression were tested in the dependent variables (i.e., epigenetic mortality risk, GrimAge, DunedinPACE, PhenoAge). Also prior to the main analyses, the amount of missing data and the percentage of cases with missing data were examined. Missing data analyses also tested whether cases with any missing data differ from cases with complete data on any variables included in the analyses using independent samples t-tests for continuous variables and chi-square tests of independence for categorical variables. Then, descriptive statistics and bivariate correlations among all variables included in the analyses were examined. All preliminary analyses were conducted in SPSS.

The main analyses involved eight hierarchical multiple regression models with three steps performed in Mplus. The first model included perceived pubertal timing as a predictor of

age-adjusted mortality risk. Step 1 included perceived pubertal timing and all covariates (early life stress, biological sex, race/ethnicity, family income at Time 1, smoking at Time 2, BMI at Time 2, and parenthood at Time 2) as predictors. In Step 2a, an interaction term of perceived pubertal timing and biological sex was added to the model. In Step 2b, a quadratic term of the standardized perceived pubertal timing variable was added to the model as an indicator of perceived off-time pubertal timing (i.e., positive and negative deviations from the mean had the same score). In Step 3ab, an interaction term of perceived off-time pubertal timing and biological sex was added to a model that also includes all effects from Step 1, Step 2a, and Step 2b so that all lower-order terms were included. Significant interaction effects were followed up with simple slope analyses. The second, third, and fourth models were set up in the same way but predicted epigenetic age acceleration using GrimAge in the second model, DunedinPACE in the third model, and PhenoAge in the fourth model.

The fifth, sixth, seventh, and eighth models paralleled the first four models but included phenotypic pubertal timing as a predictor instead of perceived pubertal timing and the interaction term of phenotypic pubertal timing and biological sex in step 2a. As in the previous models, off-time phenotypic pubertal timing was added in Step 2b and an interaction term of off-time phenotypic pubertal timing and biological sex was added in Step 3ab, which included all terms from Step 1, Step 2a, and Step 2b. The off-time pubertal timing term was created by squaring the Tanner score residuals (i.e., positive and negative deviations from the mean had the same score). The fifth model predicted epigenetic mortality risk, the sixth model predicted epigenetic age acceleration using GrimAge, the seventh model predicted DunedinPACE, and the eighth model predicted PhenoAge acceleration. All models used maximum likelihood estimation and handled missing data with Full-Information Maximum Likelihood (FIML).

Three sensitivity analyses were conducted to examine the robustness of the results under different conditions. The first sensitivity analysis was conducted with all models also adjusting for cell-type composition by including counts of CD4T, CD8T, B-cells, Monocytes, and Granular cells as additional covariates in Step 1. All cell-type scores were standardized to facilitate model convergence. While cell type composition shows inter-individual variability in saliva samples, which can drive DNA methylation, it has also been shown to mediate associations between health status and DNA methylation (Houseman et al., 2015). Moreover, cell-type composition, specifically having low cell-counts of leukocyte-type cells, is associated with cancer (Heiss & Brenner, 2017) and mortality risk (Gao et al., 2021), which may overlap with DNA methylation-based biomarkers. Due to this likely overlap in leukocyte composition and DNA methylation as markers of health, controlling for cell-type composition when using DNA methylation as a marker of health outcomes and mortality risk may be an overadjustment that potentially obscures meaningful findings (Qi & Teschendorff, 2022). Therefore, not controlling for cell-type composition does not invalidate the results when using DNA-methylation based biomarkers as markers for health outcomes or mortality risk (Zhang et al., 2016). For these reasons, cell-type counts were included as covariates in a sensitivity analysis but not in the main analysis, which is consistent with prior work that has examined links between pubertal timing and epigenetic age acceleration (Maddock et al., 2021).

To better understand the effects of pubertal timing on epigenetic health outcomes in Black youth, specifically, the second sensitivity analysis examined the main models within Black youth only (N = 279) excluding the 19% Non-Hispanic White participants and 1% participants from other races/ethnicities included in the original analyses.

Transparency and Openness

We report how the sample size is determined with all data exclusions. All measures and coding of variables are described. The data and example analysis code for one model are available in the Open Science Framework (OSF) repository using the following link [<https://osf.io/h8397>]. Data analyses were conducted in SPSS and Mplus version 8.1. This study was not preregistered.

Results

Preliminary Analyses

Results from the missing data analyses showed that 30% of participants had some missing data and 4% of the total possible data points were missing. Participants with any missing data had on average earlier perceived pubertal timing at Time 1 ($t_{(311)} = 2.11, p = .035$, Cohen's $d = 0.29$). Participants with any missing data did not differ from participants with complete data on phenotypic pubertal timing, epigenetic age acceleration and mortality risk scores, BMI at Time 2, smoking, biological sex, race/ethnicity, early life stress, family income at Time 1, or parenthood at Time 2. Descriptive statistics, skewness, and kurtosis for all variables are reported in Table 1. Skewness and kurtosis values indicated no violations of normality in either of the four dependent variables (Mortality risk, GrimAge acceleration, DunedinPACE, and PhenoAge acceleration). Plots of residuals and predicted values indicated no violations of homoscedasticity.

Results from the bivariate correlation analyses are reported in Table 2. The results showed that perceived and phenotypic pubertal timing were not significantly correlated, supporting their use as distinct measures of pubertal timing. Both perceived and phenotypic early pubertal timing were associated with higher mortality risk and GrimAge epigenetic age acceleration, whereas only phenotypic early pubertal timing was associated with accelerated DunedinPACE. Neither perceived nor phenotypic pubertal timing were linearly associated with PhenoAge epigenetic age acceleration. Finally, all covariates were correlated with at least one of the epigenetic outcomes.

Main Analyses

Results from the hierarchical multiple regression models using perceived pubertal timing are shown in Table 3. In the first model, mortality risk was uniquely predicted by perceived early pubertal timing. The results showed no effect of perceived off-time pubertal timing on mortality risk. Results from the second multiple regression model showed that perceived early pubertal timing did not predict GrimAge acceleration at Time 2 when adjusting for the covariates. The results showed no link between perceived off-time pubertal timing and GrimAge acceleration. Results from the third multiple regression model showed that neither perceived early nor perceived off-time pubertal timing were uniquely associated with

DunedinPACE. Results from the fourth multiple regression model showed that perceived early pubertal timing was not uniquely associated with PhenoAge acceleration at Time 2 but against expectations, the results showed that perceived off-time pubertal timing (i.e., early and late) was associated with lower PhenoAge acceleration. None of the effects of early or off-time perceived pubertal timing differed by sex.

Results from the hierarchical multiple regression models with phenotypic pubertal timing are shown in Table 4. In the fifth model, phenotypic early pubertal timing uniquely predicted higher mortality risk at Time 2. The results showed no effect of phenotypic off-time pubertal timing on mortality risk. Results from the sixth multiple regression model showed that phenotypic early pubertal timing did not uniquely predict GrimAge acceleration at Time 2 when adjusting for the covariates. The results also showed no link between phenotypic off-time pubertal timing and GrimAge acceleration. Results from the seventh multiple regression model showed that phenotypic early pubertal timing uniquely predicted higher DunedinPACE at Time 2. The results showed no link between phenotypic off-time pubertal timing and DunedinPACE. Results from the final regression model showed that phenotypic early pubertal timing did not uniquely predict PhenoAge acceleration. None of these effects differed by sex. However, the results showed that the effect of off-time phenotypic pubertal timing (i.e., early and late) on PhenoAge acceleration differed by sex. A plot of the interaction is shown in Figure 1. Follow-up simple slope analyses indicated that phenotypic off-time pubertal timing predicted accelerated PhenoAge in males ($\beta = 0.20$, $p = .014$) but not in females ($\beta = -0.05$, $p = .540$).

Among the covariates, higher smoking was associated with higher epigenetic mortality risk and accelerated epigenetic aging across all indicators. Higher BMI was associated with accelerated DunedinPACE and PhenoAge but also lower mortality risk. Female participants had higher DunedinPACE and PhenoAge acceleration and youth from racial/ethnic minority backgrounds had higher scores on epigenetic mortality risk as well as GrimAge and PhenoAge acceleration. Family income at Time 1, early life stress, and parenthood at Time 2 were not uniquely associated with any of the epigenetic outcomes.

Sensitivity Analyses

The first sensitivity analysis was conducted to examine the robustness of the results when including measures of leukocyte counts (i.e., CD8T, CD4T, B-cells, Monocytes, and Granular cells) as additional covariates. Results from the sensitivity analysis are reported in Tables S1 and S2 in the supplementary material. Contrary to the main results, the effect of early perceived pubertal timing on higher epigenetic mortality risk and the effects of early phenotypic pubertal timing on higher mortality risk and accelerated DunedinPACE did not reach statistical significance. Consistent with the main results, perceived off-time pubertal timing predicted lower PhenoAge acceleration, whereas phenotypic off-time pubertal timing predicted accelerated PhenoAge in males.

The second sensitivity analysis examined the hierarchical regression models only within the Black participants. Results from this sensitivity analysis are reported in Tables S3 and S4 in the supplementary material. Due to the reduced sample size, the effect of perceived early pubertal timing on mortality risk did not reach significance (at $\alpha = .05$), despite showing

a slightly larger effect size than in the main analysis. Additionally, the effect of early phenotypic pubertal timing on DunedinPACE did not reach significance. Consistent with the main analysis, phenotypic early pubertal timing predicted higher mortality risk, perceived off-time pubertal timing predicted less accelerated PhenoAge, and the effect of off-time phenotypic pubertal timing on PhenoAge acceleration differed by sex with phenotypic off-time pubertal timing predicting accelerated PhenoAge in males ($\beta = 0.26$, $p = .006$) but not in females ($\beta = -0.06$, $p = .464$).

Discussion

The present study examined the relationships of perceived and phenotypic indicators of pubertal timing with epigenetic markers of mortality risk and accelerated aging, together with sex differences in a racially diverse sample. The results from bivariate correlation analyses showed that both perceived and phenotypic early pubertal timing were correlated with higher mortality risk and accelerated epigenetic aging on the GrimAge clock. Additionally, phenotypic early pubertal timing was correlated with accelerated DunedinPACE but neither pubertal timing variable was linearly associated with PhenoAge acceleration. When adjusting for sociodemographic covariates as well as other established risk factors for epigenetic aging (i.e., early life stress, smoking and BMI), both perceived and phenotypic early pubertal timing were still uniquely associated with higher mortality risk. Moreover, early phenotypic pubertal timing was uniquely associated with accelerated DunedinPACE. However, the links between early pubertal timing and accelerated GrimAge did not reach significance when adjusting for covariates. None of the linear effects of pubertal timing on mortality risk or epigenetic age acceleration differed between males and females. Additionally, the present findings showed quadratic effects on PhenoAge that were inconsistent across perceived and phenotypic pubertal timing. Specifically, perceived off-time pubertal timing predicting less accelerated PhenoAge in both sexes and off-time phenotypic pubertal timing predicting more accelerated PhenoAge in males. In summary, most of the results support the hypothesis that early pubertal timing puts both male and female youth at higher epigenetic risk of mortality and accelerated aging but the results also highlight the possibility of off-time pubertal timing effects on PhenoAge that show some sex differences.

Pubertal Timing and Epigenetic Mortality Risk

The present findings make a novel contribution by supporting the hypothesis linking early pubertal timing with higher DNA methylation-based mortality risk during young adulthood. Since the mortality risk score used in the present study has been linked to greater risk for chronic disease (Gao et al., 2019), DNA methylation may present a mechanism through which early pubertal timing increases the risk for chronic diseases such as cardiovascular disease and cancer in racially diverse males and females (Bertrand et al., 2017; Golub et al., 2008; Lei et al., 2018). While previous research identified distinct effects of perceived and phenotypic pubertal timing on psychosocial developmental outcomes (Moore et al., 2014), the present results suggest that both perceived and phenotypic pubertal timing are linked with higher epigenetic mortality risk during young adulthood. The fact that two

distinct measures of early pubertal timing are linked with higher mortality risk increases the robustness of these results.

The present findings of both perceived and phenotypic pubertal timing being related to higher mortality risk may help explain how early pubertal timing and associated stress increases the risk for poorer health outcomes later in the lifespan. While perceiving one's own pubertal timing as earlier relative to peers may induce stress primarily on a psychosocial level (Lynne et al., 2007), earlier phenotypic pubertal timing relative to chronological age may induce stress primarily on a physiological level (Ge & Natsuaki, 2009) but also psychosocially due to being perceived as older by one's environment (Schelleman-Offermans et al., 2011). It is possible that early maturing youth are at a higher mortality risk during young adulthood due to the biological embedding of heightened experiences of both physiological and psychosocial stress during adolescence, but these potential mechanisms could not be tested in the present study. Contrary to the hypothesis regarding sex differences, links between early pubertal timing and mortality risk did not differ between males and females. Thus, the relative earlier pubertal timing of females compared to males does not seem to amplify the effect of early maturation on epigenetic mortality risk in females.

Pubertal Timing and Accelerated Epigenetic Aging

Both the bivariate correlation and adjusted regression analysis showed that phenotypic early pubertal timing predicts accelerated DunedinPACE in both males and females. This finding is consistent with prior research finding that a younger age of menarche is related to accelerated epigenetic aging in racially diverse women (Hamlat et al., 2023). The present findings suggest that the effect of pubertal timing in females replicates when measuring phenotypic pubertal timing based on Tanner stages rather than age of menarche. The present findings also advance prior research by providing initial evidence that links between early pubertal timing and accelerated epigenetic aging extend to pre-dominantly Black males and females and can be detected with the DunedinPACE indicator.

The results showed that perceived pubertal timing did not predict DunedinPACE. This may suggest that effects of early pubertal timing on accelerated epigenetic aging are better explained by physiological stress associated with early pubertal timing and being perceived as older by one's environment than peer related stress from social comparison. However, underlying mechanisms could not be examined in the present study and are subject to future research.

Consistent with previous studies that involved predominantly White samples, both early perceived and early phenotypic pubertal timing were correlated with higher GrimAge acceleration (Hamlat et al., 2021; Maddock et al., 2021). However, the association of pubertal timing with GrimAge acceleration was attenuated after adjusting for sociodemographic covariates, BMI, tobacco smoking, early life stress and parenthood and was not statistically significant (at $\alpha = 0.05$). The weaker links between early pubertal timing and GrimAge acceleration may be due to the younger chronological age of the present sample when assessing epigenetic age (mean age: 27 years) as compared to the three prior studies with mean ages of 39, 42 and 53 years, respectively (Maddock et al., 2021;

Hamlat et al., 2021; Hamlat et al., 2023). Since GrimAge was initially trained in older adult populations (Lu et al., 2019), it is possible that effects of pubertal timing on accelerated GrimAge as a marker for chronic disease risk become more pronounced at older ages when chronic disease risk is generally higher (Roberts et al., 2023). In contrast to previous findings linking early pubertal timing with higher GrimAge acceleration in females but not in males (Maddock et al., 2021), the present findings suggest that links between pubertal timing and GrimAge acceleration do not differ by sex. It is possible that previously found sex differences are at least partially driven by differences in the way pubertal timing was measured in males as compared to females. Thus, associations between early pubertal timing and accelerated GrimAge may extend to males, but further research that utilizes comparable measures of pubertal timing across males and females is needed.

Consistent with prior research, the present findings showed no linear relationship between pubertal timing and PhenoAge acceleration (Hamlat et al., 2021; Maddock et al., 2021). However, the present findings suggest that off-time phenotypic pubertal timing describing any deviation from normative pubertal timing (i.e., early and late) is associated with accelerated PhenoAge in males but not in females. While no prior studies have examined off-time pubertal timing as a predictor of epigenetic aging, there is some evidence that both early and late phenotypic pubertal timing place males at increased risk for mental health problems (Benoit et al., 2013). Thus, it is possible that both early and late pubertal timing can generate stress in adolescent males, which may in turn accelerate epigenetic aging and explain links between off-time phenotypic pubertal timing and accelerated PhenoAge in males.

Contrary to expectations, perceived off-time pubertal timing was associated with slower epigenetic aging on the PhenoAge clock in both males and females. This finding is unexpected as previous research showed that self-perceiving one's pubertal timing as either earlier or later compared to peers is associated with increased psychological stress in both males and females (Conley & Rudolph, 2009). Thus, it is unclear how perceived off-time pubertal timing could contribute to less accelerated PhenoAge. Given the opposite findings for perceived and phenotypic pubertal timing, future research is needed to better understand the effects of off-time pubertal timing on epigenetic aging on the PhenoAge clock.

There is some indication that PhenoAge is a less accurate marker of chronic disease and all-cause mortality as compared to other second-generation clocks such as GrimAge (McCrory et al., 2021), which may partially explain inconsistencies in the observed pubertal timing effects between PhenoAge and the other epigenetic markers of health. Results from the bivariate correlation analyses indeed showed stronger links between mortality risk, GrimAge acceleration, and DunedinPACE as compared to PhenoAge acceleration. This may suggest that links between pubertal timing and GrimAge, DunedinPACE, and mortality risk are more indicative of pubertal timing effects on health outcomes as compared to PhenoAge. As both early perceived and phenotypic pubertal timing were uniquely linked with higher mortality risk, correlated with accelerated GrimAge, and early phenotypic pubertal timing uniquely predicted accelerated Dunedin PACE, the consistent pattern of the present findings supports links between early pubertal timing and negative health outcomes in adulthood via accelerated epigenetic aging and mortality risk. Nevertheless, future research is needed

to better understand differences in links between pubertal timing and individual epigenetic markers of health.

It is important to note that when adjusting for estimates of cell-type composition, the effects of early pubertal timing on epigenetic mortality risk and accelerated aging were attenuated. Controlling for cell-type composition similarly attenuated links between early pubertal timing and accelerated epigenetic aging in prior research (Maddock et al., 2021). This is possibly due to cell composition representing a biological mechanism through which DNA methylation relates to health status (Houseman et al., 2015). Moreover, leukocyte composition has been associated with markers for chronic disease (Heiss & Brenner, 2017). Due to a likely overlap in leukocyte composition and DNA methylation as markers of health, controlling for cell type composition when using DNA methylation as a marker of health outcomes and mortality risk may therefore be an over-adjustment that obscures meaningful findings (Qi & Teschendorff, 2022). Controlling for cell-type composition did not affect the results of perceived off-time pubertal timing predicting lower PhenoAge acceleration and phenotypic off-time pubertal timing predicting accelerated PhenoAge in males. Thus, the effects of pubertal timing on PhenoAge may be most robust to differences in cell-type composition.

It also should be noted that only phenotypic early pubertal timing significantly predicted higher epigenetic mortality risk when excluding the Non-Black participants. However, the effect of perceived pubertal timing not reaching statistical significance in the Black-only sample can likely be attributed to the smaller sample size and reduced statistical power as the observed effect size was even slightly larger compared to the main analysis with all participants. Similarly, the effect of phenotypic early pubertal timing on accelerated DunedinPACE did not reach significance when excluding Non-Black participants from the analyses. The quadratic effects of pubertal timing on PhenoAge replicated in the Black-only sample.

Both in the present study and prior research, Black youth experienced on average earlier pubertal timing and accelerated epigenetic aging compared to White youth (Hamlat et al., 2023; Herman-Giddens et al., 2012). These racial differences reflect a history of race-related discrimination and inequities that relate to both earlier pubertal timing (Argabright et al., 2022) and accelerated epigenetic aging (Krieger et al., 2024). While the results do not suggest that the effects of early pubertal timing on epigenetic aging and mortality risk are more pronounced in Black youth, the greater prevalence of both early pubertal timing and accelerated epigenetic aging in racial/ethnic minority youth may be one factor that contributes to health disparities.

Implications

The present findings have theoretical implications by highlighting differences in the effects of pubertal timing on PhenoAge as compared to GrimAge, DunedinPACE, and epigenetic mortality risk. Thus, these findings may inform future research to focus on differences between epigenetic clocks and how these differences may explain differential effects of pubertal timing. While all of the epigenetic outcomes included in this study are epigenetic markers of chronic disease risk, they are computed differently and have shown varying

levels of associations with risk factors (Yusipov et al., 2024). Since the present results suggest that pubertal timing has a non-linear effect on PhenoAge acceleration and includes sex differences, these findings suggest that all of the three main hypotheses (i.e., *early timing hypothesis*, *deviance hypothesis*, and *gender deviation hypothesis*) need to be considered when examining links between pubertal timing and epigenetic markers of health.

The present findings may also have practical implications for identifying early adolescents at risk for epigenetic aging and mortality risk in adulthood. Specifically, the results suggest that youth who perceive their pubertal timing as early as well as youth with early phenotypic pubertal timing are at risk for poorer health outcomes in adulthood based on epigenetic markers of health and may benefit from early screenings and interventions to reduce epigenetic age acceleration and mortality risk. Assessments of DNA methylation-based epigenetic aging and mortality risk may provide opportunities to detect health risks associated with pubertal timing by young adulthood and therefore allow early implementation of interventions to buffer chronic disease risk in the long-term.

Suggesting that early pubertal timing may be a risk factor for epigenetic age acceleration, the present results also highlight the importance of developing a lifestyle during childhood and adolescence that reduces the risk for early pubertal timing and the associated risk for poorer health outcomes in adulthood. The present results show that early pubertal timing predicts higher epigenetic mortality risk and accelerated DunedinPACE in adulthood even when adjusting for early life stress and other health risks. Thus, the present findings may also inform future interventions designed to help youth cope with early pubertal timing. Despite perceived and phenotypic pubertal timing being unrelated to each other, both types of early pubertal timing were linked with poorer epigenetic health. Thus, intervention efforts need to be tailored towards both adolescents who perceive their pubertal timing as earlier than their peers and youth who phenotypically experience early pubertal timing. Youth who perceive their pubertal timing as earlier compared to their peers may benefit from interventions that reduce peer comparison and promote a positive self-image. Interventions for adolescents with earlier phenotypic pubertal timing may instead focus on strengthening abilities to cope with puberty-related hormonal stress and creating an environment in which early maturing youth are not treated differently due to their more mature appearance.

Ultimately, results from a sensitivity analysis excluding Non-Black participants did not show amplified effects of early pubertal timing on epigenetic aging. However, racial/ethnic minority youth experienced on average earlier pubertal timing and higher epigenetic aging and mortality risk compared to White youth. These findings may reflect existing health disparities and underscore the need of targeted interventions that may include monitoring and early intervention for the health risks associated with early pubertal timing and accelerated cellular aging in racially diverse youth.

Strengths and Limitations

A key strength of the present study is its longitudinal design, which enabled assessing pubertal timing during adolescence and DNA methylation-based mortality risk and epigenetic age acceleration fourteen years later during young adulthood. Another strength is the inclusion of two distinct measures of pubertal timing in the form of perceived and

phenotypic pubertal timing. Additionally, this study included the previously understudied population of predominantly Black males and females. A final strength is the inclusion of three epigenetic clocks that have been validated with physiological markers in addition to chronological age (Belsky et al., 2022; Levine et al., 2018; Lu et al., 2019) and a mortality risk score, which may arguably be the strongest DNA methylation-based marker for adverse health outcomes (Zhang, Wilson et al., 2017).

Nevertheless, some limitations need to be considered when interpreting the present results. A major limitation is that DNA methylation was not assessed at the time of puberty, which did not allow the testing of temporal precedence of early pubertal timing and changes in epigenetic mortality risk and age acceleration. Thus, the present findings cannot preclude that pre-existing differences in DNA methylation-based epigenetic aging or mortality risk before or during puberty confound the observed effects. A second limitation may be that the epigenetic clocks were initially developed in blood tissue and estimates of epigenetic aging may therefore be less accurate in saliva samples. Previous research comparing effects of socioeconomic status across tissue found consistent results but smaller effect sizes for saliva compared to blood tissue in the GrimAge, DunedinPACE, and PhenoAge clocks (Raffington et al., 2023).

A third limitation is that pubertal timing was assessed at a single time point around the age of 13 and thus may not have captured differences in the timing of pubertal changes that occurred earlier. Nevertheless, there was sufficient variability in Tanner-stage pubertal status ($SD = 0.90$, Range 1-5) and only a few of the youth (6.5%) reported the most mature pubertal status at the time of assessment. Even though the use of Full Information Maximum Likelihood minimized the bias introduced by missing data, the present findings may be less generalizable to youth with lower family income at Time 1 who were more likely to have missing data. Finally, the study is limited by not including a measure of general stress level during adolescence or puberty-related stress. It was therefore not possible to empirically test heightened stress as a mechanism explaining the links between pubertal timing and epigenetic health outcomes.

Conclusion

The present findings make novel contributions by suggesting that both male and female youth with earlier perceived and phenotypic pubertal timing are at an elevated risk of DNA methylation-based mortality risk during young adulthood even after adjusting for tobacco smoking, BMI, early life stress and socio-demographic variables. Additionally, early phenotypic pubertal timing emerged as a unique predictor of accelerated DunedinPACE in both males and females. Thus, the present findings contribute to the literature by extending links between early pubertal timing and epigenetic age acceleration to racially diverse males and females. However, the results provide no evidence for linear effects of early pubertal timing on accelerated PhenoAge during young adulthood and instead suggest that males who experience phenotypic off-time pubertal timing (i.e., early or late) are at risk for accelerated PhenoAge in adulthood. In contrast, perceived off-time pubertal timing was surprisingly associated with less PhenoAge acceleration. Despite these contradicting findings on the PhenoAge clock, findings on the three other epigenetic markers suggest that

an increased proneness to chronic disease and epigenetic mortality risk in early maturing youth may be already detectable by the third decade of life. DNA methylation-based estimates of epigenetic mortality risk and accelerated aging may present a mechanism through which early pubertal timing increases the risk for chronic disease later in life. These findings may provide interesting avenues for future research to examine mechanisms through which early pubertal timing affects health and highlight the need to identify protective factors that could mitigate the negative long-term effects of early pubertal timing on health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Public Significance Statement:

This longitudinal study overall suggests that experiencing pubertal maturation at a younger age as well as perceiving one's pubertal timing as earlier compared to peers is associated with negative health outcomes in the form of accelerated cellular aging and epigenetic mortality risk in racially diverse male and female adults. Changes in DNA methylation may present a mechanism explaining why early maturing youth are at a higher risk for chronic disease later in life.

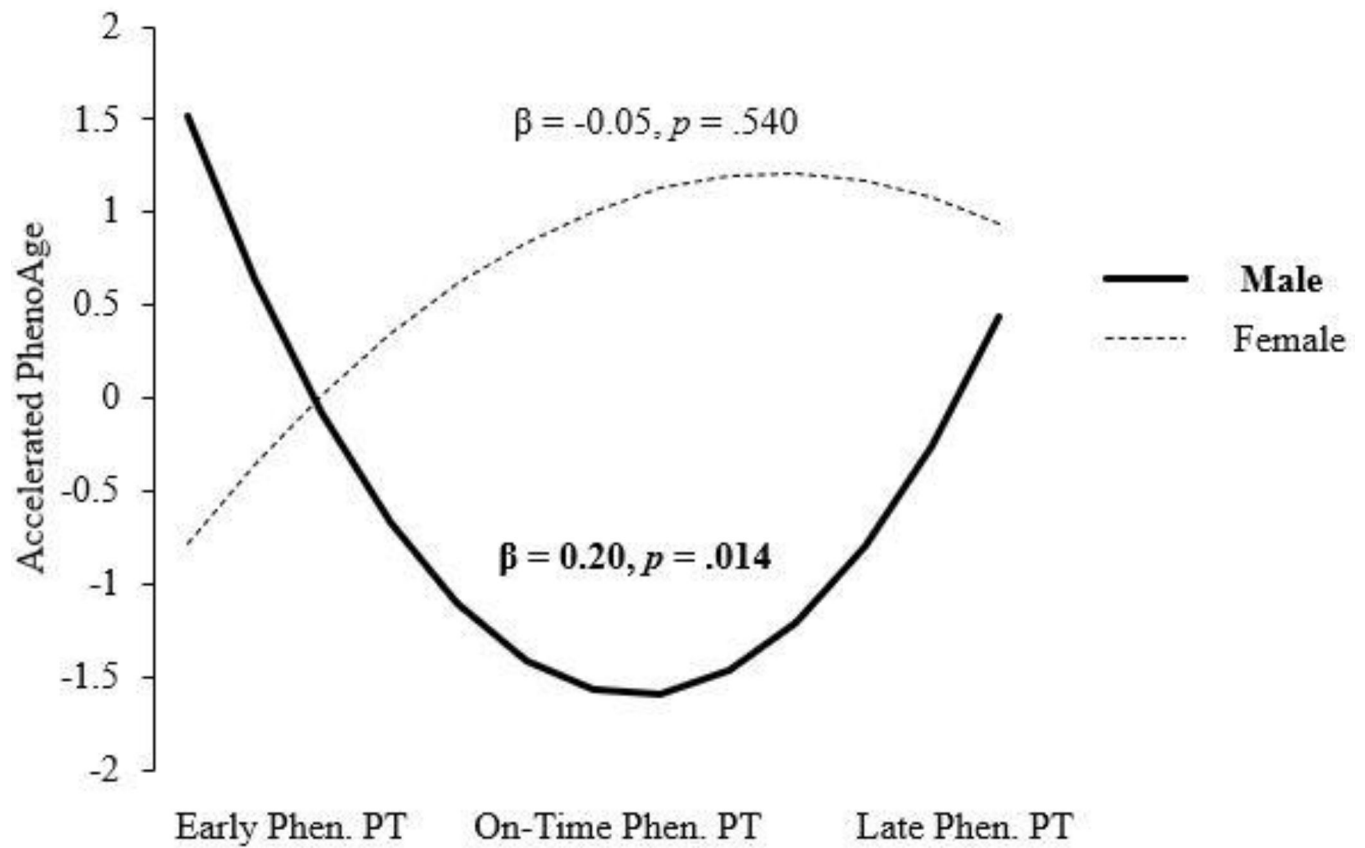


Figure 1.
Simple slopes depicting the effect of off-time phenotypic pubertal timing on accelerated PhenoAge by sex.
Note: Phen. PT – Phenotypic Pubertal Timing.

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Table 1
Descriptive Statistics and Information on Normality for the Variables Included in the Analyses

	M (SD) / %	Min, Max	Skewness, Kurtosis
Perceived Early PT (T1)	0.00 (1.03)	-2.09, 1.63	-0.13, -0.19
Phenotypic Early PT (T1)	0.00 (0.84)	-2.06, 2.18	0.02, -0.50
Mortality Risk (T2)	0.00 (0.99)	-3.78, 2.63	0.29, -0.14
GrimAge (T2)	0.00 (3.62)	-7.68, 9.39	0.37, -0.53
DunedinPACE (T2)	0.00 (0.15)	-0.68, 0.49	-0.09, 1.22
PhenoAge (T2)	0.00 (5.15)	-17.78, 14.63	0.06, -0.04
BMI (T2)	31.48 (9.55)	16.91, 70.65	1.06, 1.12
Smoking (T2)	3.87 (6.97)	0.00, 35.00	1.90, 3.06
Family Income (T1)	6.65 (3.86)	1.00, 13.00	0.02, -1.33
Early Life Stress	1.36 (0.28)	1.00, 2.64	1.32, 2.13
Racial/Ethnic Minority	81%	-	-
Female	58%	-	-
Parenthood	48%		

Note: DNAm Age variables and mortality risk adjusted for age. Early PT – Early Pubertal Timing, T1 – Time 1, T2 – Time 2.

Table 2
Bivariate Correlations between Pubertal Timing, Mortality Risk, Epigenetic Age Acceleration, and Covariates.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
1. Perceived Early PT (T1)	1.00											
2. Phenotypic Early PT (T1)	.11	1.00										
3. Mortality Risk (T2)	.14*	.15**	1.00									
4. GrimAge (T2)	.15**	.14*	.70***	1.00								
5. DunedinPACE (T2)	0.04	.19***	.36***	.46***	1.00							
6. PhenoAge (T2)	.04	.02	.29***	.20***	.32***	1.00						
7. BMI (T2)	.05	.03	-.21***	-.09	.28***	.20***	1.00					
8. Smoking (T2)	.09	.00	.44***	.44***	.10	.07	-.13*	1.00				
9. Family Income (T1)	-.03	-.09	-.07	-.18**	-.20***	.03	-.10	-.18**	1.00			
10. Early Life Stress	.16**	.10	.10	.13*	.06	.07	.07	.19***	-.24***	1.00		
11. Female	-.13*	.07	-.16**	-.12*	.26***	.15**	.16**	-.19***	-.13*	.04	1.00	
12. Racial/Ethnic Minority	.06	.26***	.13*	.19***	.23***	-.03	.08	-.08	-.32***	.02	.16**	1.00
13. Parenthood (T2)	-.01	.03	.13*	.16**	.15**	.07	-.02	.15**	-.23***	.21***	.15**	.18**

Note: All DNAm Age clocks and the mortality risk score are adjusted for chronological age, PT – Pubertal Timing, T1/2 – Time 1/2

*
p < .05,
**
p < .01,

p < .001.

Table 3
Multiple Regressions Predicting Mortality Risk and Epigenetic Age Acceleration from Perceived Pubertal Timing and Covariates.

Variable	Mortality Risk (T2) β (p)	GrimAge (T2) β (p)	DunedinPACE (T2) β (p)	PhenoAge (T2) β (p)
Step 1	$R^2 = 0.27$	$R^2 = 0.26$	$R^2 = 0.20$	$R^2 = 0.09$
Perceived Early PT (T1)	0.10 (.049)	0.10 (.060)	0.04 (.465)	0.04 (.528)
Family Income (T1)	0.05 (.356)	-0.03 (.626)	-0.04 (.487)	0.11 (.086)
Smoking (T2)	0.41 (< .001)	0.41 (< .001)	0.18 (< .001)	0.12 (.034)
BMI (T2)	-0.17 (.001)	-0.05 (.283)	0.26 (< .001)	0.20 (< .001)
Female	-0.07 (.161)	-0.06 (.211)	0.22 (< .001)	0.16 (.004)
Racial/Ethnic Minority	0.18 (< .001)	0.21 (< .001)	0.17 (.001)	-0.04 (.437)
Early Life Stress	0.00 (.970)	0.00 (.390)	-0.05 (.382)	0.04 (.501)
Parenthood (T2)	0.05 (.308)	0.06 (.234)	0.07 (.176)	0.05 (.327)
Step 2a	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$
Perceived Early PT X Female	-0.08 (.291)	0.01 (.924)	-0.06 (.433)	0.06 (.494)
Step 2b	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 = 0.03$
Perceived off-time PT	-0.04 (.421)	0.04 (.391)	0.04 (.407)	-0.17 (.002)
Step 3ab	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$
Perceived off-time PT X Female	-0.04 (.683)	-0.08 (.371)	-0.04 (.701)	-0.02 (.851)

Note: Mortality risk and epigenetic age variables are adjusted for chronological age. Standardized coefficients are shown. PT = Pubertal Timing, T1 = Time 1, T2 = Time 2. Significant effects ($\alpha = .05$) in **bold** font.

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Table 4
Multiple Regressions Predicting Mortality Risk and Epigenetic Age Acceleration from Phenotypic Pubertal Timing and Covariates.

Variable	Mortality Risk (T2) β (p)	GrimAge (T2) β (p)	DunedinPACE (T2) β (p)	PhenoAge (T2) β (p)
Step 1	$R^2 = 0.27$	$R^2 = 0.26$	$R^2 = 0.20$	$R^2 = 0.08$
Phenotypic Early PT (T1)	0.13 (.013)	0.09 (.069)	0.13 (.015)	0.01 (.884)
Family Income (T1)	0.05 (.364)	-0.03 (.637)	-0.04 (.447)	0.11 (.084)
Smoking (T2)	0.41 (< .001)	0.41 (< .001)	0.18 (< .001)	0.12 (.031)
BMI (T2)	-0.16 (.001)	-0.05 (.352)	0.26 (< .001)	0.20 (< .001)
Female	-0.09 (.076)	-0.08 (.097)	0.21 (< .001)	0.15 (.006)
Racial/Ethnic Minority	0.16 (.002)	0.19 (< .001)	0.14 (.011)	-0.04 (.460)
Early Life Stress	0.01 (.809)	0.01 (.849)	-0.05 (.326)	0.05 (.418)
Parenthood (T2)	0.05 (.336)	0.06 (.229)	0.07 (.153)	0.05 (.337)
Step 2a	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 = 0.01$
Phenotypic Early PT X Female	-0.01 (.941)	-0.01 (.928)	0.03 (.734)	0.12 (.178)
Step 2b	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 = 0.01$
Phenotypic off-time PT	0.04 (.394)	0.01 (.792)	-0.01 (.803)	0.07 (.199)
Step 3ab	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 < 0.01$	$\Delta R^2 = 0.02$
Phenotypic off-time PT X Female	-0.01 (.891)	-0.03 (.719)	0.03 (.772)	-0.21 (.027)

Note: Mortality risk and epigenetic age variables are adjusted for chronological age. Standardized coefficients are shown. PT – Pubertal Timing, T1 – Time 1, T2 – Time 2. Significant effects ($\alpha = .05$) in **bold** font.