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Childhood Adversity, Accelerated GrimAge, and Associated Health Consequences

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Abstract

Objective: Childhood adversity is linked to psychological, behavioral, and physical health problems, including obesity and cardiometabolic disease. Epigenetic alterations are one pathway through which the effects of early life stress and adversity might persist into adulthood. Epigenetic mechanisms have also been proposed to explain why cardiometabolic health can vary greatly between individuals with similar Body Mass Index (BMIs).

Methods: We evaluated two independent cross-sectional cohorts of adults without known medical illness, one of which explicitly recruited individuals with early life stress (ELS) and control participants (n=195), and the other a general community sample (n=477). In these cohorts, we examine associations between childhood adversity, epigenetic aging, and metabolic health.

Results: Childhood adversity were associated with increased GrimAge Acceleration (GAA) in both cohorts, both utilizing a dichotomous yes/no classification (both p<0.01) as well as a

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continuous measure using the Childhood Trauma Questionnaire (CTQ) (both $p<0.05$). Further investigation demonstrated that CTQ subscales for physical and sexual abuse (both $p<0.05$) were associated with increased GAA in both cohorts, whereas physical and emotional neglect were not. In both cohorts, higher CTQ was also associated with higher BMI and increased insulin resistance (both $p<0.05$). Finally, we demonstrate a moderating effect of BMI on the relationship between GAA and insulin resistance where GAA correlated with insulin resistance specifically at higher BMIs.

Conclusions: These results, which were largely replicated between two independent cohorts, suggest that interactions between epigenetics, obesity, and metabolic health may be important mechanisms through which childhood adversity contributes to long-term physical and metabolic health effects.

Keywords

Epigenetic aging; GrimAge; childhood trauma; childhood adversity; health outcomes

Introduction

Early life stress has well documented long-lasting deleterious effects on physical and psychological health[1–6]. Although progress has been made toward uncovering molecular mechanisms of how early life stress impacts future medical conditions, more research is needed to inform the development of personalized, mechanistically-based interventions[7]. One of the etiological pathways underlying the long-term effects of early life stress on future functioning is alterations in molecular pathways and epigenetic profiles. It is hypothesized that epigenetic modifications during early life may be stable throughout adulthood[8]. DNA methylation, whereby a methyl-group is added to CpG sites, can regulate expression of nearby genes, and changes in DNA methylation is known to be associated with a number of biological processes such as aging and development[9,10]. Thus, epigenetic changes such as DNA methylation could be mechanistic causes of the downstream health effects of early life stress. Recently, DNA methylation has been associated with accelerated biological aging as well as stress and adversity[11,12]. Prior analyses have also demonstrated that exposure to early adversity is associated with reduced telomere length[13–16], the repetitive regions at the end of chromosomes which can be an index of biological aging.

There are multiple ways to estimate an individual's biological age, and over the past decade methodologic advances have led to the development of epigenetic clocks, which correlate closely with measures of long-term health[17–20]. Epigenetic clocks provide an estimate of biological age ("epigenetic aging") by assessing DNA methylation at sites across the genome at sites associated with aging. One of these epigenetic clocks, "GrimAge", was trained through the selection of DNA methylation sites that predict morbidity and mortality through several "sub-clocks" correlated with known biomarkers of health and aging. GrimAge incorporates over 1000 methylation sites in addition to gender and chronologic age, and has shown the ability to predict mortality and multiple serious health conditions, including coronary heart disease and cancer[18,21–23]. GrimAge has been associated with adverse stressful and social exposures like SES and education[24] as well as better morbidity and mortality prediction[22], and thus we selected it as our primary outcome measure.

Adjusting for chronological age yields an index of epigenetic age acceleration, which captures the difference between an individual's chronological age and their epigenetic age.

While epigenetic clocks primarily serve as markers of biological aging, they may also relate to the development of chronic conditions such as cardiometabolic disease. Epigenetic changes are thought to play an important role in both the development and maintenance of cardiometabolic disease[25,26], which are influenced by both genetic and environmental factors[27–31], and may be modifiable either directly or through other interventions[32–35]. Obesity and dysregulation in eating behaviors are associated with early life stress[36–40], and stress- and BMI-related changes in metabolic and appetite-related hormones are associated with alterations in eating behaviors, which may perpetuate and exacerbate weight gain and metabolic disturbances[41,42]. Prior work in both mice and humans has demonstrated interactions between stress, obesity, and specific methylation sites[29,43,44], although not all have found statistically significant effects[45].

The relationship between obesity and an individual's metabolic health can vary substantially. Recent work has attempted to study the variability in metabolic health among individuals with obesity[46–51]. This variability may be associated with differences in muscle mass and health behaviors such as diet and exercise[52–54]. Epigenetic changes, including those seen in epigenetic aging, may represent a biological mechanism that determines which individuals with obesity are at increased risk for developing metabolic disease[55,56], and recent work by Kim et al in the CARDIA study suggest that GrimAge may play an important role in the relationship between obesity and diabetes risk in middle-aged individuals[57]. However, what role GrimAge plays in the relationship between obesity and insulin resistance at younger ages has not been studied.

This study utilizes two independently recruited cohorts of adults without known chronic medical illness from separate sites and research groups to investigate relationships between childhood adversity, epigenetic age, and metabolic health. The focus on adults without known chronic medical illness complements other studies which typically include these participants[58–60], minimizing potential confounding effects of medications and medical conditions. This study represents the first assessment of epigenetic aging in the LIFE Study, and further expands upon prior work in the Yale Stress Center cohort where we previously found that accelerated GrimAge was linked to higher lifetime (not childhood) adversity[12]. To our knowledge, the LIFE Study is the first investigation of epigenetic aging using the Childcare and Abuse Interview for the identification of childhood adversity. Additionally, while we identified relationships between GrimAge and insulin resistance, how BMI interacts with these variables remains unclear both in individuals with and without childhood adversity. Thus, the purpose of this cross-sectional study was threefold: using two independent cohorts, we (1) characterized the relationship between childhood adversity and accelerated aging (GrimAge) in adulthood, (2) examined the relationship of childhood adversity with metabolic risk markers, and (3) tested moderator effects of accelerated aging on the association between BMI and insulin resistance in each cohort separately. We hypothesized that childhood adversity would be associated with accelerated epigenetic aging and increased metabolic risk, and that epigenetic changes associated with aging may be important moderators of the relationship between BMI and insulin resistance.

Materials and Methods

Cohort Recruitment

The Lifestyle Influences of Family Environment (LIFE) Study cohort included young adults without known medical illness aged 18–40 as previously described[61]. Briefly, participants were from the greater Providence, RI area and were recruited using internet and community advertisements seeking healthy individuals with stable two-parent households and those with a history of early life stress (ELS), including parental loss and maltreatment between Octoboer 2014 and March 2020. The Childhood Care and Abuse (CECA) Interview was used to identify those with early life stress. the ELS group had at least moderate severity of one or more maltreatment domain within the CECA prior to age 18. Phone screens evaluated prospective participant eligibility. The sample was selected to exclude individuals with medication use and diagnosed medical conditions to avoid confounding and to examine specific physiological effects of childhood adversity in individuals without known illness. Voluntary written and informed consent was obtained, and the study was approved by the Butler Hospital Institutional Review Board. Participants who had Peripheral blood mononuclear cell (PBMC) DNA methylation array data (N=195) were included in this analysis. Participants with ELS (n=115 cases) had a history of moderate-to-severe childhood maltreatment, and most also experienced childhood parental death or prolonged separation. Participants in the control group (n=80 controls) had no history of childhood maltreatment or psychiatric disorder and were raised in two-parent homes without parental separation, divorce, or loss. Acute and chronic medical conditions, pregnancy, and use of daily medications other than hormonal contraceptives were exclusionary. Thirteen Participants who endorsed major acute stressors, illness, recent vaccination, or sleep loss were rescheduled to participate after these issues were resolved. Positive drug screens or current substance use disorders were initially exclusionary, but in order to support adequate recruitment, this criterion was adjusted to include ELS participants who reported using marijuana 3 or more times per week or who tested positive for tetrahydrocannabinol (THC, n=15) and n=1 control participant who also tested positive for THC. In addition, ELS participants who met the criteria for a current substance use disorder were included if usage patterns suggested a low risk of either intoxication or withdrawal during study participation (n=7 cannabis only, n=4 alcohol only, and n=2 with both disorders). Individuals with primary obsessive-compulsive disorder, bipolar disorder, and psychotic disorders were excluded. As medical conditions and/or medications are known to be linked to biological parameters of health, including accelerated epigenetic aging[62,63], the inclusion/exclusion criteria were aimed at minimizing the effects of these potential confounders and better isolating the effects of ELS on our outcomes interest. Participants with current substance use disorders were included to support adequate enrollment only if their usage patterns suggested a low risk of withdrawal or intoxication affecting the outcome variables of interest.

The Yale Stress Center cohort described previously[12], included adults between the ages of 18–50 (n = 477) in the greater New Haven, CT area who volunteered to participate in a study examining the role of stress and self-control at the Yale Stress Center. Participants were recruited via advertisements online, in local newspapers, and at a community center between 2008 and 2012. Participants were excluded if they had an active psychiatric or substance use disorder (not including nicotine) as assessed via the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (SCID-I for DSM-IVTR,[64]), were pregnant, had a chronic medical condition (e.g., hypertension, diabetes, hypothyroidism), or were unable to read English at or above the 6th grade level. Participants on medications for any chronic medical conditions were excluded. Participants were also excluded if they had a head injury or were using any prescribed medications for any psychiatric or medical disorders. Participants were screened for substance use via breathalyzer and urine toxicology tests. The sample of 477 came from a total of 1000 participants who provided physiologic and behavioral data, and epigenetic data were only available on 477, who comprised the current sample. All participants provided written and verbal informed consent to participate, and the research protocol was reviewed and approved by the Yale IRB.

Early adversity, psychiatric and metabolic assessments

LIFE study participants met with study staff during two study visits for a fasting blood draw, evaluation of medical history with a laboratory-developed standardized interview, identification of DSM-V psychiatric illnesses with the SCID-5-RV, assessment of early life stress with the Childhood Care and Abuse (CECA) Interview, and completion of self-report questionnaires as previously described[61]. Prior to the release of the SCID-5, a subset of participants (n =35) had psychiatric diagnoses assessed using the Mini-International Neuropsychiatric Interview for the DSM-IV (MINI), which was adapted to include information for DSM-5 diagnoses[65]. Height and weight were measured and used to calculate body mass index (BMI, weight [kg]/height [m^2]). A fasting venous blood sample was collected at the first study visit at approximately 8:30 AM and assessed for insulin and glucose levels. Participants were financially compensated for participating in the study.

The Yale Stress Center study had all eligible participants meet with a research assistant for two study visits with a structured clinical interview for diagnoses of DSM-IVTR psychiatric illnesses[64], a cumulative stress interview using the Cumulative Adversity Index (a 140-item structured interview of life events and subjective stress[66]), medical history, self-report questionnaires, and a separate morning biochemical evaluation after fasting overnight as previously described[12]. Height and weight were measured and used to calculate BMI. Fasting venous blood samples to measure insulin and glucose were obtained at approximately 7:30 AM. Participants were financially compensated for participating in the study.

The Childhood Trauma Questionnaire (CTQ), a common and well-validated 28-item assessment of a variety of traumatic experiences prior to the age of 18[67], was used in both studies. The CTQ contains 5 subscales assessing Physical, Emotional, and Sexual Abuse, and Physical and Emotional Neglect on a 5-point Likert scale. The total CTQ score was calculated by summing the responses for the subscales of Physical Abuse, Emotional Abuse, Sexual Abuse, Physical Neglect, and Emotional Neglect, and regression analyses regarded the CTQ as a continuous variable. For the LIFE Study, Chronbach's alpha was 0.90 for the full scale, and as follows for each of the subscales: Physical Abuse (0.88), Emotional Abuse

(0.91), Sexual Abuse (0.96), Physical Neglect (0.87), and Emotional Neglect (0.94). For the Yale Stress Center study, Chronbach's alpha was 0.92 for the full scale, and as follows for each of the subscales: Physical Abuse (0.75), Emotional Abuse (0.86), Sexual Abuse (0.95), Physical Neglect (0.65), and Emotional Neglect (0.91).

Both studies also used the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated using the equation fasting serum insulin (μU/ml) × fasting plasma glucose (mmol l-1)/22.5)[68,69].

The Childhood Experiences of Care and Abuse (CECA[70,71]) is a semi-structured interview that assesses physical abuse, sexual abuse, parental neglect, psychological abuse, and antipathy. It also assesses for childhood parental loss and violence between parents. Interviews were conducted by a trained interviewer, and scores were independently reviewed by a second scorer. Unclear cases were settled with consensus scoring. Individuals in the ELS group had at least moderate severity of one or more maltreatment domain within the CECA prior to age 18.

DNA Methylation and epigenetic clock analysis

For the LIFE Study, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and stored at −80°C prior to isolating DNA for epigenetic analysis and assay with the Illumina Infinium Human MethylationEPIC (EPIC) BeadChip. The EPIC array contains over 850,000 probes and shows cross-platform reliability with its precursor, the Illumina Infinium HumanMethylation450 BeadChip[72]. Quality control, including the removal of cross-reactive probes, was performed[72].

For the Yale Stress Center study, DNA for epigenetic analysis was collected from whole blood samples as previously described[73]. Briefly, all samples were profiled using the Illumina Infinium HumanMethylation450 BeadChip, which covers 96% of CpG islands and 99% of RefSeq genes. Quality control was performed as previously published[73].

For both studies, after obtaining beta values, epigenetic clock analysis was performed. For GrimAge and other clocks using traditional calculation methods, analyses were performed as described in Lu et al. using the New Methylation Age Calculator at https://dnamage.genetics.ucla.edu/new[23]. The data were normalized as per the Lu et al. protocol, and the advanced analysis option was used. Secondary analyses (see supplementary tables 5 and 10) utilized principal component-based calculation methods. These analyses were performed as described by Higgins-Chen et al. using code provided by the authors[74]. In an effort to focus analyses in a hypothesis-driven manner, our primary analyses utilized GrimAge acceleration (defined as the residuals of a linear regression of GrimAge on chronologic age) due to its relationship to morbidity and mortality and prior data demonstrating a link between GrimAge and lifetime adversity in the Yale cohort[12]. For GrimAge Acceleration, positive values suggest that the participant is biologically older than would be expected for their chronologic age, whereas negative values suggest they are biologically younger than their chronologic age.

Statistical analysis

The LIFE Study cohort and Yale Stress Center cohort were assessed independently. In the LIFE Study cohort, all Control (non-ELS) participants had a CTQ of 38 or less. Thus, for the Yale Stress Center cohort (which was not selected or grouped on the basis of childhood adversity), we used a cutoff of CTQ ≥ 39 to define the "High Childhood Adversity" group. Thus, the "Low Childhood Adversity" group had a CTQ of 38 or less.

Data organization and analysis were conducted using R 3.6.3[75] and RStudio. R scripts used for analysis are available from author ZMH upon request. When comparing groups (i.e., case/control for ELS in the LIFE Study cohort, high/low childhood adversity in Yale Stress Center cohort) without covariates, Wilcoxon rank-sum tests were used to account for non-normality (Data found to be non-normal by Shapiro-Wilks test). Analyses with GrimAge Acceleration as the dependent variable used univariate linear regression, then multivariate linear regression to incorporate age, self-reported sex, self-reported race, current smoking status, and blood cell count proportions as covariates where indicated in the text. As we are not ranking predictors, unstandardized coefficients are reported to allow direct interpretation of how changes in the independent variable impact the dependent variable. For the LIFE study, cell count proportions were determined by laboratory blood count, dropping neutrophils to avoid overfitting. As blood counts were not available for the Yale Stress Center cohort, cell count proportions were determined by the Houseman method[76], dropping granulocytes to avoid overfitting. To assess for moderating relationship between GrimAge Acceleration and BMI on HOMA-IR, interaction terms were incorporated into regression analyses. Analyses with HOMA-IR as the dependent variable used multivariate linear regression to incorporate age, self-reported sex, self-reported race, and current smoking status as covariates. ANOVA was used to compare whether incorporation of the interaction terms significantly improved the variance prediction of the models.

Results

Demographics and clinical characteristics

As shown in Table 1, the LIFE Study cohort and the Yale Stress Center cohort had similar age and BMI but differed in racial and gender composition as well as trauma exposure. As would be expected given the sampling methodology, participants in the LIFE Study cohort had a higher overall childhood trauma burden. The LIFE Study also had a higher proportion of women and white participants, and fewer individuals who smoke cigarettes.

Childhood adversity is associated with increased GrimAge and GrimAge Acceleration in the LIFE Study Cohort

We first evaluated whether Early Life Stress (ELS) group was associated with increased epigenetic age in adulthood in the LIFE Study Cohort. We found that participants with ELS (Cases) had higher GrimAge than Controls (those without ELS) (Figure 1A, beta = 1.61, $p = 0.0001$ accounting for chronologic age). We next evaluated the residuals for GrimAge after regression to chronologic age (e.g., GrimAge Acceleration). GrimAge Acceleration represents the difference between an individual's chronologic age and epigenetic age, with a positive value indicating that their epigenetic age is higher than expected based on their chronologic age. We found that those with ELS had higher GrimAge Acceleration (Figure 1B, $p = 3.01e\text{-}5$ via Wilcoxon test) than Controls. Those with ELS also had increased GrimAge Acceleration after accounting for age, sex, race, smoking status, and cell type proportions (beta = 1.05, $p = 0.0076$, see Supplementary table 1). This association remained significant when also accounting for SES by including education level and income level as covariates (beta = 0.85, $p = 0.037$, see Supplementary table 2). When clocks other than GrimAge were examined, traditional calculation methods identified significant raw differences between those with ELS and those without in Hannum and Skin-Blood clocks, and near-significant differences in PhenoAge and DNAmTL clocks (Supplementary table 4). Using principal-component based methods, all clocks showed significant raw differences between those with ELS and those without (Supplementary table 5).

While the method of recruitment and classification of those with ELS versus Controls in the LIFE study was binary, the CTQ was available as a quantitative method of assessing childhood adversity. We observed a significant relationship between the total CTQ score and GrimAge Acceleration after accounting for age, sex, race, smoking status, and cell type proportions (Figure 1C, "all", beta = 0.0192, $p = 0.0217$, see supplementary table 3). As would be expected, there was a large and highly significant difference in CTQ score between those with ELS and Controls ($p < 2.2e\text{-}16$ via Wilcoxon test).

Childhood adversity is associated with increased GrimAge and GrimAge Acceleration in the Yale Stress Center cohort

We next asked whether GrimAge and GrimAge Acceleration was also increased with childhood adversity with the cohort from the Yale Stress Center. Based on the LIFE Study CTQ values, we used a CTQ cutoff of ≥ 39 to define the "High Childhood Adversity" group. Using this grouping, we observed similar findings as in the LIFE Study cohort. The High Childhood Adversity group had higher GrimAge (Figure 1D, beta = 1.03, $p = 0.0007$) when compared to the Low Childhood Adversity group after accounting for age. We also observed higher GrimAge Acceleration in the High Childhood Adversity group (Figure 1E, $p = 0.00249$ via Wilcoxon test) compared to the Low Childhood Adversity group. This finding was maintained after accounting for age, sex, race, smoking status, and cell type proportions (beta = 0.637, $p = 0.0090$, see Supplementary table 6). Again, this association remained significant after accounting for years of education and total income (beta = 0.518, $p = 0.0351$, see Supplementary table 7). When using traditional calculation methods, other clocks did not detect differences between the high and low childhood adversity groups (Supplementary table 9). Using principal-component based methods, PC-GrimAge was significant in all models, and PC-PhenoAge was significant when including all covariates except smoking (Supplementary table 10).

When we utilized the CTQ score as a quantitative measurement in the Yale Stress Center cohort, we again observed a significant relationship between total CTQ score and GrimAge Acceleration (Figure 1F, "all", beta = 0.026, $p = 0.00541$ after accounting for age, smoking status, race, sex, and cell type proportions, see Supplementary table 8).

Childhood physical and sexual abuse were associated with increased GrimAge Acceleration in both cohorts

The CTQ score assesses multiple aspects of childhood adversity, including physical, emotional, and sexual abuse, as well as physical and emotional neglect. We next analyzed whether each of these subscales individually correlate with GrimAge Acceleration, after controlling for covariates. In the LIFE Study cohort, subscale scores for Physical Abuse (beta = 0.087, $p = 0.0192$) and Sexual Abuse (beta = 0.079, $p = 0.0148$) each were associated with higher GrimAge Acceleration when accounting for age, sex, race, smoking status, and cell type proportions. (Table 2).

In the Yale Stress Center cohort, we again found that both Physical Abuse (beta = 0.117, $p = 0.0090$) and Sexual Abuse (beta = 0.171, $p < 0.0001$) were associated with higher GrimAge Acceleration (Table 3). In the Yale Stress Center cohort, we also found a relationship between Emotional Abuse and GrimAge Acceleration (beta = 0.065, $p = 0.0195$). Neither the LIFE Study cohort nor the Yale Stress Center cohort showed a significant relationship of Physical Neglect or Emotional Neglect with GrimAge Acceleration (p values > 0.05).

Adversity history is associated with elevated metabolic risk factors in both cohorts

While both cohorts had no known chronic medical illnesses at the time of study participation, childhood adversity is associated with increased cardiometabolic risk. In the LIFE Study cohort, those with ELS had greater BMI ($p = 0.0027$ via Wilcoxon test), waist circumference ($p = 0.0488$ via Wilcoxon test), and HOMA-IR ($p = 0.0023$ via Wilcoxon test) in comparison to Controls. Similarly, higher CTQ score was associated with higher BMI (beta = 0.056, $p = 0.0025$), higher waist circumference (beta = 0.0396, $p = 0.0423$), and higher HOMA-IR (beta = 0.011, $p = 0.031$) in univariate regression analyses. Examining the CTQ subscales individually, each subscale except physical neglect ($p = 0.068$) was significantly associated with BMI (p values < 0.02 for each subscale). For waist circumference, all individual CTQ subscales were not significant (0.06 < all subscale p values < 0.14). When examining the subscale relationships with HOMA-IR, emotional abuse (beta = 0.044, $p = 0.021$), emotional neglect (beta = 0.040, $p = 0.048$), and sexual abuse (beta = 0.050, $p = 0.014$) were significantly associated with HOMA-IR, while physical abuse and neglect were not (p values > 0.1).

In the Yale Stress Center cohort, the High Childhood Adversity group had significantly higher BMI than the Low Childhood Adversity group ($p = 1.65e-5$ via Wilcoxon test), though HOMA-IR was not significantly different between groups ($p = 0.148$ via Wilcoxon test). In univariate regression analyses, the continuous CTQ score was associated with both higher BMI (beta = 0.10, $p = 7.8e-8$) and higher HOMA-IR (beta = 0.022, $p = 0.0036$). Each individual CTQ subscale also showed a significant association with BMI (p values < 0.03 for each subscale). However, only physical abuse (beta = 0.11, $p = 0.0012$) and sexual abuse (beta = 0.11, $p = 0.0004$) were significantly associated with HOMA-IR.

GrimAge Acceleration moderates the relationship between BMI and insulin resistance in both cohorts

Recent work indicates that epigenetic mechanisms may underlie relationships between early adversity and metabolic health outcomes[77,78]. Thus, we next asked whether GrimAge Acceleration was associated with insulin resistance. In the LIFE Study cohort, we identified a significant relationship between GrimAge Acceleration and HOMA-IR (beta = 0.161, p = 0.0010) after accounting for sex, age, smoking status, and race.

Elevated BMI is a well-known risk factor for insulin resistance, and other work has suggested GrimAge may play a role in the relationship between obesity duration and the development of metabolic disease[57]. Indeed, in the LIFE Study cohort there is a significant univariate relationship between higher BMI and elevated HOMA-IR (beta = 0.132, p = 1.1e-11) and higher waist circumference and HOMA-IR (beta = 0.113, p = 2.93e-9), but research suggests that some individuals with obesity may not have increased risk for metabolic dysfunction[47,50]. Some work has suggested epigenetic signatures may assist with prediction of metabolic health in obesity[56]. Thus, we next asked whether GrimAge Acceleration improved prediction of HOMA-IR when accounting for BMI. When compared to a model of HOMA-IR with predictors BMI, sex, age, smoking status, and race, the addition of GrimAge Acceleration did not explain significantly more variance (ANOVA: F = 2.14, p = 0.145; adjusted R2 w/ GAA: 0. 232; adjusted R2 without GAA: 0.227). However, when we incorporated an interaction between GAA and BMI, the model explained significantly more variance (ANOVA: F = 8.84, p = 0.003; adjusted R2 w/ interaction term: 0.264; adjusted R2 without interaction term: 0. 232). In this model, lower GrimAge Acceleration was associated with lower insulin resistance in those with overweight or obesity (interaction term: beta = 0.018, p = 0.0034, Figure 2A, see Supplementary table 9). The addition of adversity history did not improve prediction of the model when analyzed as either CTQ score (ANOVA: F = 0.442, p = 0.507; adjusted R2 including CTQ score: 0.262; adjusted R2 without CTQ score: 0. 264) or Case/Control grouping (ANOVA: F = 1.50, p = 0.222, see Supplementary table 10; adjusted R2 including Case/Control factor: 0.266; adjusted R2 without Case/Control factor: 0. 264). Similar analyses using waist circumference instead of BMI demonstrate consistent results (GrimAge Acceleration: Waist Circumference interaction term: beta = 0.0154, p = 0.0218, adjusted R2 including interaction term: 0.233).

In the Yale Stress Center cohort, we previously demonstrated a relationship between GrimAge Acceleration and HOMA-IR[12]. However, an interaction with BMI had not been examined. The Yale Stress Center cohort did demonstrate a significant univariate relationship between BMI and HOMA-IR (beta = 0.188, p < 2e-16). In this cohort, the simple addition of GrimAge Acceleration as a predictor to a model of HOMA-IR with covariates BMI, sex, age, smoking status, and race did explain significantly more variance (ANOVA: F = 7.26, p = 0.0073; adjusted R2 w/ GAA: 0. 270; adjusted R2 without GAA: 0.260). Inclusion of an interaction term further improved the model (ANOVA: F = 8.18, p = 0.0044; adjusted R2 w/ interaction term: 0.281; adjusted R2 without interaction term: 0.270). Similar to the LIFE Study cohort, lower GrimAge Acceleration was associated with lower insulin resistance in those with overweight or obesity (interaction term: beta = 0.012,

$p = 0.0044$, Figure 2B, see Supplementary table 11). Again, the addition of adversity history via either CTQ score (ANOVA: $F = 0.433$, $p = 0.511$; adjusted R2 including CTQ score: 0.281; adjusted R2 without CTQ score: 0.281) or High/Low Childhood Adversity grouping (ANOVA: $F = 1.09$, $p = 0.298$, see Supplementary table 12; adjusted R2 including yes/no adversity factor: 0.282; adjusted R2 without yes/no adversity factor: 0.281) did not improve the prediction of the model.

Discussion

In this cross-sectional study, we used two independently recruited community samples of adults without known medical illness to demonstrate that childhood adversity is associated with increased epigenetic age acceleration (as measured by GrimAge Acceleration) in adulthood, and that increased epigenetic age acceleration interacts with BMI to predict higher insulin resistance. In both cohorts, defining childhood adversity via either a dichotomous variable or using the continuous CTQ measure demonstrated similar results: both were associated with increased epigenetic age. In addition, in both cohorts, childhood physical and sexual abuse were each significantly associated with epigenetic age acceleration, whereas physical and emotional neglect were not. Childhood adversity was also associated with higher BMI and insulin resistance in both cohorts. Notably, both cohorts also demonstrated a significant interaction between GrimAge Acceleration and BMI in their relationship to HOMA-IR, where lower GrimAge Acceleration was associated with lower HOMA-IR among those with overweight or obesity. When accounting for epigenetics and BMI, childhood adversity no longer significantly predicted HOMA-IR. These results demonstrate that childhood adversity is associated with greater metabolic risk, increased obesity, and accelerated epigenetic aging. Strikingly, in both cohorts interactions between epigenetic indices, obesity, and metabolic health suggest potential mechanisms through which long-term adverse physical health effects may manifest.

A major strength of this study is the consistency of the findings between two similar, yet independently-recruited cohorts of generally healthy adults. This is particularly notable given recent concerns about replicability in science. Both cohorts had similar age ranges, had no known chronic medical illness, and were not on daily prescription medications for any chronic medical or psychiatric illness. The recruitment protocols were distinct, with the LIFE Study cohort recruited in a case/control fashion, whereas the Yale Stress Center study cohort did not have a case/control recruitment, and instead was advertised as a study examining the impact of stress in healthy individuals. The distinct recruitment periods (2014–2020 for the LIFE study, and 2008–2012 for the Yale Stress Center Study) suggest that these results, which are largely consistent across two different recruitment periods, are independent of specific historical contexts. These differences in recruitment, along with the replication of the majority of findings between cohorts provide support for the potential generalizability of this work.

While the replication of the major findings between the LIFE study and the Yale Stress Center cohort is notable, there are also potentially important differences. In the LIFE study, the Hannum and Skin-Blood clocks demonstrated significant differences between the early life stress and controls in both traditional and principal component-based calculation methods, which was not found in the Yale Stress Center cohort. This may be a result of the differences in recruitment and inclusion/exclusion criteria, with the case/control design of the LIFE study identifying starker contrasts by including participants who experienced higher levels of early life adversity. This hypothesis is supported by the higher average CTQ score and higher variance of the CTQ in the LIFE study. While GrimAge has been shown to better predict morbidity and mortality[22], these differences between clocks may have implications regarding their underlying mechanisms[79]. Improved understanding of the epigenetics of aging may help tease apart the subtle distinctions between epigenetic clocks.

These results build upon the existing literature that suggests stress and trauma may lead to accelerated epigenetic aging[59,80]. Prior studies of adversity have often included individuals with existing health problems, studied children or adolescents, or specifically focused on mental health diagnoses such as depression[81–85]. Our prior work with the Yale Stress Center study cohort demonstrated that cumulative stress was associated with epigenetic age in a putatively healthy population[12], but did not examine childhood adversity. The current results show that, even while outwardly appearing to be medically healthy and having no known medical conditions at evaluation, adults with childhood adversity bear epigenetic marks that may predict increased morbidity and mortality in the future. The specific mechanisms through which this occurs are unclear and likely multifactorial, related to a complex interplay of neuroendocrine, inflammatory, developmental, and behavioral processes that all influence (and are influenced by) aging, adversity, and resilience. For instance, recent work has demonstrated that early life adversity may lead to accelerated aging through earlier onset of menarche[58]. In another example, childhood sexual abuse appears to drive changes in resting-state cortisol, which was in turn associated with accelerated epigenetic aging[86]. Others evidence indicates that early life adversity causes immune dysregulation[87] and accelerates aging through low-grade chronic inflammation sometimes called “inflammageing”[88], though such chronic inflammation may represent a distinct mechanism of aging from epigenetic clocks[89]. Health behaviors are also associated with both childhood adversity[90,91] and epigenetic aging[92–95], providing another plausible link. Mechanistic studies may help identify specific pathways and methylation sites linking these complex processes to childhood adversity and aging.

We also observed distinct results based on the type of abuse or neglect experienced. The physical and sexual abuse subscales of the CTQ were associated with GrimAge Acceleration in both cohorts, and while most subscales were associated with BMI, only sexual abuse was associated with elevated insulin resistance in both cohorts. This work is consistent with prior studies suggesting abuse and neglect have differential effects on epigenetic aging[81,96], although it remains difficult to isolate the effect of one type of abuse from another, as multiple forms of abuse/neglect are often present[97,98]. As physical and sexual abuse are generally less common than emotional abuse/neglect[99–101], these results are also consistent with cumulative theories of the effects of childhood adversity[102], as those who experience physical and/or sexual abuse may simply have experienced more childhood adversity overall. Future longitudinal work may help better identify any distinct effects of abuse versus neglect.

The relationships among GrimAge Acceleration, insulin resistance, and measures of obesity is also notable. Our results indicate that GrimAge Acceleration is only associated with elevated HOMA-IR (which indicates higher insulin resistance) in those with overweight or obesity, and that among those with overweight or obesity, those with low GrimAge acceleration do not have substantial insulin resistance. While analyses in the Yale Stress Center Cohort was limited to BMI, In the LIFE study, this interaction was consistent across two separate measures of obesity: BMI and waist circumference. This finding is consistent with prior work suggesting potential interactions between GrimAge, obesity, and diabetes[57]. It is possible that synergistic effects between epigenetic changes and obesity may contribute to the development of insulin resistance, although bi-directional relationships could complicate interpretation of these results and given the cross-sectional nature of this study, we are not able to test alternate models. The replication of these moderating effects in both samples is remarkable, though the cross-sectional nature of both cohorts limits our ability to draw causative inferences. These results do suggest that, in individuals with overweight or obesity, epigenetic markers may be useful for differentiating healthy versus unhealthy obesity. In the future, longitudinal studies would enable prospective assessments of whether epigenetic changes might drive the development of insulin resistance and diabetes.

Strengths of this study include the use of two similar samples of adults with overlapping measures, allowing for replication in two independent cohorts. Attempting to replicate results in such a manner contributes to an ongoing effort of the scientific community to improve reproducibility in science[103]. The participants had no known medical conditions and were not currently taking medications, suggesting that the findings cannot be explained by confounding effects of medications or chronic illness. As this work utilized the childhood trauma questionnaire, a widely used measure of childhood adversity, the findings can be compared to other studies of childhood adversity[104,105]. Finally, the use of GrimAge for analysis increases applicability to long-term morbidity and mortality outcomes compared to other epigenetic clocks[18,23].

These findings need to be interpreted in the context of study limitations that provide important direction for future research. The cross-sectional design precludes drawing any conclusions regarding the temporal order or directionality, and future research utilizing longitudinal designs is warranted. As adults with current or chronic medical conditions (e.g., heart disease, diabetes) and/or who are on medications other than hormonal birth control were excluded from this study, the findings may not generalize to such individuals or those who have a well-managed medical diagnosis that does not require current medication use. Future research with broader inclusion/exclusion criteria is warranted to explore whether the significant findings are maintained in other populations, though this may come at the expense of possible confounding factors. While the CECA (an interview-based approach) was used to classify early life adversity in the LIFE study, we also used the CTQ in both the LIFE and Yale Stress Center cohorts. As a retrospective self-report of childhood adversity, the CTQ may introduce recall bias and could be confounded by unmeasured confounding variables, such as childhood SES[106], which could also be addressed with future prospective, longitudinal studies. Additionally, as previously mentioned, both populations were putatively healthy and had no known serious medical conditions, which could have restricted the range of severity for BMI and insulin resistance. There were also some differences in the cohorts, including that the LIFE study allowed some individuals with cannabis use. Both cohorts were also predominantly white, non-hispanic. Future research with more diverse samples and including assessment of effects of racial and ethnic discrimination, and with a wider range or severity of health conditions is needed to examine how these findings generalize to diverse populations and those with more health problems.

In sum, we found that childhood adversity was linked to increased epigenetic age in two independent samples of adults. Specifically, physical and sexual trauma, but not neglect, were related to accelerated epigenetic aging. Childhood adversity was also associated with greater BMI and insulin resistance. Lastly, GrimAge correlated with metabolic health (as measured by insulin resistance) in those with overweight and obesity. These findings highlight a potential role of early life stress and epigenetics in predicting future health problems. Epigenetic changes seem to be associated with metabolic health in those with overweight and obesity, and this association could inform future research targeting modifiable risk factors, including accelerated aging and BMI. Future studies may use longitudinal methods to investigate epigenetics as a potential tool for differentiating metabolically healthy and unhealthy obesity, particularly in those with childhood adversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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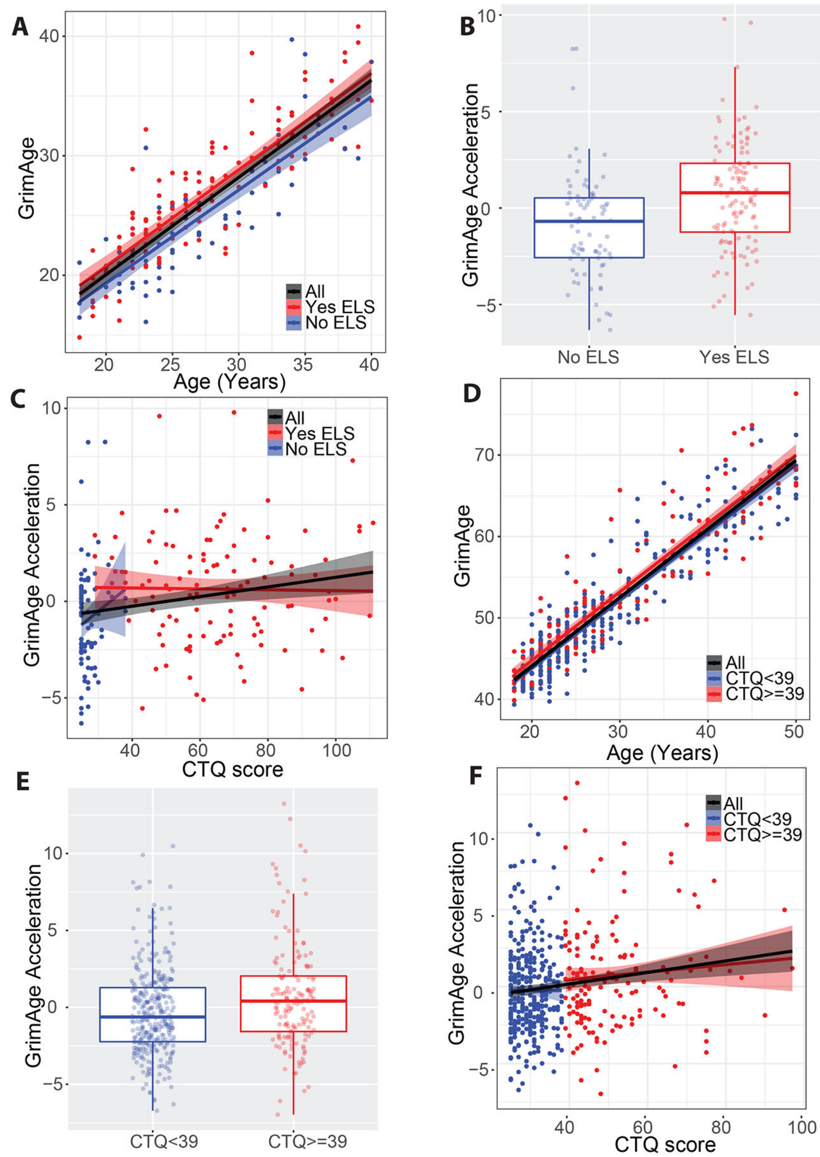

Figure 1:

(A) In the LIFE study, GrimAge is strongly correlated with Chronologic Age in both Cases (red, those with ELS) and Controls (blue, those without ELS). Individuals with ELS Have higher GrimAge than those without. (B) In the LIFE study, GrimAge Acceleration is elevated in Cases compared to Controls. (C) In the LIFE study, The Childhood Trauma Questionnaire (CTQ) score is positively associated with GrimAge Acceleration. As would be expected, Cases (red) have higher CTQ scores than Controls (blue). (D) In the Yale Stress Center study, GrimAge is strongly correlated with Chronologic Age in both individuals with high (red) and low (blue) childhood adversity. High childhood adversity does correlate with higher GrimAge. (E) In the Yale Stress Center study, High childhood adversity correlates with higher GrimAge Acceleration. (F) In the Yale Stress Center study, the CTQ score is positively associated with GrimAge Acceleration.

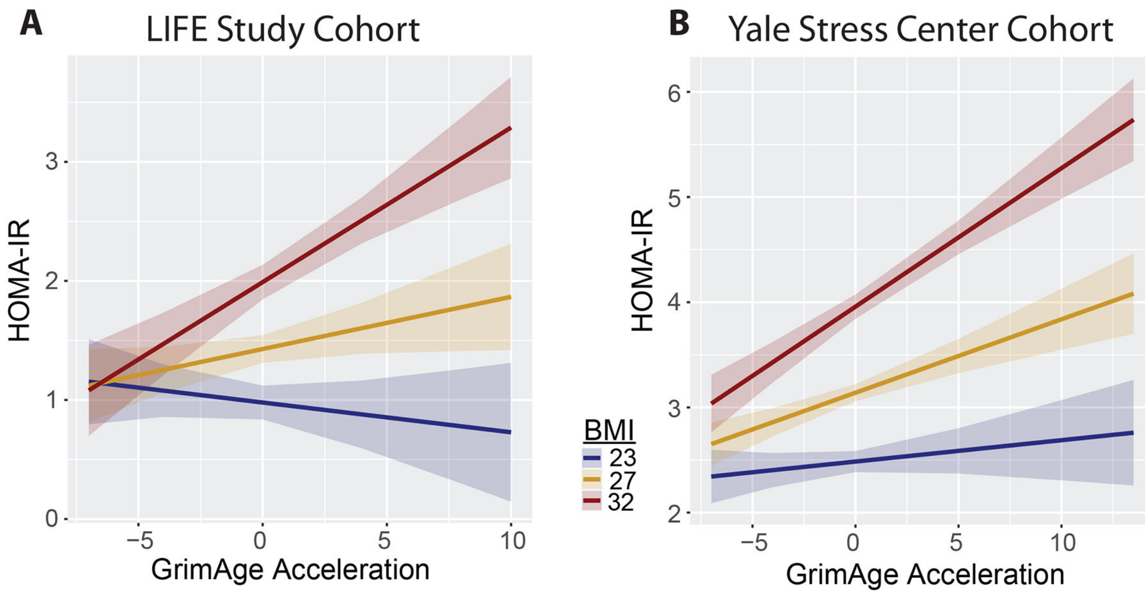

Figure 2:

(A) In the LIFE study, GrimAge Acceleration is positively associated with HOMA-IR in individuals with elevated BMI (interaction term: beta = 0.018, p = 0.0034). (B) In the Yale Stress Center study, GrimAge Acceleration is associated with elevated HOMA-IR in individuals with elevated BMI (interaction term: beta = 0.012, p = 0.0044). Plots represent the relationship between GrimAge Acceleration and HOMA-IR at representative values of BMI modeled as a continuous variable (HOMA-IR ~ GAA*BMI) in individuals with obesity (red), overweight (yellow), and those without overweight or obesity (blue).

Table 1:

Characteristics of the LIFE Study and Yale Stress Center study cohorts

Category		LIFE Study Cohort (n = 195)	Yale Stress Center Study Cohort (n = 477)	P value
Early Life Stress (n)	Yes	115 (59%)	155 (32%)	< 0.00001[a]
	No	80 (41%)	322 (68%)	
Sex (n)	Female	133 (68%)	267 (56%)	0.0034[a]
	Male	62 (32%)	210 (44%)	
Race (n)	Black or African American	19 (10%)	93 (19%)	< 0.00001[a]
	White	134 (69%)	343 (72%)	
	Asian	13 (7%)	41 (9%)	
	American Indian or Alaska Native	4 (2%)		
	>1 Race	16 (8%)		
	Unknown	9 (5%)		
Age (years)		27.41 +/− 0.41	28.81 +/− 0.40	0.9911[b]
BMI (kg/m^2)		27.6 +/− 0.45	27.1 +/− 0.25	0.5061[b]
CTQ score (total score)		49.71 +/− 1.76	36.96 +/− 0.58	0.00004[b]
Current cigarette smoker		24 (12%)	103 (22%)	0.0048[a]

Statistical tests for categorical variables (a) utilized chi-square tests, and numbers represent the number of individuals in that category and the percentage of the total n for that group. Statistical tests for quantitative variables (b) utilized Wilcoxon tests due to non-normal distributions, though the number represents the mean +/− the SEM.

Table 2:

Physical and Sexual Abuse Are Associated with GrimAge Acceleration in the LIFE Study

Independent Variable	GrimAge Acceleration
CTQ Total	0.172 *
PhysAb	0.175 *
EmotAb	0.125
SexAb	0.183 *
PhysNeg	0.091
EmotNeg	0.115

Cohen's partial f demonstrating the effect size of the relationship between variables (model accounts for age, sex, race, smoking status, and cell count proportions).

Legend: *: $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 3:

Physical, Emotional, and Sexual Abuse Are Associated with GrimAge Acceleration in the Yale Stress Center Study

Independent Variable	GrimAge Acceleration
CTQ Total	.130 **
PhysAb	0.122 **
EmotAb	.109 *
SexAb	.203 ***
PhysNeg	0.040
EmotNeg	0.040

Cohen's partial f demonstrating the effect size of the relationship between variables (model accounts for age, sex, race, smoking status, and cell count proportions).

Legend: *: $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$