Associations Among Early Adversity, Child Epigenetics, And Behavioral and Cognitive Development Across the First Few Years of Life

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Abstract

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Developmental psychobiologists theorize that epigenetic pathways play a crucial role in explaining the biological embedding of socioeconomic disadvantage and early life stress on health and well-being across the lifespan. Biological aging as indexed via epigenetics is a promising biomarker of both early life adversities and deleterious health outcomes across multiple domains, and recent advances suggest these associations are detectable within childhood itself. However, little is understood about when these associations emerge during development, under what conditions, and the meaning of such biomarkers for young children's psychological development. The present dissertation examined disparities in epigenetic aging using a diverse sample of mother-infant dyads followed from pregnancy through toddlerhood. Study 1 found that socioeconomic and stress-related perturbations in epigenetic aging are not robustly detectable at birth, but appear to emerge by 30-months postpartum. Study 2 found no differences in child epigenetic aging as a result of exogenous exposure to the social experience of the COVID-19 pandemic. Study 3 found that child epigenetic aging was not a reliable indicator of children's concurrent cognitive or behavioral development. Collectively, these findings suggest that adversity-related disparities in biological aging are detectable within the first few years of life; are not affected by the social experience of the pandemic; and are not informative for psychological development in real-time. Implications for the biological embedding of adversity, the application of epigenetic clocks to pediatric samples, and future research are discussed.

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Dedication

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Chapter 1: Introduction

Social scientists have long observed that children raised under conditions of socioeconomic disadvantage tend to have poorer outcomes compared to their higher income peers (Shonkoff et al., 2012). These outcomes are present across multiple domains, including cognitive, behavioral, and physical health, and are detectable across the lifespan (Schuurmans et al., 2022; Shonkoff et al., 2012). Similar outcomes have been observed for children born and raised under conditions of stress (e.g., Troller-Renfree et al., 2022; Tung et al., 2024). Importantly, these negative outcomes are not fully accounted for by individual health or risk-taking behaviors and are detectable at the earliest stages of development (Shonkoff et al., 2012). Developmental psychologists have studied many potential pathways by which socioeconomic disadvantage and early life stress may lead to such outcomes, including parenting behaviors, reduced access to high quality childcare, and exposure to environmental toxicants, among others. Nonetheless, accounting for such pathways alone does not fully explain the link between early adversity and long-term outcomes – implicating another potential source of variation.

These observations have led researchers to examine epigenetic pathways as a possible biological mechanism to explain the link between early adversity and life-long functioning. DNA methylation (DNAm) is an epigenetic process that is sensitive to both adverse experiences and may also lead to transcriptional changes that impact physiological, neurobiological, and immune pathways with implications for health and well-being (Weaver, 2014). One promising avenue of inquiry is the study of epigenetic age, which reflects an individual's 'biological' age, as calculated by a 'clock' trained on typical trajectories of DNAm patterns over time. An accelerated epigenetic age – i.e., a more advanced biological age than one's chronological age – is thought to measure physiological wear and tear on the body as it is a robust predictor of both

adverse life experiences and deleterious health outcomes in adults (Horvath & Raj, 2018). Indeed, an accelerated epigenetic age is robustly correlated with adverse exposures such as traumatic life events and socioeconomic disadvantage (Belsky et al., 2022), as well as their associated health outcomes, like cancer, cardiovascular disease (Oblak et al., 2021), and poorer cognition (McCartney et al., 2022).

Recent studies have extended application of these epigenetic clocks developed in adult samples to pediatric populations. These early studies have reported similar associations among accelerated aging and adverse exposures in middle childhood, including socioeconomic disadvantage (Raffington et al., 2021) and racially-intrusive encounters with police (Del Toro et al., 2024). Similarly, these clocks also appear to detect some signal with concurrent child health, with accelerated epigenetic aging predictive of higher body mass index (Raffington et al., 2021) and poorer self-reported health (Del Toro et al., 2024). However, it remains unknown *when* these associations first emerge in development. Tracking the emergence of these biomarkers is useful to understanding how early adversity may biologically embed to impact later functioning. Longitudinal research demonstrates there is considerably less stability in epigenetic age during childhood (deSteiguer et al., 2025). Therefore, examining the "long-arm" of early life adversity in real-time – that is, tracking the emergence of adversity-related biomarkers prior to their correlated manifestations on physical health and well-being – may allow for the identification of those most at-risk of experiencing deleterious outcomes. In the most optimistic scenario, it could possibly be leveraged to identify periods for early intervention to improve long-term outcomes (though it should be noted that low inter-timepoint stability does not necessarily imply sensitivity to intervention).

The COVID-19 pandemic underscores the importance of examining the biological embedding of adversity in young children. Indeed, the pandemic caused sudden and massive disruptions to typical child-rearing contexts. The unexpected and global experience of the pandemic provides an opportunity to examine the effects of these disruptions through a quasi-experimental design. There is a need for research into the long-term effects of exposure to the socialized experience of the pandemic on children's developmental trajectories.

Crucially, little work to date has rigorously assessed the validity of epigenetic algorithms in pediatric samples. One barrier is that most epigenetic clocks were developed in adult samples using blood tissue, which is difficult to collect in children. To date, several studies have applied these measures in adolescent samples using saliva tissue and successfully replicated associations observed in adult samples (e.g., socioeconomic disadvantage and accelerated aging), indicating construct validity of the measure in children. However, it is unknown if such patterns will extend to early childhood. Indeed, an important question remains regarding the functional significance – or the ability to predict outcomes in real-time – of epigenetic aging during childhood. For example, while it is well-established that accelerated aging in adulthood is associated with physical and cognitive health (Horvath & Raj, 2018), it is not clear if these patterns will persist into the first few years of life.

Early childhood is a period of rapid maturation for both the methylome and other developmental traits, making it critical to validate these measures in order to draw inferences from adversity-related patterns. Therefore, examining these biomarkers during early childhood fulfills two scientific aims: one, to prospectively examine the emergence of adversity-related indicators; and two, to examine the construct validity of this measure during this age range. Indeed, the detection of a biological signature correlated with early life adversity during early

childhood suggests that these epigenetic markers of well-being in adults are detectable in the first few years of life, and that they can be applied across the lifespan.

The Present Study

The present dissertation seeks to address these knowledge gaps. Chapter 2 provides the theoretical underpinnings which motivate the research questions examined. First, I review the relevant theories and hypothetical mechanisms which link early exposure to stress and socioeconomic disadvantage to adverse developmental outcomes. Second, I argue for the utility of epigenetic aging as a potential biomarker of these early life experiences and the value of identifying their emergence. Third, I discuss potential challenges with applying these algorithms to pediatric populations and propose methods of assessing their validity in this age range.

Chapters 3 – 5 detail the methods, results, and discussion of Studies 1 through 3. In Study 1, I examined the emergence of stress and socioeconomic-based perturbations in epigenetic aging across the first few years of life. This was achieved by assessing epigenetic age longitudinally from infancy to toddlerhood in a socioeconomically- and racially-diverse sample of children. Study 2 examined a sub-cohort of these children who were naturally exposed to differing amounts of the COVID-19 pandemic in the first year of life. I leveraged this natural experiment design to examine the impact of exposure to the pandemic on epigenetic aging and developmental outcomes in toddlerhood. Study 3 tests the observed correlates of epigenetic aging with behavioral and cognitive outcomes in this same sample of toddlers to reveal the functional significance of epigenetic aging. Finally, I provide an overall Conclusion to interpret these findings collectively. This dissertation makes an important and novel contribution to the literature by deepening the understanding of the "biological embedding" of adversity during early childhood.

Chapter 2: Theoretical Overview of Epigenetic Aging, Adversity, and Development

Exposure to early life stress and socioeconomic disadvantage are robust predictors of worse health and well-being across multiple domains. For example, children of mothers who report higher levels of stress tend to demonstrate poorer behavioral and cognitive development (e.g., Troller-Renfree et al., 2022). In addition, decades of research have documented that children born into poverty are prone to lower performance on measures of cognitive and socioemotional functioning, as well as worse physical and mental health, compared to their higher-income peers (Noble et al., 2021).

These effects emerge during the first few years of life, and are persistent across the lifespan (Hertzman & Power, 2004). As such, several theories have emerged to explain these social determinants of health. It is argued that exposure to adverse conditions early in life, such as socioeconomic disadvantage and high-stress environments, may alter an individual's biological functioning in ways that shape life-long trajectories of well-being. Subsequently, researchers have investigated early biomarkers of both adverse exposures and of altered developmental trajectories to understand how these processes shape life course well-being.

2.1 Biological Embedding of Childhood Adversity

The concept of "biological embedding" formulated over the last few decades as scientists observed socially-stratified gradients in public health. Primarily, they observed that the stratification of physical, behavioral, and cognitive development began in childhood and was detectable across societies and the lifespan (Hertzman, 2012). Hertzman (2012) describes the present model of biological embedding as relying on four key principals: 1) experiences alter human biological processes in ways that "get under the skin"; 2) systematic differences in early

experiences lead to systematic differences in biological functioning; 3) these differences are stable and enduring; and 4) these changes may impact health and well-being across the lifespan.

The hypothesized pathways by which adverse exposures biologically embed varies by the timing and type of exposure. Indeed, the umbrella term 'childhood adversity' often refers to many different kinds of experiences, such as abuse, neglect, caregiver psychopathology, and deprivation, and can span from the prenatal to adolescent period (Berens et al., 2017). For the purposes of this dissertation, I define childhood adversity as exposure to higher levels of stress (perceived and physiological) and socioeconomic disadvantage across the prenatal period and the first few years of postnatal life. While these exposures are theorized to frequently co-occur, the present dissertation examines these as separate experiences.

Developmental Origins of Behavior, Health, and Disease Hypothesis

It is hypothesized that the biological embedding of adversity begins via prenatal, and possibly even pre-conception, experiences. Epigenetic modifications such as DNA methylation are one potential pathway by which early adverse experiences may exert these effects. The Developmental Origins of Behavior, Health and Disease (DOBHaD) hypothesis proposed by Van den Bergh (2011) argues that prenatal insults, such as poor nutrition, toxic stress, and environmental toxicants, shape developing fetal systems via epigenetic pathways. For example, families residing in low-income neighborhoods tend to have reduced access to healthy foods (Vilar-Compte et al., 2021), and poor prenatal nutrition has subsequently been linked to epigenetic alterations in offspring (Koemel & Skilton, 2022). Similarly, poor mental health and greater levels of perceived stress during pregnancy have also been linked to alterations in infant DNA methylation, possibly explained by changes to placental regulation of glucocorticoids (Champagne et al., 2024).

Numerous studies have documented poorer outcomes for children born after adverse in-utero exposures. Indeed, a natural experiment following pregnant women during severe flooding in Ecuador found in-utero exposure to the natural disaster was associated with poorer cognitive abilities several years later (Rosales-Rueda, 2018). Additionally, a recent meta-analysis found that prenatal stress robustly predicted emotional and behavioral problems in childhood, above and beyond postnatal levels of distress (Tung et al., 2024). Evidence of similar patterns is also present in rodent models via experimental studies, and epigenetic changes appear to explain some of these effects. For example, offspring of rats experimentally exposed to stress during pregnancy demonstrate behavioral and cognitive changes much like those observed in human offspring, such as greater internalizing symptoms and declines in cognitive functioning (Weinstock, 2017), and subsequent epigenetic changes (Blaze & Roth, 2015). Combined, this evidence suggests that some kind of epigenetic link may be responsible for these patterns, as postnatal experiences alone do not fully account for these associations.

According to the DOBHaD hypothesis, these 'programmed' fetal systems subsequently interact with the postnatal environment to produce individual susceptibility to disease. Though these adaptations may carry risks for overall health and behavior, they are not deterministic; indeed, they may also co-occur with *improved* performance in ecologically relevant domains, such as emotion detection, attention, and memory (see Ellis et al., 2020 for further discussion). It is also important to acknowledge that the factors that influence stress and social disadvantage are interconnected and tend to co-occur within individuals: mothers experiencing social disadvantage tend to also experience greater negative life events, report higher levels of distress (Lefmann & Combs-Orme, 2014) and demonstrate increased chronic physiological stress (Ursache et al., 2017). Furthermore, cumulative lifetime stress impacts both modern-day stress

appraisals and the stress-response system, shaping psychological experience and physiological functioning, and ultimately, health and wellbeing (Epel et al., 2018). Therefore, identifying unique pathways between such exposures and differential outcomes remains challenging. Nonetheless, the accumulating evidence presented under the DOBHaD hypothesis posits that prenatal exposures set the foundation for the biological embedding of adversity, potentially shaping later health and well-being. However, the extent to which these epigenetic biomarkers are detectable at the beginning of postnatal life, versus later in childhood, remains largely unknown.

Of note, it is possible these effects emerge even prior to conception: experimental evidence from rodent models (Blaze & Roth, 2015) and correlational work in humans (e.g., Dye et al., 2024) suggests that maternal preconception experiences, like adverse early childhood experiences, may also impact future offspring's development via epigenetic pathways. However, causal evidence to support these intergenerational pathways remains scarce and highly contested in humans (see Scorza et al., 2019 for greater discussion). It is necessary to first elucidate associations and potential causal pathways within the same individuals before extending across generations.

Weathering Hypothesis

Additional hypotheses attempted to explain the biological embedding adversity via an accumulation of lifetime experiences. The sociologist Arline Geronimus (1992) first introduced the idea of biological 'weathering' to explain differential birth outcomes for infants born to Black and White mothers. Geronimus observed that infants born to Black teen mothers tended to be healthier than infants born to older Black women, which functioned counter to the pattern observed in White mothers. Geronimus argued that this racial variation in pregnancy outcomes

reflected an accumulation of adverse experiences: Black women's health deteriorated due to accumulating exposure to adversities such as socioeconomic disadvantage, racism, and systemic inequality, resulting in poorer health outcomes for infants born to women during their 'prime' childbearing years (e.g., 20's to 30's).

Numerous studies have tested, and extended, this hypothesis since its inception (e.g., Forde et al., 2019). Psychologists have argued that an accumulation of early childhood experiences such as a lack of warm parenting, poverty, maternal depression, and chaotic social environments may also result in physiological weathering. Alternative names assigned to this phenomenon include "allostatic load," which refers to chronic activation of the stress response system. Allostatic "overload" is defined as strain on the body's regulatory systems in response to environmental challenges, resulting in a hyper-activated state of toxic stress (Lucente & Guidi, 2023). Frequently, researchers study biomarkers of the hypothalamus-pituitary-adrenal (HPA) axis, or stress-response system, as evidence of allostatic load. For example, adverse childhood experiences are associated with multiple biomarkers of allostatic overload during childhood and adolescence, and these biomarkers subsequently predict deleterious physical, emotional, and cognitive health outcomes (Lucente & Guidi, 2023).

2.2 Epigenetics, Biological Aging, and Adversity

Epigenetic changes are thought to be a key pathway to explain how adverse exposures biologically embed, or "get under the skin," to impact life-long well-being. Epigenetics is defined as the study of changes to gene function or activity without change to the DNA sequence itself (Moore et al., 2013). The most widely-studied epigenetic index is DNA methylation (DNAm), which is thought to regulate gene expression by "silencing," or down-regulating, gene transcription. These transcriptional changes subsequently alter gene expression, with potential

consequences for physiological systems such as neurobiological and immune functioning (Weaver, 2014). Critically, patterns of DNAm are both genetically and environmentally mediated (Duan et al., 2022). Recent advances in epigenome sequencing have revealed that patterns of DNAm reliably change over the life course (Duan et al., 2022), leading to developments in the measurement and conceptualization of biological aging.

Biological age reflects age at a cellular level, indicating an individual's physiological functioning relative to their chronological age (Duan et al., 2022). While the methylome reliably changes with age, these patterns also exhibit individual variation across the population. This led to the creation of DNAm algorithms, or "clocks", able to predict chronological age and other clinical markers of aging. An accelerated epigenetic age – a more advanced epigenetic age relative to one's chronological age – has emerged as a robust predictor of deleterious health and disease in adulthood (Oblak et al., 2021). Indeed, Horvath & Raj (2018) posit that accelerated epigenetic aging in adults may be a biomarker of physiological weathering given that it is associated with both adverse lifetime exposures and deleterious health outcomes. For example, numerous studies report associations between adverse experiences in childhood – such as trauma and domestic violence (Dye et al., 2024), sexual abuse (Lawn et al., 2018), and poverty (McCrory et al., 2022) – and accelerated aging in adulthood. Importantly, these findings are inconsistent across correlational studies and rely on retrospective reporting, highlighting the need for prospective designs to improve temporal inference.

Emerging evidence suggests that the associations among adverse experiences, epigenetic age, and health may be detectable within childhood itself. For instance, Raffington and colleagues (2021) found that greater socioeconomic disadvantage and body mass index were independently associated with accelerated aging during middle childhood and adolescence,

suggesting a link between early adverse exposures and health. Similarly, del Toro and colleagues (2024) observed that greater racially-intrusive encounters with police and poorer self-reported health were associated with accelerated aging in racially minoritized youth. However, it is unknown when epigenetic signals of biological weathering are first detectable in development. Identification of the emergence of such biomarkers may provide insight regarding the extent to which early experiences shape epigenetic variation and subsequent outcomes. For example, it is possible that these biological signals may emerge prior to their associated health outcomes. Therefore, examining their emergence may help identify populations most at-risk of deleterious outcomes.

Recent research has extended these investigations even earlier in life, but with mixed findings. McGill and colleagues (2022) found that prenatal maternal anxiety was associated with accelerated aging across early childhood, but this effect was primarily seen in boys. In contrast, a multi-cohort study of nearly 4,000 newborns reported no association between prenatal perceived stress and epigenetic aging (Murgatroyd et al., 2024). Differences in the types of epigenetic clocks used may partially explain these divergent findings. Next, I will describe the various types of epigenetic clocks, and the most recent evidence for studying the biological embedding of socially-stratified constructs like stress and socioeconomic disadvantage in children.

The Evolution of Epigenetic Clocks

Initial investigations using DNAm as a measure of biological aging focused on the prediction of chronological age using specific cytosine-phosphate-guanine (CpG) sites across the genome. This type of algorithm became known as a first-generation clock. The most widely used of these algorithms is the Horvath clock, which is a multi-tissue predictor of chronological age developed across the lifespan and found to be a robust predictor of health outcomes in adults

(Horvath, 2013; Horvath & Raj, 2018). Using these clocks, researchers find associations between accelerated aging in adulthood following exposure to trauma (Boks et al., 2015), childhood adversity (Sumner et al., 2019), childhood economic disadvantage (Hughes et al., 2018), and cumulative life stress (Zannas et al., 2015).

However, there is inconsistent evidence of these effects in early childhood. For example, although some studies report associations between accelerated aging in infants and prenatal exposures such as psychological distress and socioeconomic disadvantage using these first-generation clocks (e.g., Euclydes et al., 2022; Javed et al., 2016; McGill et al., 2022), other studies find that the same exposures predict *decelerated* aging (Appleton et al., 2022; Folger et al., 2024; Katrinli et al., 2023; Laubach et al., 2024; McKenna et al., 2021; Pilkay et al., 2024; Suarez et al., 2018). Still other studies report null associations (Khouja et al., 2018; McKenna et al., 2021; Murgatroyd et al., 2024; Popovic et al., 2021). The most decisive evidence to date comes from a meta-analysis, which concludes that first-generation clocks are not reliable indicators of socially-stratified epigenetic profiles (Willems et al., 2025) .

Building on this early literature, other approaches were developed to estimate biological age with greater precision. Second-generation clocks are designed to predict clinical markers of aging, rather than chronological age itself (e.g. PhenoAge, GrimAge). These clocks leverage multiple markers of health and well-being to estimate biological age via DNAm, such as organ system integrity and mortality risk. More recently, a third-generation clock has been developed using repeated measures within longitudinal samples. The DunedinPACE clock quantifies the pace of biological aging – that is, the rate of aging for every chronological year of life (Belsky et al., 2022). DunedinPACE also appears to be sensitive to socioeconomic disparities (Raffington et al., 2021) and stressful exposures in childhood (Del Toro et al., 2024). Of the studies that have

examined these clocks in childhood, a clearer picture is emerging: a meta-analysis of 140 studies by Willems and colleagues (2025) found that second- and third-generation clocks reliably detect socioeconomic disparities in cohort studies. This suggests that these newer algorithms hold promise for detecting the biological embedding of social disadvantage. Notably, the third-generation clock (DunedinPACE) emerged as the only reliable predictor of socioeconomic disparities in pediatric cohorts in this meta-analysis, though the frequency of studies in this age range is small (Willems et al., 2025). Nonetheless, for both the second- and third-generation clocks, it is unknown how these effects manifest during the first few years of life.

2.3 Application in Pediatric Samples

A remaining question for the field is identifying *when* disparities in epigenetic aging are detectable in development. However, applying adult-based epigenetic clocks to pediatric samples introduces several methodological challenges. First, the majority of epigenetic clocks were developed using blood tissues. These samples are difficult to collect in young children, where saliva or buccal tissues are preferred. Previous work shows low to moderate cross-tissue correlation between blood and saliva estimates of epigenetic age (Apsley et al., 2025; Raffington et al., 2023), highlighting the need to develop oral-based or pan-tissue clocks.

Second, there is a relatively small correlation between epigenetic age and chronological age during early childhood (e.g., Bozack et al., 2023; deSteiguer et al., 2023; Laubach et al., 2024; Simpkin et al., 2017), which limits the ability of researchers to confirm the accurate measurement of epigenetic age in this population. Pediatric-specific clocks have since been created to increase accuracy (e.g., PedBE, a first-generation clock), but the extent to which this is accurate in newborns is less clear due to limited research in this age range. Given the success of second- and third-generation clocks in detecting socioeconomic disparities in epigenetic aging,

there is also a need to verify the measurement validity of these algorithms in younger samples in order to extend the detection of biological embedding to earlier in life.

The examination of these measures in young children provides the rare opportunity to fulfill two scientific aims: first, to test the validity of the measure itself in this population, and second, to test the hypothesized process being examined. First, detecting patterns of epigenetic aging in early childhood that mimic those observed in adulthood provides convergent validity evidence for epigenetic clocks in pediatric cohorts. Establishing validity is critical in order for future scientists to apply these measures in very young children to answer future research questions. Secondly, establishing whether adversity-related perturbations in epigenetic age are present in early childhood also provides evidence for the biological embedding of adversity. Therefore, examining such associations within the first few years of life is a novel contribution to the field and fulfills dual scientific aims.

2.4 COVID-19, Epigenetics, and Development

The COVID-19 pandemic caused large disruptions to the typical child rearing environment. It is reasonable to expect that the environmental conditions created by the pandemic resulted in stressful experiences for many families, with potential consequences for children's developmental trajectories. For example, pandemic-related stress during pregnancy has been associated with poorer self-regulatory capacities (Bradley et al., 2023; Provenzi, Grumi, et al., 2021), sociocognitive skills (Nazzari et al., 2023), and neurodevelopment in small, infant cohort studies (Jiang et al., 2024; Shuffrey et al., 2021; Werchan et al., 2024). One study found that pandemic-related stress during pregnancy predicted greater DNAm of the serotonin transporter gene, which subsequently predicted infant temperament at 3-months postpartum (Provenzi, Mambretti, et al., 2021).

In the context of COVID-19 infection, recent epigenome-wide association studies have identified DNAm differences in patients infected with the virus (Corley et al., 2021; Huoman et al., 2021), with variation in DNAm able to predict infection even one year after recovery from severe illness (Balnis et al., 2022). Less work has sought to examine epigenetic differences based on exposure to the *social* experience of the pandemic, rather than viral infection. Indeed, if DNAm is responsive to adverse early experiences, it may be possible to detect biomarkers of exposure to pandemic-related conditions. For example, environmental disruptions, social isolation, economic uncertainty, and other pandemic-related stressors within families may leave measurable imprints on stress-sensitive processes like DNAm. Identifying such biomarkers may be useful in identifying those children most at-risk of experiencing delays due to pandemic-related disruptions. One longitudinal study of older children and adolescents tested repeated measures of DNA methylation clocks collected pre- versus post-pandemic, and found no differences in the rank-order stability of biological aging (deSteiguer et al., 2025). However, it is unknown if these null findings will persist in younger children.

There has been mixed evidence to date regarding the effect of the pandemic on young children's development. In some cases, there is evidence of deleterious effects. For example, a scoping review by Zuniga-Montanez and colleagues (2024) finds that young children's language-learning environments, and subsequent language skills, declined modestly due to the pandemic. Other systematic reviews find similar deleterious effects of the pandemic on the mental health of children and mothers (Panchal et al., 2023; Penna et al., 2023). A longitudinal study from Japan found young children aged 3 to 5 years old who had been exposed to the pandemic scored lower on a parent-reported measure of development indexing language, socioemotional development, and motor skills compared to a pre-COVID comparison group

(Sato et al., 2023). However, results were inconsistent across age groups and assessment types. Indeed, younger children in this same study (aged 1 to 3 years old) seemed to perform slightly *better* in these skills compared to their pre-pandemic peers (Sato et al., 2023).

There is also evidence of heterogeneity across demographic subgroups. It is well established that socioeconomically disadvantaged communities were more likely to experience higher levels of infection, mortality, and economic challenges (Abrams et al., 2022). However, there is also some evidence to suggest positive family-level effects of pandemic restrictions for these subgroups. For example, studies found that some low-income families with young children reported more positive parent-child interactions (Kalil et al., 2020), improved maternal mental health (Premo et al., 2023), and improved maternal sleep quality (Premo et al., 2023) during the pandemic compared to pre-pandemic cohorts.

Critically, the majority of this literature relies on cross-sectional and self-reported data, which may prematurely raise the alarm for children's developmental outcomes due to biased estimates (see Firestein et al., 2022 for expanded discussion). In contrast, studies that examine pre- versus post-pandemic cohorts of children and longitudinal assessments suggest a more optimistic view. In a longitudinal study of NYC-based families, I found that greater exposure to the pandemic during the first year of life was not associated with maternal reports of infant language, socioemotional problems, or maternal mental health across the first two years of life (Sperber et al., 2022). In addition, a recent investigation comparing over 50,000 births from the years 2018-2022 found significant but small effects of the pandemic on infant developmental milestones, with declines of 2-3% in the domains of communication, problem-solving, and personal-social skills (Johnson et al., 2024). Quasi-experimental designs with longitudinal data

are necessary to improve our understanding of the pandemic on the youngest generation of children.

With the appropriate data, the universal yet unexpected nature of the pandemic creates a suitable environment with which to study the effects of this global stressor using a natural experiment design. Specifically, scientists can better understand the transmission of maternal stress and children's developmental trajectories. While it is crucial to understand the impacts of the pandemic to mitigate possible deleterious effects on development, this global event also provides a unique opportunity to inform basic science. Furthermore, there is a need for objective, lab-based assessments to definitively assess the effect of the pandemic on families. Identifying potential biomarkers of this global catastrophe via epigenetics improves our understanding of both the pandemic and developmental science at large.

2.5 Functional Relevance in Childhood

Little is understood about the significance of deviations in epigenetic age during early childhood. Limited work suggests that accelerated aging at birth is associated with behavior problems at age 3 (Suarez et al., 2018), and with brain structure during both infancy (Gomaa et al., 2022) and adolescence (Hoare et al., 2020). However, this literature is limited and inconsistent. Horvath and Raj (2018) also speculated that evidence for epigenetic age as an indicator of physiological wear-and-tear is inconsistent in early childhood and do not reflect meaningful differences in development.

More recent evidence from an older cohort of children suggests otherwise. Raffington and colleagues (2022) found that amongst a large sample (n > 1,000) of children aged 8 to 19 years old, socioeconomic disadvantage was associated with epigenetic profiles that, in previous studies of adults, were predictors of poorer health and cognitive functioning. Furthermore, these

epigenetic profiles were associated with concurrent measures of cognitive abilities in children. This particular study applied a novel clock called "Epigenetic-g" to their sample, which reliably predicts cognitive abilities in older adults independent of polygenic scores of intelligence (McCartney et al., 2022). Findings from the Raffington et al. (2022) study indicate that a DNAm-based predictor of cognitive abilities demonstrated concurrent validity during middle childhood and adolescence, while simultaneously reflecting socioeconomic disparities in health and cognition. Of course, not all children exposed to stressful environments experience cognitive and behavioral difficulties; therefore, deviations in epigenetic age may be an important biomarker of children who are most impacted by adversity.

Importantly, it is unclear if the directionality of effects observed in middle childhood and adolescence will extend to toddlerhood. While adult research has observed a consistent pattern of accelerated epigenetic aging following exposure to trauma (Boks et al., 2015), childhood adversity (Sumner et al., 2019), childhood economic disadvantage (Hughes et al., 2018), and cumulative life stress (Zannas et al., 2015), this may not hold true for the first few years of life. While previous work concludes that epigenetic age is quite stable in adults (Reed et al., 2022), the methylome is rapidly developing during the first few years of life. Recent work suggests epigenetic age is much less stable in middle childhood and adolescence (deSteiguer et al., 2025), suggesting that the functional significance of epigenetic age at one moment in time for a child's development may be short-lived. Indeed, it is possible that advanced biological maturation could be a *positive* outcome at the very beginning of life. Examining the concurrent correlates of accelerated aging in very young children is an important step for establishing the expected trajectory of epigenetic aging and development across childhood. In addition, it would provide further evidence for the validity of these measures in pediatric populations.

Baby's First Years

Emerging research from the Baby's First Years (BFY) study sheds additional light on the functional relevance of epigenetic age in early childhood. BFY is the first randomized control trial of poverty reduction in the United States, providing causal estimates of unconditional cash transfers on epigenetic changes for families living in poverty. In this study, 1,000 mothers living below the poverty line were recruited in the hospital after giving birth to their child. After consenting to participate in a prospective child development study, mothers were randomly assigned to either receive a high-cash ($333/month, or $4,000/year) or low-cash ($20/month, or $240/year) gift for the first few years of their child's life. At four years postpartum, mothers and children completed a battery of cognitive and behavioral measures, and provided a saliva sample to assess epigenetic age.

Preliminary results demonstrate that children of families who received the high-cash gift demonstrated a slower pace of biological aging according to the DunedinPACE clock, and a lower Epigenetic-g score (notably, the association with Epigenetic-g was counter to the hypothesized direction, but not statistically significant; Raffington et al., under review). In contrast, no difference in epigenetic age was detectable in mothers. There were also no observable differences in children's behavior and cognitive development based on the cash transfers, and these epigenetic biomarkers did not robustly correlate with children's cognition, health, or behavior. This indicates that at this developmental stage, epigenetic patterns may not hold signal for children's concurrent development. In addition, Epigenetic-g in these mothers was not associated with well-validated cognitive assessments, raising doubts as to the generalizability of this measure to other populations.

The findings from this seminal study raise important questions for both epigenetic measurement and application. Primarily, the slowing of DunedinPACE in children as a result of the cash transfers implies that epigenetic age is sensitive to changes in socioeconomic position, though additional follow-up is necessary to determine if this effect will persist over time. The mechanistic pathway that explains this physiological change remains unknown; for example, it is possible that greater consumption of fresh produce in the high-cash gift group accounted for this effect (Sperber et al., 2023). Nonetheless, the interpretation of this finding within the context of the broader study remains contested. Indeed, equal interpretive weight must be given to the marginally significant yet unexpected negative pattern of Epigenetic-g in children. It is possible this may reflect an iatrogenic consequence of the intervention; a lack of understanding about how epigenetic clocks manifest in young children; or statistical noise due to invalid measurement in this population. The lack of association between Epigenetic-g and cognition in mothers raises further concerns as to the external validity of this measure.

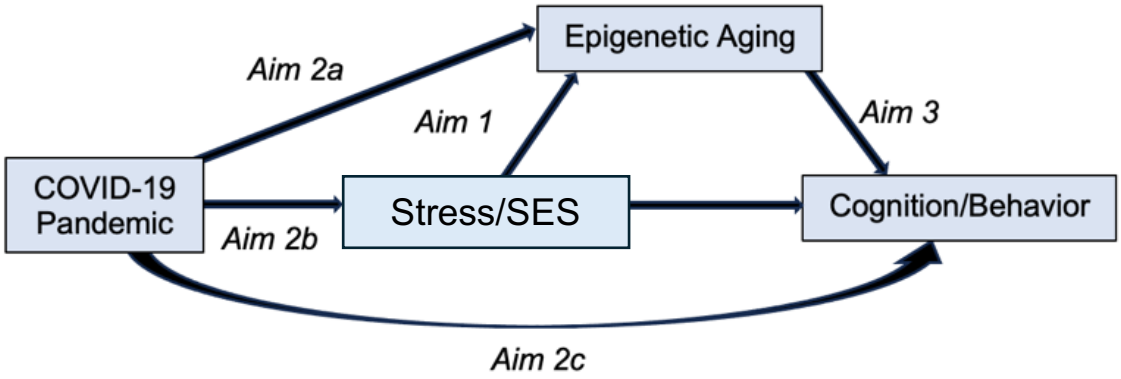
Nonetheless, the randomized nature of this study denotes a causal link between a poverty reduction intervention and children's epigenetic aging. It is especially notable that maternal epigenetic scores remained unchanged across groups, highlighting early childhood as a sensitive period for physiological changes. Furthermore, the lack of group differences in children's cognitive or behavioral outcomes suggests that epigenetic changes may occur independently of observable differences in development. Therefore, it is plausible that epigenetic age may serve as an early indicator of adverse experiences, with potential implications for later health and well-being, yet hold little significance for concurrent developmental outcomes.

2.6 Summary and Limitations of Current Research

Though it is well-documented that socioeconomic disadvantage and early life stress are associated with adverse developmental trajectories, it is necessary to identify the pathways by which these exposures predict later disparities. Epigenetic aging may serve as a possible pathway, yet few studies have investigated these effects longitudinally in pediatric populations. Though accelerated aging is robustly associated with deleterious health and well-being in adulthood, the functional relevance of these effects in the first few years of life is unknown. The recent global exposure to a public health crisis highlights the need to examine the effects of such experiences on children's biological and developmental trajectories. Additionally, tracking the emergence of such biomarkers deepens our understanding of the biological embedding of adversity and it's impacts of life-long trajectories of health and well-being.

Figure 1-1 demonstrates the theoretical overview of the present dissertation. Using a three-study design, I examined the associations among early adversity (defined as low SES and high stress), epigenetic aging, and developmental outcomes during early childhood. Study 1 examined the associations among stress and socioeconomic status in a diverse sample of newborns and toddlers to examine when, and if, these associations first emerge in the first few years of life (Aims 1a and 1b). Next, Study 2 leveraged the exogenous shock of the COVID-19 pandemic to examine the effect of lockdown on children's epigenetic aging (Aim 2a), stress physiology (Aim 2b), and developmental outcomes (Aim 2c). Finally, Study 3 tested the associations between epigenetic aging and developmental outcomes to determine the functional relevance of epigenetic aging in toddlerhood.

Figure 1-1.
Theoretical Model



Chapter 3: Associations Among Early Adversity and Epigenetic Aging Across Early Childhood

*Analyses for Research Question 1a were preregistered with *Open Science Framework*: Sperber (2024). *Prenatal Stress & Infant Epigenetics*. https://doi.org/10.17605/OSF.IO/5E4KC

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Research Question and Hypotheses

Little work has examined the presence of adversity-related epigenetic markers very early in childhood. Thus, Study 1 examined the associations between early adversity and epigenetic aging across the first few years of life. Since the literature of these effects during childhood is sparse, I developed hypotheses in line with those effects observed in older children and adult samples. Thus, I hypothesized that higher maternal stress and socioeconomic disadvantage during pregnancy and across the first few years of life will be associated with accelerated epigenetic aging in infancy and toddlerhood.

Numerous clocks were considered and examined in the present dissertation. For analyses during infancy (i.e., Study 1a), two epigenetic clocks were preregistered as the primary outcomes of interest due to their popularity in the literature: the Horvath and PedBE clocks. However, after pre-registration was complete, a meta-analysis preprint revealed that a more recently developed clock, DunedinPACE, demonstrated great promise for detecting socioeconomic disparities in epigenetic aging within pediatric samples (Willems et al., 2025). Due to my interest in examining socioeconomic disparities, I included the DunedinPACE clock as a secondary

outcome of interest in addition to the pre-registered Horvath and PedBE clocks in Study 1a. These three clocks are reported in the main text below. Additional clocks examined during infancy (PhenoAge, GrimAge, and Epigenetic-g) are reported in the Appendix.

Analyses using measures from toddlerhood (i.e., Study 1b) were not pre-registered. Therefore, I shifted to solely focus on DunedinPACE as the primary outcome of interest for this research question given the evolving nature of the literature. I continued to examine associations with Horvath, PedBE, and the other epigenetic clocks to maintain alignment with Study 1a as secondary outcomes. These results are reported in the Appendix.

Research Question 1a: Does prenatal greater stress and lower SES predict accelerated aging in newborns? Ordinary least squares (OLS) regression with robust standard errors was used to examine the continuous associations between each measure (assessed during the prenatal period) with epigenetic scores at 1-month. Analyses and hypotheses for Research Question 1a were preregistered on Open Science Framework (OSF; https://osf.io/5e4kc) and findings are published in *Psychoneuroendocrinology* (Sperber et al., 2025).

Research Question 1b: Does greater cumulative maternal stress and lower SES across the first few years of life predict accelerated aging in toddlers? OLS regression with robust standard errors was used to examine the continuous associations between each measure (assessed across the prenatal and postnatal period) with epigenetic age scores at 30-months.

3.1 Methodology

Participants

This study utilized data from the Building Understanding of Developmental Differences across Years (BUDDY) dataset, a prospective study of child development from birth to age three. 209 participants from New York City were recruited during their last trimester of

pregnancy to participate in this longitudinal study. Participants were recruited via local prenatal clinics, online advertising, flyers, and community events. To be included, mothers had to: 1) be fluent in either English or Spanish; 2) have a fetus with no known developmental disorders; 3) be at least 35 weeks pregnant with a singleton pregnancy; 4) live within a 1-hour radius of the laboratory by public transportation; and 5) be at least 18 years of age. Infants were formally enrolled in the study if they were born at least 37-weeks' gestation with no birth complications or known neurological illnesses.

Due to restrictions related to the COVID-19 pandemic, participants were recruited in two cohorts. Cohort 1 (n = 93) was recruited between June 2019 and March 2020, and Cohort 2 was recruited between August 2021 and September 2022 (n = 116).

Procedure

Upon providing informed consent, eligible participants completed a prenatal visit during which they completed surveys on their demographics, perceived stress, and provided a hair sample to assess physiological stress. Mothers were then invited to complete follow-up lab visits with their children at 1-month postpartum (55% female; n = 193) and then every 6 months thereafter until 36-months of age. At the 1-month and 30-month visits, a saliva sample was collected from children to assess epigenetic age. At the 6-month, 12-month, and 24-month visits, mothers again completed surveys of their demographics and perceived stress. A second hair sample was collected from children at the 30-month visit to assess their physiological stress. All procedures were approved by the Institutional Review Board of Teachers College, Columbia University.

Measures

Perceived Stress Component

I utilized principal component analysis (PCA) to reduce two survey-based measures into a single "perceived stress" component. These measures are described in detail below. Surveys administered at the prenatal timepoint loaded onto a single component with an eigenvalue of 1.53 and factor loadings of 0.71 (n = 201; "Prenatal Perceived Stress"). This component explained 77% of the variance and was extracted to predict 1-month epigenetic aging (i.e., Research Question 1a). A second component was generated using surveys from the prenatal, 6-month, 12-month, and 24-month appointments ("Cumulative Perceived Stress"). A single component with an eigenvalue of 3.72 emerged, with factor loadings ranging from 0.36 – 0.41 (Table A1-1; n = 149). This component explained 53% of the variance and was extracted to predict 30-month epigenetic age (i.e., Research Question 1b). Two values from this component were winsorized to match the next highest value 3 SD's from the mean before using in linear analyses. The two perceived stress factors correlated at 0.82 ($p < 0.001$).

Perceived Stress Scale (Cohen & Williamson, 1988). The Perceived Stress Scale (PSS) was administered at the prenatal timepoint, 6-months, 12-months, and 24-months postpartum. The PSS is a 10-item scale assessing the degree to which participants have perceived situations as stressful within the last month. Participants responded to each item using a 5-point Likert scale, ranging from 1 (*never*) to 5 (*very often*). Four items were positively stated and thus reverse scored before summing the items together. Participants needed to respond to at least 80% of items to receive a valid score. Mean imputation was used to account for missingness on individual items.

Perceived Impact of Negative Life Events (Sarason et al., 1978). The Life Experiences Survey (LES) was administered at the prenatal timepoint, 12-months, and 24-months postpartum. Participants were presented with 44 life events and asked if any had occurred within the past

year. For each event that participants endorsed as occurring, participants then rated the perceived impact of the event on their life on a 7-point Likert scale, ranging from -3 (extremely negative) to +3 (extremely positive). The absolute value was calculated for events endorsed as negative and these values were summed together to generate a Perceived Impact of Negative Life Events score.

Physiological Stress

Hair cortisol concentration is a measure of cumulative cortisol secretion over an extended period and is traditionally considered a measure of chronic HPA axis activation. One centimeter of hair is associated with approximately 1 month of cortisol output, with the 3cm closest to the root used to index cortisol output over the last three months (Meyer & Novak, 2021). A hair sample was collected from mothers at the prenatal timepoint to index infant exposure to physiological stress in utero (n = 161). At the 30-month visit, child physiological stress was assessed directly by collecting a sample of the child's own hair (n = 108). Six additional children donated a hair sample at the 36-month appointment; these samples were retained to maximize sample size.

Upon providing informed consent, a trained research assistant cut a 3cm segment of hair from the posterior vertex of the participant's scalp. Samples were frozen at -80°C then sent to either University of Massachusetts-Amherst or Yale Child Study Center for analysis. Across all participants and timepoints, 39 mother samples and 5 child samples were excluded from processing due to too small of a sample being retained. The remaining samples were analyzed using previously described methods (see Meyer et al., 2014). Briefly, samples were weighed, washed twice with isopropanol and allowed to air dry, ground to a powder, and hormone was extracted with methanol. The methanol extract was evaporated, redissolved in an assay buffer,

and samples were analyzed by an enzyme-linked immunosorbent assay according to standard quality controls (DetectX® Cortisol enzyme immunoassay kit #K003-H1W/H5W, Arbor Assays, Ann Arbor, MI). Values were set to missing if the participant reported using a corticosteroid (e.g., cream, medication) or applying one to another person within the last 3 months (n = 7 mothers; n = 11 children). Values were also set to missing if they were deemed biologically implausible in line with previous research (≤ 0 or ≥ 750 pg/mg; n = 2 mothers; n = 5 children; Magnuson et al., 2024) or greater than three standard deviations (SDs) from the mean (n = 1 mother; n = 3 children). To address the skew in the raw data and normalize the distribution, 112 maternal hair cortisol values from the prenatal timepoint and 84 child samples from the 30-month/36-month timepoint were log-transformed (ln) and standardized.

Due to cultural differences in values surrounding touching/cutting hair, challenges obtaining a large enough hair sample for analysis, and sample attrition, there were high levels of missingness on hair cortisol concentration values (n = 48 missing mother samples; n = 95 missing child samples). This missingness was not completely random according to Little's MCAR test at the prenatal timepoint (X^2 (17) = 29.97, p = 0.03) or the 30-months timepoint (X^2 (27) = 45.11, p = 0.02). Mothers who identified as Black were more likely to have a missing hair cortisol value for themselves at the prenatal appointment (β = 0.39, SE = 0.09, p < 0.001). Similarly, children of mothers who identified as Black (β = 0.22, SE = 0.08, p = 0.02), some other race (β = 0.18, SE = 0.08, p = 0.03), or reported lower incomes (β = -0.03, SE = 0.01, p = 0.03) were less likely to have donated a hair sample at the 30-month/36-month appointment.

To adjust for missingness in analyses with maternal hair cortisol data (i.e., Research Question 1a), I implemented multiple imputation with auxiliary variables and chained equations to allow for more flexible imputation estimation. I also report non-imputed results in the

Appendix, which aligned with the imputed results reported below. In contrast, the high rate of missingness on child hair cortisol data complicated the ability to reliably impute missing values for Research Question 1b. Therefore, I restricted the analytic sample for these analyses to those children with a valid hair cortisol sample; these analyses are presented as exploratory and are reserved for the Appendix.

Socioeconomic Status

Income-to-Needs. Participants reported their annual household income and the number of adults and dependent children living in their household at the prenatal, 12-month, 18-month, and 24-month appointments. These data were used to calculate an income-to-needs ratio, which reflects a household's economic well-being relative to the federal poverty line. For example, a family income-to-needs ratio of 2 means that a family has an annual income of 200% the federal poverty threshold. Due to a large right skew, values greater than 3 SDs from the mean were winsorized to match the next highest value within 3 SD of the mean. A value of $1 was added to participants who reported zero dollars in income before log-transforming (ln) values for analysis. For analyses predicting 1-month epigenetic age (i.e., Research Question 1a), the prenatal income-to-needs value was used (n = 192). For analyses predicting 30-month epigenetic age (i.e., Research Question 1b), I averaged across all the reported income-to-needs values, log-transformed, and then standardized the values (n = 203).

Maternal Education. Participants reported the years of education that they had attained at the prenatal visit. All participants provided this information; there were no missing data. Values were standardized for analyses.

Epigenetic Age Calculation

Sample Collection and Processing

At 1-month and 30-months of age, children were brought into the lab and saliva samples were collected using Oragene DNA kits. A total of 185 saliva samples were collected at the 1-month timepoint, and 125 samples were collected at the 30-month timepoint. An additional 11 children donated a saliva sample at the 36-month appointment; these samples were retained to maximize sample size. A dummy indicator reflecting timepoint of data collection was included in analyses to adjust for this.

Upon confirming that the child had not eaten for at least 30 minutes prior to the appointment, a research assistant swabbed the inside of the infant's mouth for 20 seconds to collect 1 mL of saliva. Samples were frozen at -80°C and then shipped for processing at The University of Texas at Austin. DNA from saliva was extracted using the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit by following the high-throughput automated DNA purification workflow on the KingFisher™ Flex System. DNA from samples was extracted and eluted to 90 μL. At this stage, several samples (n = 21 1-month samples and n = 6 30-month samples) were deemed unusable due to a low concentration of DNA in the sponge (<6 ng/μL). Retained samples underwent sodium bisulfite conversion and processed on the Beadchip using the Illumina Infinium HD Methylation Assay protocol (Moran et al., 2016; Pidsley et al., 2016).

Due to the multiple cohorts and phases of data collection in the present study, saliva samples were processed on different types of EPIC arrays. All 1-month samples from both cohorts (n = 164) and 30-month samples from Cohort 1 (n = 52) were processed on Infinium MethylationEPIC v1 BeadChip (850K) arrays, while 30-month samples from Cohort 2 (n = 78) were processed on EPIC v2 arrays (Illumina, Inc., San Diego, CA, USA). These updated arrays provide greater coverage of the genome via a larger probe count while also retaining a majority of probes used in previous epigenetic clocks (Kaur et al., 2023). A recent examination found

minimal systematic differences in epigenetic age calculated using these two arrays, with correlations between epigenetic age estimates all above 0.80 (Tay et al., 2025). Nonetheless, several adjustments occurred to account for the different structure of EPICv2 data. At the recommendation of other researchers (e.g., Sugden, 2023), I removed the newly added suffixes from the CpG sites of EPIC v2 data, averaged together duplicate probes, and removed discordantly named probes to align CpG names across array types.

Pre-processing Pipeline

DNA methylation levels were assayed and stored as intensity data (IDAT) files. Beadchips were scanned with the Illumina NextSeq 550 at the Genomic Sequencing and Analysis Facility to assess fluorescence intensities and an average methylation value for each CpG site was obtained. Methylation data was pre-processed using the *minifi* Bioconductor package (Aryee et al., 2014) and examined for quality control using the *ewastools* package according to Illumina quality control metrics (Just & Heiss, 2023). After ensuring each sample passed the bisulfite conversion threshold of 0.08, samples were examined for predicted sex against maternal report data and examined for genetic relatedness. At this stage, 3 samples were excluded due to a discrepancy between predicted and reported sex, and 3 samples were excluded as they were determined to be duplicate samples.

Next, methylation beta values (via IDAT files) were filtered for background fluorescence using detection p-values and number of hybridizing beads at each CpG site (Heiss & Just, 2019) and dye-bias correction was implemented using preprocessNoob (Triche et al., 2013). Importantly, sample normalization occurred separately for the Cohort 1 and Cohort 2 samples at 30-months due to the different array types. Next, sex probes, cross-reactive probes, and SNPs were removed. PCA was used to extract the proportion of cell type to account for heterogeneity

in epithelial and immune cells in saliva using previously established methods (Middleton et al., 2022). Ultimately, 159 samples from the 1-month timepoint and 128 samples from the 30-month timepoint were retained for the calculation of epigenetic age.

Epigenetic Clocks

The Horvath and PedBE epigenetic clocks were calculated using the *methylclock* package (Pelegí-Sisó et al., 2021). The PhenoAge, GrimAge, and DunedinPACE clocks were calculated using the *dnaMethyAge* package (Wang et al., 2023). Epigenetic-g was calculated using publicly available code (McCartney et al., 2022). After calculating the clocks, each value was residualized for technical artifacts (i.e., slide, array, plate) and cell type composition (i.e., proportion of leukocytes vs. epithelial cells). Samples collected at the 30-month timepoint were additionally residualized for child chronological age at the time of data collection. After these adjustments, all epigenetic values were standardized for analysis.

Horvath Clock

The Horvath (2013) multi-tissue clock has been trained to predict chronological age using thousands of samples across the lifespan. Developed using elastic net regression, it examines 353 CpG sites to estimate an individual's epigenetic age. Though it is the most widely-researched epigenetic clock, the Horvath clock has an error rate of 3.6 years, and therefore may not be suitable for pediatric populations. Outliers were winsorized to 3 SD's from the mean (n = 2)

PedBE Clock

The Pediatric-Buccal-Epigenetic (PedBE; McEwen et al., 2020) clock was trained on a sample of children aged 0-20 years using buccal cells and is strongly correlated with chronological age across this age range. It utilizes 94 CpG sites to calculate epigenetic age and has demonstrated robustness with saliva samples. The PedBE clock is preferred in pediatric

populations due to its low error rate of 0.35 years. The 30-month Cohort 2 DNA methylation data were missing 21% of the necessary probes to calculate PedBE due to the change in probes to EPIC v2 arrays, which is slightly higher than the recommended threshold of 20% missingness. In line previous studies with a different epigenetic clock (e.g., Sugden, 2023), I continued with calculation of PedBE for this sample subset by changing the missingness threshold. Outliers were winsorized to 3 SD's from the mean (n = 2).

DunedinPACE

The DunedinPACE is a third-generation clock designed to predict the rate of biological aging (Belsky et al., 2022). Developed from blood samples collected over two decades from the Dunedin Birth Cohort, DunedinPACE was trained to predict decline in organ system integrity using 19 clinical indicators, including biomarkers from cardiovascular, metabolic, and immune systems. The clock is scaled such that a value of 1 reflects one year of biological aging for each chronological year. The validation sample consists entirely of adults, though subsequent studies have applied this clock to pediatric populations. In line with a previous investigation comparing EPIC v1 and v2 data (Sugden, 2023), greater missingness was observed on DunedinPACE data resulting from EPIC v2 arrays (28%). At Sugden's (2023) recommendation, I altered the missingness threshold to 30% due to this known loss of data. No adjustments were made to the DunedinPACE distribution.

PhenoAge

The PhenoAge clock is a second-generation clock designed to use blood samples to predict phenotypic age (Levine et al., 2018), which reflects differences in health and lifespan of similarly-aged individuals. Using a set of clinical biomarkers collected from thousands of adults, the PhenoAge clock accurately estimates all-cause mortality, cognition, physical health, and

lifestyle factors. I calculated a principal components version of the clock, which has been shown to improve reliability in technical replicates (Higgins-Chen et al., 2022). Outliers were winsorized to 3 SD's from the mean (n = 8).

GrimAge

The GrimAge clock is a second-generation clock designed to use blood samples to predict mortality risk (Lu et al., 2019). The algorithm was developed using DNA methylation-based biomarkers of smoking pack-years and plasma proteins previously associated with stress and physiological risk factors, such as C-reactive protein. The algorithm was validated on a sample of older adults (mean age = 66) and scores reflect an individual's estimated biological age. While a robust predictor of lifespan, little research has applied this clock to pediatric populations. I calculated a principal components version of the clock, which is shown to improve reliability in technical replicates (Higgins-Chen et al., 2022). No adjustments were made to the GrimAge distribution.

Epigenetic-g

The Epigenetic-g clock is a blood-based clock developed to predict general cognitive ability in older adults (McCartney et al., 2022). Previous work has identified socioeconomic and racial disparities in Epigenetic-g in older children (Raffington et al., 2022). Scores are standardized within each sample, with higher values associated with better cognitive abilities. Publicly available code was used to calculate the clock (https://gitlab.com/danielmccartney/ewas_of_cognitive_function).

Covariates

Research Question 1a

A set of demographic variables were preregistered to examine as potential confounders for analyses with epigenetic data at 1-month. Variables that were associated with either the PedBE or Horvath clock with a Pearson's correlation coefficient ≥ 0.10 were retained in linear analyses to maximize degrees of freedom. Ultimately, the demographic covariates retained for analyses included: maternal race and ethnicity, gestational age at birth, whether the infant was exclusively breastfed or not, and maternal report of alcohol consumption during pregnancy. In analyses with prenatal hair cortisol, I also included a covariate for the mother's week of gestation at the time the hair sample was collected and a categorical variable reflecting the lab in which the hair sample was processed; this later covariate was unnecessary at the 30-month timepoint as all of these samples were processed at the same lab facility. This covariate was not listed in the original preregistration plan. Greater detail on the covariates examined are reported in the preregistration document (https://osf.io/5e4kc).

Research Question 1b

The following covariates were included for analyses using 30-month epigenetic data: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Analyses with child hair cortisol also included a dummy indicator of the timepoint of hair sample collection.

Analytic Plan

To estimate the effect of early adversity on epigenetic aging across early childhood, I leveraged OLS regression with robust standard errors. All variables were standardized for linear analysis, so coefficients can be interpreted as standardized betas. DNA methylation data were processed and epigenetic age was calculated using RStudio. Subsequent analyses were conducted in Stata v.18.

Research Question 1a: Prenatal Exposures and 1-month Epigenetic Aging

These analyses were restricted to those infants with a valid epigenetic age estimate at 1-month (n = 159). Multiple imputation with chained equations (ICE) and 25 iterations was used to account for missing data on independent variables and covariates.

The pre-registered analytic plan utilized OLS regression with robust standard errors to estimate the associations among the Prenatal Perceived Stress component, maternal hair cortisol, prenatal income-to-needs, and maternal education with Horvath and PedBE scores in newborns. I deviate from this plan in several ways. First, I pre-registered the cell type-adjusted epigenetic clock calculated by the *methylclock* package as the dependent variable (i.e., AgeAccel3). Upon further investigation, I observed that this package did not fully adjust for cell composition in the data. To correct for this, I manually calculated the cell-adjusted residuals and discarded the package-generated variables. At this stage, I opted to further adjust for batch effects (i.e., slide, array, and plate) using partial correlations, which allowed for the interpretation of the associations between the variables of interest without further adjustment for technical characteristics. Beyond these adjustments, I followed the preregistration plan's approach of utilizing regression to adjust for demographic characteristics in the prenatal analyses.

The first set of models estimated the association between each independent variable of interest and epigenetic aging, residualized for cell composition and technical artifacts (Model 1). Next, I covaried for child chronological age (Model 2). A significant association in this model would provide evidence that adversity-related disparities in epigenetic age are detectable at the beginning of postnatal life, above and beyond the influence of arbitrary factors like technical confounds and chronological age. However, this estimate also reflects all other environmental and genetic confounders that predict both adverse exposures and epigenetic aging.

Next, I adjusted for the pre-registered demographic characteristics listed under "*Covariates – Research Question 1a"* (Model 3). This coefficient further refines the estimate to examine if such associations are independent of other prenatal experiences that are associated with both greater adversity and epigenetic aging, such as maternal alcohol consumption during pregnancy, infant gestational age, and whether an infant is breastfed. It is possible that such factors may serve as mediating pathways between adversity and infant outcomes, and adjusting for such covariates may reduce the observed effect. If this is the case, I would observe a change in the coefficient from Model 2 to Model 3 as these covariates were introduced. This is valuable to observe as I seek to test if the proposed association between prenatal adversity and epigenetic age exists independently of such factors. Indeed, the DOBHaD hypothesis posits that experiences such as hyper activation of the HPA axis, environmental toxins, and poor nutrition, among other exposures, are accountable for the epigenetic programming of health and disease.

Finally, in line with the pre-registration, I conducted a multiple regression analysis to examine the association between all prenatal predictors and epigenetic age at 1-month, adjusting for child age and demographic covariates. Further refining the estimate in Model 3, this coefficient reflects the independent effect of each prenatal adversity, holding constant all other measured exposures. This model informs whether each exposure exerts independent effects on infant biological aging, and is informative for theory as I attempt to elucidate the pathways by which prenatal experiences may lead to epigenetic signatures at the beginning of postnatal life.

Research Question 1b: Early Life Adversity and 30-month Epigenetic Aging

Analyses using 30-month epigenetic data were not pre-registered. Recent meta-analytic findings suggest a robust association between SES and accelerated aging in pediatric samples

when using the DunedinPACE clock (Willems et al., 2025); therefore, I focus on DunedinPACE as the primary outcome of interest for Study 1b; all other clocks are reported in the Appendix.

To estimate the association between cumulative adverse exposures and epigenetic aging in toddlerhood, I again utilized OLS regression with robust standard errors. The Cumulative Perceived Stress component, average family income-to-needs across early childhood, and maternal education were regressed separately onto epigenetic age at 30-months. Epigenetic age scores were residualized for technical artifacts (i.e., slide and array), cell type composition, and child chronological age (Model 1). For these analyses, I adjusted for chronological age at the onset rather than maintaining the pre-registered approach in Research Question 1a of adjusting for it in a separate step (this is because the field's preferred specification is to residualize the variance of chronological age first in order to observe the raw association between epigenetic age and the outcome of interest). These coefficients indicate whether adversity-related perturbations in epigenetic age are detectable in toddlerhood, inclusive of all other factors that may also account for this association. These estimates cannot isolate the exact exposure, or timing of exposure, that predict such biological signatures; rather, they serve as a general indicator of the presence of such biomarkers.

Next, I covary for the covariates listed under "*Covariates – Research Question 1b"* (Model 2). To select these covariates, I conducted a thought experiment: if a hypothetical intervention or policy were to reduce early adversity (e.g., reduce poverty via an increased child tax credit, or reduce maternal perceived stress via mindfulness classes) without changing any other aspect about a family's circumstances, would subsequent changes be observed in children's epigenetic age? If so, such an effect would need to persist above and beyond characteristics like child sex. While there may be epigenetic differences based on child sex, I seek to examine if

these associations persist above and beyond such qualities. This is informative to identifying if such an intervention would impact all families uniformly.

Notably, additional demographic covariates were included in a previous iteration of this analysis, including maternal age, maternal race and ethnicity, and maternal receptive vocabulary scores (as an indicator of general cognitive abilities). These covariates were preselected under the same logic as described earlier (e.g., an intervention that improved maternal stress would not subsequently alter maternal race). However, upon investigation, several of these variables were correlated with the independent variables of interest at moderate effect sizes (r's range 0.29 – 0.56; see Table A1-5). Therefore, including these demographic covariates in the regression model led to the inflation of standard errors, which resulted in less precise estimates. To empirically answer the primary question of this study (i.e., "*are epigenetic signatures of SES and stress detectable in toddlers*"), I chose to exclude these demographics from the final model specifications and retain covariates that are less strongly associated with the independent variables examined (e.g., child sex).

Continuing this thought experiment, I further adjusted for epigenetic age at 1-month in Model 3. These estimates reflect the effect of each exposure above and beyond a child's biological 'baseline' (i.e., pace of aging at birth), isolating the effect of our theoretical intervention to the postnatal period. Indeed, if a poverty reduction intervention were to occur after birth (e.g., Baby's First Years), these coefficients should reflect the effect of such an intervention holding constant prenatal experiences and one's biological 'starting point.' While unmeasured variable bias in correlational models is a perpetual concern, this estimate is likely most informative to our hypothetical intervention, as it holds constant many confounding biological and family-level factors that influence both early adversity and epigenetic aging.

Finally, I conducted a multiple regression analysis to examine the association amongst all predictors and epigenetic age at 30-months, adjusting for demographics and 1-month epigenetic scores. These coefficients reflect the independent effect of each exposure on postnatal patterns of epigenetic aging, holding constant all other measured exposures. These analyses reveal, for example, if a poverty reduction intervention would predict changes to the pace of aging if no other aspect of family functioning was altered. This is also an informative estimate for our thought experiment, as it assumes a policy or intervention like BFY does not lead to changes in other aspects of early adversity (e.g., maternal perceived stress or years of education).

In addition to the above, an exploratory analysis examined the association between child hair cortisol concentration and epigenetic aging at 30-months. These models were restricted to those children with both a valid hair cortisol value and epigenetic age estimate at 30-months (n = 77). Model specifications and covariates align with the other 30-month analyses described above. I also include a dummy indicator reflecting whether the hair sample was collected at the 30-month or 36-month visit.

All analyses were restricted to children with a valid epigenetic age estimate at 30-months (n = 128). Missing data was handled via multiple imputation estimated with chained equations and 25 iterations. DNA methylation data and clock calculation occurred in R. Subsequent analyses occurred in Stata v.18.

3.2 Results

Descriptive statistics

Descriptive statistics for the analytic sample are reported in Table 1-1. At birth, infants were of normal weight (M = 7.4 lbs, SD = 1.0) and full term (M = 39.4 weeks, SD = 1.0). Approximately 43% of the infants were male. Mothers identified as Asian (8%), Black (24%),

Other/Mixed/Unknown Race (30%), and White (38%). About half of the sample (44%) identified as Hispanic. At the prenatal visit, mothers were on average 32.4 years old (SD = 5.7), attained 15.3 years of formal education (SD = 3.5), and reported an income-to-needs ratio of 6.8 (SD = 9.3). Maternal hair cortisol values for the sample (pg/mg; M = 12.7; SD = 19.5) fell within the expected values for pregnant women in the third trimester (Marceau et al., 2020). Similarly, mothers' average perceived stress levels across all assessments (M = 11.6, SD = 5.7) aligned with normative PSS scores for adult females (M = 13.7, SD = 6.6; Cohen & Williamson, 1988).

Table 1-2 reports the raw epigenetic age estimates obtained at both 1-month and 30-months, and the results of a t-test examining differences between these two means. The pace of biological aging increased from 1-month (M = 1.34, SD = 0.07) to 30-months (M = 1.49, SD = 0.10, $p < 0.001$) according to DunedinPACE estimates. According to first-generation clocks, the average epigenetic age of the sample was overestimated according to the pan-tissue Horvath clock (M = 1.44 years, SD = 3.7) and PedBE clocks (M = 0.29 years, SD = 0.23) at the 1-month appointment. These estimates substantially improved at the 30-month timepoint ($M_{Horvath}$ = 3.26 years, SD = 1.46; M_{PedBE} = 3.35 years, SD = 0.83). In contrast, the blood-based PhenoAge and GrimAge clocks provided epigenetic ages that extremely overestimated the chronological age of the sample at both timepoints (means ranged 42.59 – 51.81 years). This divergence is likely due to differences in the tissues used to generate these clocks and those examined by the present study. Notably, the variance (measured via standard deviations) in all of the clocks examined increased substantially from 1-month to 30-months. In addition, there was no difference observed in average epigenetic age by child sex at either timepoint (Table A1-2).

Correlations among key variables

Table A1-3 reports the bivariate correlations among the key variables of interest related to epigenetic aging at 1-month. Lower family income-to-needs, but not maternal education, was weakly correlated with higher perceived stress during pregnancy (r = -0.17, p = 0.04). Notably, maternal hair cortisol was not significantly associated with any of the predictors during pregnancy, including perceived stress. Although each epigenetic age measure overestimated the mean age of the 1-month infants, all the measures were significantly correlated with infant chronological age, though to varying degrees. The strongest correlations were detected for the PedBE and PhenoAge clocks (r's range 0.42 – 0.47, p's < 0.001). The Horvath, DunedinPACE, and GrimAge clock each demonstrated a weak association with chronological age (*r's* range 0.16 – 0.17, p's = 0.04). This result from the Horvath clock aligns with a previous study in newborns (Bozack et al., 2023), while the DunedinPACE result aligns with work conducted in both adults (Raffington et al., 2023) and older children (deSteiguer et al., 2025). Interestingly, we observed few significant associations among the epigenetic clocks themselves, possibly due to the few overlapping CpG's across clocks. As clocks trained to predict chronological age, PedBE and Horvath were moderately correlated with each other (r = 0.50, p < 0.001). The only other clock to significantly correlate with every other clock was PhenoAge *(r's* range 0.20 – 0.35, *p's* < 0.05).

Table A1-4 presents bivariate correlations among the key variables of interest related to epigenetic aging at 30-months, as well as associations among epigenetic age estimates at each timepoint. At 30-months of age, the PedBE (r = 0.25, p < 0.01) and GrimAge (r = 0.38, p < 0.001) clock were the only epigenetic clocks associated with child chronological age at the time of the assessment. Amongst the epigenetic clocks at 30-months, correlations ranged from -0.08 to 0.40, with 7 out of 14 coefficients significantly associated with other clocks. For within-clock

correlations across time, a higher GrimAge ($r = 0.39$, $p < 0.001$), PhenoAge ($r = 0.20$, $p = 0.04$), and Epigenetic-g ($r = 0.22$, $p = 0.03$) at 1-month was associated with a higher score for that same clock at 30-months, suggesting limited stability in epigenetic age across the first few years of life. Amongst the variables of interest, greater Cumulative Perceived Stress was associated with higher child hair cortisol concentrations ($r = 0.25$, $p = 0.04$) at 30-months. Notably, higher child hair cortisol was significantly associated with lower maternal education ($r = -0.22$, $p = 0.04$) and marginally associated with lower family income ($r = -0.19$, $p = 0.08$).

Table A1-5 presents pairwise correlations among epigenetic age at 30-months, the independent variables of interest, and demographic characteristics. Notably, cumulative perceived stress was not associated with either family income-to-needs ($r = -0.09$, $p = 0.32$) nor maternal years of education ($r = 0.01$, $p = 0.87$). In contrast, family income and maternal education were strongly correlated with demographic characteristics like maternal race, ethnicity, age, and receptive vocabulary scores (see Table A1-5). Higher cumulative perceived stress was only associated with the likelihood of a participant identifying as Black ($r = 0.19$, $p = 0.02$).

OLS Regression Results: Research Question 1a

Table 1-3 reports the coefficients from OLS models estimating the association between prenatal adversity and accelerated aging in newborns according to the Horvath, PedBE, and DunedinPACE clocks. These coefficients are presented as forest plots in Figure 1-2 and Figure 1-3. All other epigenetic clocks examined at 1-month are presented in Table A1-6 and presented visually in Figures A1-1 and A1-2. Non-imputed estimates are reported in Table A1-7.

Prenatal Perceived Stress and 1-month Epigenetic Aging

I observed scant evidence of an association between prenatal perceived stress during pregnancy and infant epigenetic aging. Only 1 of the 6 pre-registered models (ie., Horvath and

PedBE) emerged as significant, with greater perceived stress associated with an accelerated Horvath age only when adjusting for demographic characteristics ($\beta = 0.15$, SE = 0.07, $p = 0.03$). Of all the clocks examined, effect sizes ranged from -0.17 to 0.20 across models, with the majority indicating that greater perceived stress was associated with accelerated aging. However, few were statistically significant.

Prenatal Hair Cortisol and 1-month Epigenetic Aging

I observed little evidence of a robust association between maternal prenatal hair cortisol concentration and infant epigenetic aging, as none of the pre-registered outcomes were statistically significant. In partial correlations, greater maternal hair cortisol during pregnancy was associated with an accelerated DunedinPACE ($\beta = 0.20$, SE = 0.09, $p = 0.03$). However, this effect diminished to trend-level significance when adjusting for infant chronological age ($\beta = 0.17$, SE = 0.11, $p = 0.10$). Across the other clocks, the magnitude and directionality of estimates varied widely across models (β*'s* range -0.04 to 0.20).

Prenatal SES and 1-month Epigenetic Aging

Overall, I did not observe robust evidence of an association between prenatal income-to-needs and epigenetic aging in newborns. Out of the 6 pre-registered models, two emerged as statistically significant: lower income-to-needs was associated with accelerated PedBE age when adjusting for chronological age ($\beta = -0.15$, SE = 0.08, $p = 0.05$) and demographics ($\beta = -0.18$, SE = 0.08, $p = 0.03$). After adjusting for covariates, the association between family income and DunedinPACE was negatively valanced, but statistically non-significant ($\beta = 0.13$, SE = 0.08, $p = 0.11$). Across the other clocks examined, coefficients were small in magnitude yet negative in directionality (β*'s* range -0.18 to 0.08), suggesting that lower income-to-needs was associated with accelerated aging.

Similarly, there was little evidence of an association with maternal education. None of the pre-registered clocks were significantly associated with maternal education across the 6 models tested. After adjusting for demographics, a higher DunedinPACE was marginally associated with greater years of education (β = -0.17, SE = 0.12, p = 0.16). Across the other models examined, effect sizes ranged from -0.17 to 0.12, with the majority of models indicating that lower maternal education was associated with accelerated epigenetic aging.

Joint Prediction Model

Table 1-4 presents multiple regression results of the preregistered clocks and DunedinPACE, while Table A1-8 reports these results for all clocks examined. Non-imputed estimates for these models are reported in Table A1-9.

When all stress and SES predictors were entered into the model, none of the coefficients for DunedinPACE were statistically significant. Of the pre-registered clocks, greater perceived stress continued to predict trend-level accelerated Horvath aging at a similar magnitude as OLS models above (β = 0.14, SE = 0.08, p = 0.08). Additionally, lower income-to-needs continued to predict an accelerated PedBE age in newborns (β = -0.20, SE = 0.09, p = 0.04). However, none of the other predictors maintained statistical significance for any other clock.

OLS Regression Results: Research Question 1b

The next set of analyses examined the association among cumulative measures of stress and SES (i.e., measured across the prenatal period and first few postpartum years) with epigenetic age in toddlers. Here, I focus on DunedinPACE as the primary outcome of interest as emerging work suggests this clock to be preferred for examining these constructs in pediatric samples (Willems et al., 2025; all other clocks are reported in the Appendix). Table 1-5 reports the coefficients from linear regression models, and Figures 1-4 through 1-6 present these

coefficients as forest plots with various model specifications. All other clocks examined are reported in Table A1-10 and presented visually in Figures A1-3 through A1-5. Non-imputed estimates are reported in Table A1-11.

Cumulative Perceived Stress and 30-month Epigenetic Aging

Higher Cumulative Perceived Stress across the first few years of life was associated with a faster DunedinPACE score in toddlers, even after adjusting for demographic characteristics and epigenetic age at 1-month (β = 0.22, SE = 0.09, p = 0.02). No other epigenetic clock examined maintained a statistically significant association with perceived stress after adjusting for demographics. Additionally, these coefficients varied in magnitude and directionality.

Early Life SES and 30-month Epigenetic Aging

There was modest evidence of an association between SES and DunedinPACE scores at 30-months. A lower family income-to-needs was associated with an accelerated DunedinPACE after adjusting for covariates (β = -0.24, SE = 0.12, p = 0.05). When adjusting for baseline DunedinPACE, the coefficient maintained the same strength but dropped to marginally significant (β = -0.24, SE = 0.12, p = 0.06). Some evidence of a signal with family income was present in the other clocks examined, particularly for the PhenoAge and GrimAge clocks. Of these second-generation clocks, all coefficients maintained a negative valance and 4 out of 6 coefficients were statistically significant. Notably, the PhenoAge clock maintained significance even after adjusting for baseline epigenetic age (β = -0.27, SE = 0.11, p = 0.02).

For maternal education, all DunedinPACE coefficients were negatively valanced, and p-values ranged from 0.06 to 0.11 depending on model specifications. When examining the other epigenetic clocks, PhenoAge and GrimAge demonstrated a similar level of association, with greater years of education associated with decelerated epigenetic aging. For these clocks, all

coefficients a negative directionality after adjustment for covariates and baseline epigenetic age, with 5 out of 6 coefficients being statistically significant across model specifications.

Child Hair Cortisol and 30-month Epigenetic Aging

Exploratory analyses estimated the association between child hair cortisol concentrations and epigenetic age at 30-months. Table A1-12 reports the imputed coefficients, while Table A1-13 reports the non-imputed coefficients. Overall, no evidence of an association was observed across all of the clocks examined. Beta values ranged between -0.15 to 0.17 (SE's ranged 0.07 – 0.10) with none of the coefficients emerging as statistically significant.

Joint Prediction Model

Finally, all the measures of cumulative early adversity, demographic covariates, and baseline measures of epigenetic age for each clock were entered into a regression model to predict toddler epigenetic age. Results for DunedinPACE are presented in Table 1-6, and all other clocks are reported in Table A-14. Non-imputed estimates are reported in Table A1-15.

Aligned with the findings from above, greater perceived stress continued to predict accelerated pace of aging when adjusting for other adversities at the same strength as bivariate models ($\beta = 0.22$, SE = 0.09, $p = 0.02$). Additionally, a higher income-to-needs was associated with slower DunedinPACE at a similar effect size as bivariate models, but at a diminished significance level ($\beta = -0.20$, SE = 0.16, $p = 0.21$). Among the other clocks examined, higher maternal education continued to predict a lower GrimAge in the joint model ($\beta = -0.24$, SE = 0.10, $p = 0.02$).

3.3 Discussion

Overall, the evidence for adversity-related perturbations in epigenetic aging at birth was limited. No robust associations were detected with the pre-registered clocks (Horvath and

PedBE). For the clock most reliable at detecting socioeconomic disparities in pediatric samples (DunedinPACE), no coefficients with perceived stress, maternal HPA axis activation, or SES were statistically significant, though all effects aligned in the hypothesized direction. Of the second-generation clocks sensitive to socioeconomic disparities in adult samples (PhenoAge and GrimAge), results were also inconsistent and varied depending on the construct examined, suggesting some of the detected effects may be spurious. Overall, I interpret these results as largely null: the epigenetic effects of perceived stress, maternal HPA axis activation, and social disadvantage are not robustly detectable at birth in this sample.

In contrast, stronger associations between epigenetic age and early adversity emerged over the following years. By 30-months postpartum, greater perceived stress was robustly associated with an accelerated DunedinPACE regardless of model specifications. This suggests that the rate of biological aging in children increased in the first few years of life as mothers reported greater perceived stress levels. This was also reflected in a multiple regression analysis, which found greater perceived stress to be associated with higher DunedinPACE at 30-months, holding constant other socioeconomic adversities. To our knowledge, this is the first reported association amongst these factors at this developmental stage. However, I did not observe an association between child HPA axis activation assessed via hair cortisol and epigenetic aging.

For socioeconomic constructs, a similar association emerged with DunedinPACE at 30-months, though these effects were less robust than perceived stress. A higher family income was significantly associated with an accelerated DunedinPACE across model specifications. A similar direction of effects was observed with maternal education, but these results were not statistically significant. Among the second-generation clocks, this pattern of effects was somewhat detectable with the PhenoAge and GrimAge clocks, which aligns with recent research

of socioeconomic disparities in epigenetic aging (Willems et al., 2025). Collectively, this evidence indicates that stress-based and socioeconomic perturbations in biological aging are detectable as early as the first few years of life.

As described earlier, previous model specifications found that, when adjusting for other demographic characteristics (e.g., maternal race and ethnicity, maternal receptive vocabulary, and maternal age), the standard errors of these coefficients in toddlerhood increased substantially, resulting in imprecise estimates. This indicates that the correlation between the independent variable and each covariate was stronger than the association with the dependent variable. This is important to note as it is unclear what may be accounting for the effect between early adversity and epigenetic aging in children. While these covariates could indeed reflect confounders of the detected associations, they may also serve as potential mediators (e.g., younger mothers may parent in such a way that results in accelerated epigenetic aging). Disambiguating the complex factors that may account for this association is beyond the scope of this dissertation. Nonetheless, it is important to acknowledge the constraints of the reported effects and maintain transparency on the various model specifications examined.

Another notable pattern observed is the increased variance in epigenetic age scores from infancy to toddlerhood. This aligns with previous longitudinal work from birth to later childhood (Simpkin et al., 2017). One interpretation of this pattern is that typically-developing children tend to be born with relatively similar epigenetic age profiles, but these begin to diverge with the accumulation of childhood experiences. Alternatively, it could indicate that these clocks are not equipped to detect real variation at the beginning of life and are therefore inappropriate for application to infant samples.

Collectively, these findings provide suggestive evidence that epigenetic signatures of socioeconomic disadvantage and early life stress may emerge over the first few years of life. This finding aligns with the biological weathering hypothesis, which posits that an accumulation of adverse experiences results in a physiological wear-and-tear on the body. Future research is necessary to understand how these epigenetic patterns biologically embed in young children, e.g., via poor nutrition, exposure to environmental toxicants, or other sources.

Validity in Newborn Samples

To date, few studies have critically examined the validity of applying epigenetic clocks designed for adult populations to infant samples. This concern is underscored by the poor chronological age estimates of the Horvath clock at 1-month of age, and reflected in the non-significant correlations amongst several of the epigenetic clocks at the same timepoint. These findings highlight the need to further consider the validity of applying epigenetic clocks designed for adults at such an early moment in life. Indeed, it is possible that an adverse prenatal environment may set the stage for perturbations in epigenetic aging, but the present measures are not able to detect such perturbations in infants.

Some of the detected estimates were aligned in directionality and magnitude with those produced by other studies, providing some positive indication for the application of these epigenetic clocks in newborn populations. For example, the association between lower maternal education and faster DunedinPACE in the present study align with previously reported correlations in adult (Effect Size [ES] = 0.15; Maunakea et al., 2024) and pediatric samples (ES = 0.18; Raffington et al., 2021). In addition, the correlations observed in toddlers provides a positive sign for the validity of these measures at 30-months of age.

Another falsifiability test is the examination of epigenetic age by sex. The adult literature consistently demonstrates that men have an accelerated epigenetic age compared to women (e.g., Belsky et al., 2022). This shorter lifespan could largely be due to life course factors that shape health and mortality risk (e.g., risk-taking propensity, substance use; Horvath et al., 2016). However, it is possible that this effect could be detectable early in life as testosterone is thought to accelerate biological maturation. I observed little evidence that males had a higher epigenetic age at either 1-month or 30-months. This finding could be evidence for the importance of accumulating life-course factors on ageing, or it could further underscore concerns regarding the validity of these measures at this developmental stage.

One way to interpret these findings is that epigenetic clocks hold little to no measurement validity in newborns, and thus are not appropriate indicators at this early stage of development. Alternatively, it is plausible that the biological embedding of adversity has not yet occurred at birth, but accumulates via postnatal experiences. This could also explain why associations emerged at the 30-month timepoint. Since I am unable to draw conclusions to this question with the present data, additional research to validate epigenetic clocks in this age range.

Strengths and Limitations

A strength of this study is the use of a socioeconomically- and racially-diverse cohort of children, longitudinal data, and the pre-registered analysis plan. A limitation is the relatively small sample size, which may have limited our power to detect small effects, and the use of saliva samples with epigenetic clocks that were predominantly validated using blood tissues. This later concern was addressed via the use of a cell-type reference panel specifically designed for pediatric saliva samples to improve cell composition estimation (Middleton et al., 2022). Nonetheless, greater research on the validity of epigenetic clocks in pediatric samples, as well as

the creation of saliva-specific clocks, are necessary to improve the generalizability of this work. In addition, the use of maternal hair cortisol during pregnancy may not fully reflect maternal HPA axis activation due to interference from the developing fetus: placental corticotrophin releasing hormone (CRH) during the third trimester is thought to drive the release of cortisol from the mother's HPA axis (Magiakou et al., 1996), resulting in a stress-response system during pregnancy that is independent of the mother's own experience. Future work should evaluate hormone-based assessments of stress in infants themselves to clarify these associations.

Table 1-1
Descriptive Statistics of Study 1 Analytic Sample

	N	Mean	SD	Min	Max
Sample Characteristics at Prenatal Timepoint					
Maternal Age	159	32.37	5.69	19	45
Income-to-Needs Ratio *(winsorized)*	146	6.82	9.23	0	44
Maternal Years of Formal Education	159	15.26	3.46	6	22
Perceived Stress Scale Total	158	13.25	6.98	0	33
Perceived Impact of Negative Life Events Score Total	154	5.22	6.74	0	46
Maternal Hair Cortisol Concentration *(pg/mg; winsorized)*	112	12.69	19.46	0.10	160
Infant Birth Characteristics					
Birth Weight *(in grams)*	159	3348.99	464.79	2268	4508
Gestational age at birth *(in weeks)*	158	39.41	1.0f3	37	42
Infant Chronological Age *(in months)*	159	1.37	0.58	0	3
Infant is Male	158	43%			
Maternal Race & Ethnicity					
Hispanic	159	44%			
Asian	159	8%			
Black	159	24%			
Other/Mixed/Unknown Race	159	30%			
White	159	38%			
Sample Characteristics at 30-month Timepoint					
Income-to-Needs Ratio [a] *(winsorized)*	127	6.2	7.33	0.01	30.95
Perceived Stress Scale Total [b]	128	11.6	5.72	0.80	32.4
Perceived Impact of Negative Life Events Score Total [c]	127	4.89	5.51	0	41
Child Hair Cortisol Concentration [d] *(pg/mg; winsorized)*	84	49.68	85.31	2.53	356.9
Observations	159				

Note. Sample descriptives at 30-months restricted to those with valid epigenetic age values.
[a] Values were averaged across prenatal, 12-month, 18-month, and 24-month appointments
[b] Values were averaged across prenatal, 6-month, 12-month, and 24-month appointments
[c] Values were averaged across prenatal, 12-month, and 24-month appointments
[d] Assessed at the 30-month appointment. Includes participants with at least 1 valid measure of epigenetic age at either timepoint.

Table 1-2
Descriptive Statistics of Epigenetic Clocks

	Raw Epigenetic Age Estimates						
	1-month			30-months			
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	p-value
First Generation Clocks (scaled in years)							
Horvath	1.44 (0.31)	0.84	2.72	3.26 (1.46)	1.11	7.10	<0.001
PedBE	0.29 (0.23)	0.051	2.29	3.35 (0.83)	1.95	6.13	<0.001
Second Generation Clocks (scaled in years)							
PhenoAge	47.29 (4.89)	13.80	53.59	46.58 (8.52)	21.61	62.61	0.52
GrimAge	42.59 (1.60)	34.34	45.55	51.81 (8.88)	34.72	65.17	<0.001
Epigenetic-g [a]	0.00 (0.18)	-0.63	0.61	0.00 (0.17)	-0.40	0.50	
Third Generation Clock (pace of aging)							
DunedinPACE	1.34 (0.07)	1.04	1.56	1.49 (0.10)	1.20	1.70	<0.001
N	159			128			

Note. P-value reflects the results of a t-test examining the difference between the two means.

[a] Epigenetic-g scores were standardized within each timepoint

Table 1-3
Prenatal Adversity and Epigenetic Aging in Newborns

	Horvath	PedBE	DunedinPACE
Cumulative Perceived Stress Component			
1. Partial Correlation	0.11 (0.06)	-0.06 (0.07)	0.09 (0.08)
2. Adjusts for Age	0.11 (0.06)	-0.07 (0.07)	0.08 (0.08)
3. Adjusts for Demographics	0.15* (0.07)	-0.00 (0.07)	0.13 (0.09)
Maternal Hair Cortisol Concentration			
1. Partial Correlation	0.02 (0.09)	0.14 (0.08)	0.20* (0.09)
2. Adjusts for Age [a]	-0.02 (0.11)	0.03 (0.09)	0.17 (0.11)
3. Adjusts for Demographics [a]	-0.04 (0.11)	-0.01 (0.09)	0.20 (0.12)
	Horvath	PedBE	DunedinPACE
Income-to-Needs			
1. Partial Correlation	-0.02 (0.11)	-0.12 (0.08)	-0.09 (0.06)
2. Adjusts for Age	-0.04 (0.11)	-0.15 (0.08)	-0.11 (0.06)
3. Adjusts for Demographics	-0.06 (0.12)	-0.18* (0.08)	-0.13 (0.08)
Maternal Education			
1. Partial Correlation	0.02 (0.08)	-0.07 (0.08)	-0.10 (0.07)
2. Adjusts for Age	0.02 (0.08)	-0.05 (0.08)	-0.09 (0.07)
3. Adjusts for Demographics	0.06 (0.10)	-0.06 (0.09)	-0.17 (0.12)
Observations		159	
Cumulative Perceived Stress Component			
1. Partial Correlation	0.11 (0.06)	-0.06 (0.07)	0.09 (0.08)
2. Adjusts for Age	0.11 (0.06)	-0.07 (0.07)	0.08 (0.08)
3. Adjusts for Demographics	0.15* (0.07)	-0.00 (0.07)	0.13 (0.09)
Maternal Hair Cortisol Concentration			

1. Partial Correlation	0.02 (0.09)	0.14 (0.08)	0.20* (0.09)
2. Adjusts for Age [a]	-0.02 (0.11)	0.03 (0.09)	0.17 (0.11)
3. Adjusts for Demographics [a]	-0.04 (0.11)	-0.01 (0.09)	0.20 (0.12)
Income-to-Needs			
1. Partial Correlation	-0.02 (0.11)	-0.12 (0.08)	-0.09 (0.06)
2. Adjusts for Age	-0.04 (0.11)	-0.15 (0.08)	-0.11 (0.06)
3. Adjusts for Demographics	-0.06 (0.12)	-0.18* (0.08)	-0.13 (0.08)
Maternal Education			
1. Partial Correlation	0.02 (0.08)	-0.07 (0.08)	-0.10 (0.07)
2. Adjusts for Age	0.02 (0.08)	-0.05 (0.08)	-0.09 (0.07)
3. Adjusts for Demographics	0.06 (0.10)	-0.06 (0.09)	-0.17 (0.12)
Observations		159	

*Note. *p<0.05*. Model 1 reflects partial correlations between the outcome of interest and each biological clock, residualized for technical artifacts (slide, array, and plate) and cell composition. Model 2 adjusts for infant chronological age at the time of sample collection. Model 3 adjusts for the following demographic characteristics: gestational age at birth, maternal race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standardized betas. Robust standard errors are reported in parentheses.

[a] Hair cortisol estimates in Models 2 and 3 further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Table 1-4
Prenatal Adversity and Epigenetic Age in Newborns: Joint Prediction

	Horvath	PedBE	DunedinPACE
Prenatal Perceived Stress Component	0.14 (0.08)	-0.03 (0.08)	0.12 (0.09)
Maternal Hair Cortisol Concentration [a]	-0.04 (0.11)	-0.02 (0.09)	0.20 (0.12)
Income-to-Needs	-0.07 (0.15)	-0.20* (0.09)	-0.07 (0.09)
Maternal Education	0.10 (0.12)	0.03 (0.11)	-0.14 (0.13)
Observations		159	

	~~Horvath~~ Horvath	~~PedBE~~ PedBE	~~DunedinPACE~~ DunedinPACE
Prenatal Perceived Stress Component	0.14 (0.08)	-0.03 (0.08)	0.12 (0.09)
Maternal Hair Cortisol Concentration [a]	-0.04 (0.11)	-0.02 (0.09)	0.20 (0.12)
Income-to-Needs	-0.07 (0.15)	-0.20* (0.09)	-0.07 (0.09)
Maternal Education	0.10 (0.12)	0.03 (0.11)	-0.14 (0.13)
Observations		159	
Prenatal Perceived Stress Component	0.14 (0.08)	-0.03 (0.08)	0.12 (0.09)
Maternal Hair Cortisol Concentration [a]	-0.04 (0.11)	-0.02 (0.09)	0.20 (0.12)
Income-to-Needs	-0.07 (0.15)	-0.20* (0.09)	-0.07 (0.09)
Maternal Education	0.10 (0.12)	0.03 (0.11)	-0.14 (0.13)
Observations		159	

Note. *$p<0.05$. All predictors were simultaneously entered into the model to predict biological age. Epigenetic clock variables were residualized for cell type composition and technical artifacts (slide, array, and plate). Model adjusts for infant chronological age at the time of sample collection and the following demographic characteristics: gestational age at birth, maternal race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standardized betas. Robust standard errors in parentheses.

[a] Hair cortisol estimates further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Table 1-5
Early Adversity and Epigenetic Aging in Toddlers

	DunedinPACE
Cumulative Perceived Stress Component	
1. Partial Correlation	0.22* (0.09)
2. Adjusts for Covariates	0.22* (0.09)
3. Adjusts for 1-month Epigenetic Age	0.22* (0.09)
Income-to-Needs	
1. Partial Correlation	-0.25* (0.12)
2. Adjusts for Covariates	-0.24* (0.12)
3. Adjusts for 1-month Epigenetic Age	-0.24 (0.12)
Maternal Education	
1. Partial Correlation	-0.16 (0.09)
2. Adjusts for Covariates	-0.15 (0.09)
3. Adjusts for 1-month Epigenetic Age	-0.14 (0.09)
Observations	128

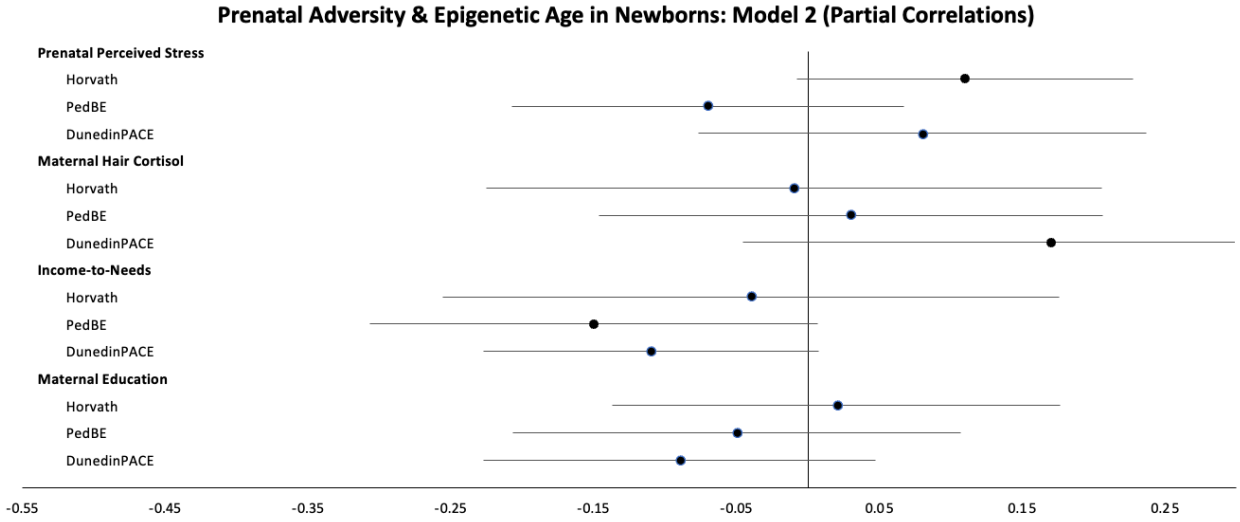
Note. *$p<0.05$. Model 1 reflects partial correlations between the variable of interest and epigenetic age, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table 1-6
Early Adversity and Epigenetic Aging in Toddlers: Joint Prediction

	DunedinPACE
Cumulative Perceived Stress Component	0.22*
	(0.09)
Income-to-Needs	-0.20
	(0.16)
Maternal Education	-0.04
	(0.12)
Observations	128

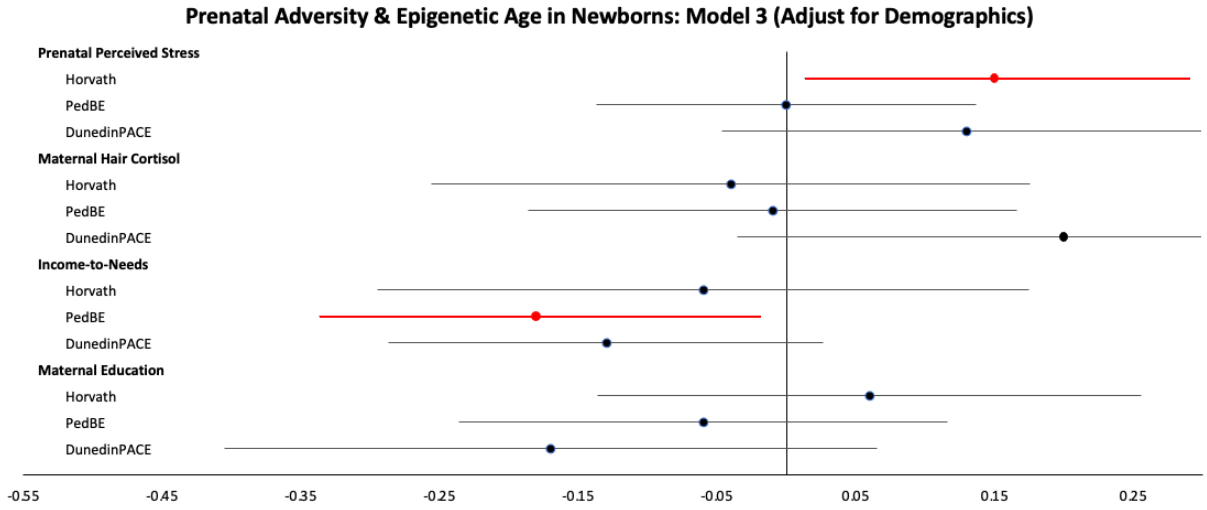
Note. *$p<0.05$. Epigenetic age has been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Models adjust for epigenetic age at 1-month and the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Figure 1-2
Forest Plot: Prenatal Adversity and Epigenetic Aging in Newborns (Model 2)



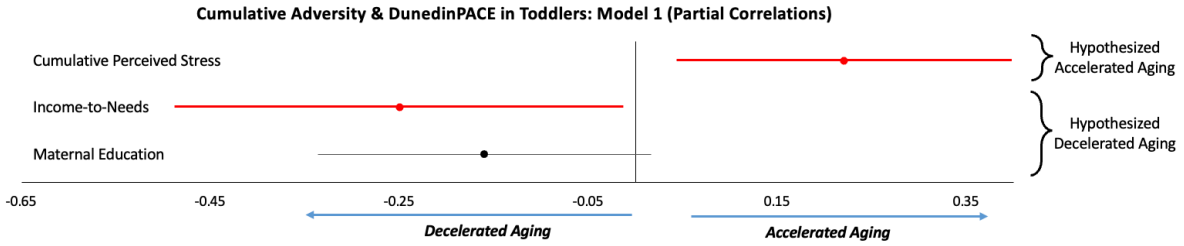
Note. N=159. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the partial correlation between each construct and the epigenetic clock of interest, residualized for technical factors and chronological age (i.e., Model 2 in Table 1-3).

Figure 1-3
Forest Plot: Prenatal Adversity and Epigenetic Aging in Newborns (Model 3)



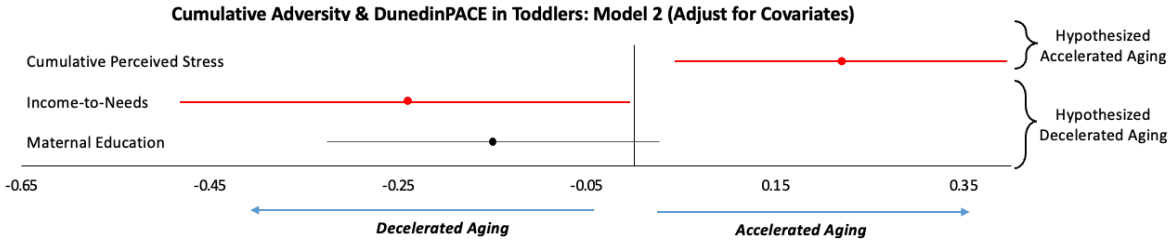
Note. N=159. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, and demographic covariates (i.e., Model 3 in Table 1-3).

Figure 1-4
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 1)



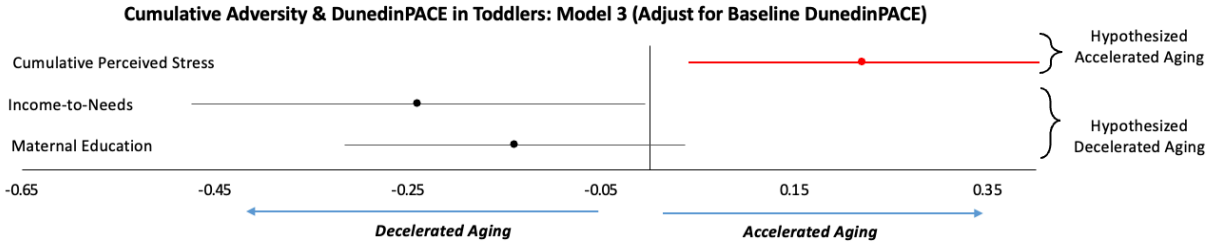
Note. N=128. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age (i.e., Model 1 in Table 1-5).

Figure 1-5
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 2)



Note. N=128. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, and demographic covariates (i.e., Model 2 in Table 1-5).

Figure 1-6
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 3)



Note. N=128. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age, covariates, and epigenetic age at 1-month of age (i.e., Model 3 in Table 1-5).

Chapter 4: Effect of the COVID-19 Pandemic on Epigenetic Aging

***** The manuscript resulting from these analyses is currently under revision at *Child Development*: Sperber, J.F., Miller, M., Bellatin, A., Dettmer, A.M., Champagne, F.A., Noble, K.G. (under revision). The effect of the COVID-19 pandemic on trajectories of biological aging across early childhood. Under revision at *Child Development*.

Research Question and Hypotheses

Little is understood about the long-term physiological and psychological impacts of the COVID-19 pandemic on children's development. Through a natural experiment design and correlational analyses, Study 2 examines the effect of the pandemic on children's epigenetic aging, stress physiology, and development across the first few years of life. Critically, this study leverages longitudinal samples collected at both pre-pandemic and post-pandemic timepoints. Informed by the findings from Study 1b, I focused my analyses on DunedinPACE as the index of epigenetic age given its sensitivity to maternal perceived stress. Results for the other clocks examined in this study are presented in the Appendix and described briefly below. I hypothesized that greater exposure to the pandemic in the first year of life and greater parent-reported disruptive life events would be associated with accelerated pace of biological aging, disrupted stress physiology, and poorer developmental outcomes at 30-months.

Research Question 2a: Does greater exposure to the pandemic in the first year of life and greater disruptive life events predict accelerated pace of aging across the first few years of life? OLS regression with robust standard errors estimated the association between each pandemic measure and DunedinPACE at 30-months of age, adjusting for pre-pandemic (i.e., 1-month) scores.

Research Question 2b: Does greater exposure to the pandemic in the first year of life and greater disruptive life events predict disrupted stress physiology in children? OLS regression with robust standard errors estimated the association between each pandemic measure and hair cortisol concentrations at 30-months of age.

Research Question 2c: Does greater exposure to the pandemic in the first year of life and greater disruptive life events predict developmental outcomes in toddlerhood? OLS regression with robust standard errors estimated the association between the pandemic measures and a series of cognitive and behavioral assessments.

4.1 Methods

Participants & Procedure

Only Cohort 1 described in Study 1 was included in the present analysis. Between June 2019 and March 2020, about half (n = 93) of the full sample described above had been recruited prior to the beginning of lockdown restrictions in New York City (mid-March, 2020). Most (*n* = 82) of the infants in this cohort provided a saliva sample at 1-month of age prior to the pandemic lockdown restricting in-person data collection procedures. During pandemic restrictions, data collection moved to remote procedures, whereby mothers completed surveys on their own stress and their child's development every 6 months. Once pandemic restrictions were lifted, participants safely returned to complete in-person lab visits.

The next possible timepoint by which families could safely return to the lab for in-person assessments was June 2021, when the oldest infants in the study were 30-months of age. At this visit, a second sample of saliva was collected from children to assess epigenetic age and a hair sample was collected from children to assess hair cortisol concentrations. Some children also completed a battery of cognitive tests, and mothers completed surveys of their child's

development. All study procedures were approved by the Institutional Review Board of Teachers College, Columbia University.

Many families moved out of NYC during the pandemic, which substantially reduced the number of participants who returned for in-person assessments: of the initial 93 families in the cohort, 66 families completed at least one follow-up measure at the 30-month visit. For the present study, I restrict analyses to these participants that returned for the 30-month appointment, and implement weighting procedures to reduce bias from differential attrition.

Measures

Pandemic Indicators

Duration of Pandemic Exposure

Details on the pandemic exposure measure have been described in detail elsewhere (see Sperber et al., 2022). Briefly, for this cohort of participants, all infants were born before the pandemic, but reached their first birthday during the pandemic. Thus, there was exogenous variation in their exposure to the pandemic during the first year of life. We selected a cut-off date of March 22nd, 2020 to signify the start of lockdown, as this was the first day that NYC enacted social distancing requirements and required all non-essential personnel to remain home. Using the infant's birthday, I then calculated the number of days each family would experience the first postpartum year under pandemic conditions, with higher scores indicating greater pandemic exposure. The distribution was such that infants experienced a range of 2.5-12 months of pandemic conditions during the first year of life (see Figure 2-1). This variable was available for all participants; there was no missing data.

In previous work, I demonstrated that this indicator was unassociated with observable participant characteristics, thus making it a reasonably exogenous indicator of pandemic

exposure in this sample (Sperber et al., 2022). I replicated this analysis to determine if there was evidence of differential attrition at 30-months based on this same indicator, which is critical to ensuring the exogeneity persists into later waves of data collection (for example, it is possible that families with older children at the start of the pandemic were more likely to leave New York City and thus attrit from the sample at 30-months). A logistic regression found no significant difference in the likelihood of attrition based on duration of pandemic exposure (OR = 1.38, SE = 0.33, $p = 0.18$), indicating that this measure was not differentially impacted by participant attrition (Table A2-1).

Disruptive Life Events

During the 12-month remote visit, mothers completed a modified version of the Epidemic-Pandemic Impact Inventory (Grasso et al., 2020; $n = 61$). Participants were presented with a checklist of 71 events that may or may not have happened to them or someone in their household due to the COVID-19 pandemic. Items ranged in their severity across 7 different domains, including Work or Employment (e.g., "Laid off from job or had to close own business"), Home Life (e.g., "More conflict with child or harsher in disciplining child or children"), and Infection History (e.g., "Death of close friend or family member from this disease"). Mothers indicated whether each item impacted themselves, a person in their home, or none of the above. A composite score was generated by summing across all positively endorsed items experienced by either the participant or someone in their home. One participant's score was deemed invalid as they endorsed the most extreme answer choices for each item on the scale. This observation was set to missing before standardizing scores across the sample ($n = 60$).

Epigenetic Age

Due to challenges in getting families to return to the lab after pandemic-related lockdowns, some children provided their saliva sample several months after the intended collection date. Specifically, 8 children provided a saliva sample during the 36-month lab visit (M = 38.3 months; SD = 1.8 months). These observations are retained for analyses with epigenetic age to maximize sample size. The methods used to process DNA methylation data and calculate epigenetic age are the same as those described above in Study 1.

After obtaining the estimated epigenetic age according to each clock's specifications, values were inspected for their distribution. One value of the PedBE clock was winsorized due to a low value. Next, each clock was residualized for technical artifacts (i.e., plate, slide, and array), child age at the time of data collection, and cell type composition (proportion of leukocytes vs. epithelial cells) to estimate accelerated aging. At this stage, the clock distributions were re-investigated and 1 value on the PhenoAge clock was winsorized due to a low outlier. Finally, the epigenetic age values were standardized for analysis.

Developmental Outcomes

Behavior Problems

Mothers completed the Brief Infant-Toddler Social Emotional Assessment at the 30-month visit (BITSEA; Briggs-Gowan et al., 2004; n = 64). The BITSEA is a clinical screener to assess for delays in socioemotional development in children ranged 12-36 months of age. For the present study, I utilized the BITSEA Problems Subscale, which consists of 31 items where mothers rated the frequency in which their child engaged in problem behaviors on a 3-point Likert scale (0 = not true/rarely, 1 = sometimes true/often, 2 = very true/often; e.g., "Is restless and can't sit still"). Items were summed to create an index where higher scores indicated greater problem behaviors (α = 0.87). Mothers needed to respond to at least 80% of the items to receive

a valid score. Upon review of individual scores, 1 score was deemed invalid due to a mother endorsing the most extreme values for each item of both the Problems and Competency subscales. These values were set to missing and then all scores were standardized for analyses (*n* = 63).

Language Ability

Mothers completed the LENA Developmental Snapshot survey at the 30-month timepoint to assess child language ability (*n* = 62). The LENA Snapshot is a 52-item standardized measure of language development designed for children aged 2-36 months of age (Gilkerson et al., 2017). Mothers were asked to respond to each item if their child was consistently able to demonstrate that ability or not, with items steadily progressing in difficulty until a ceiling is reached (e.g., "When you point to pictures in a book, does your child name them?"). A standardized score is then calculated based on the caregivers' responses which is shown to be highly correlated with other performance-based assessments (Gilkerson et al., 2017). LENA Snapshot scores were standardized to the sample for linear analyses.

Receptive Vocabulary

Children completed the Receptive One-Word Picture Vocabulary Test during the 30-month lab visit with a trained experimenter in their preferred language (ROW-PVT; Brownell, 2010). Children were presented with four pictures on a flipbook and asked to point to the image that best aligned with the word the experimenter stated. Due to challenges getting each child to reach the ceiling for a score to be obtained, about half of the sample received a valid score (n = 31). For analyses, I use the Conceptual Score which reflects the common items administered from both the Spanish and English versions of the assessment. Scores were standardized to the sample.

Executive Function

Children's cognitive ability was assessed via the Minnesota Executive Function Scale (MEFS App; Carlson & Zelazo, 2017). MEFS is a tablet-based app designed to assess executive function in children aged 24 months and older. The app delivers an adaptive version of the dimensional card-sorting task and has been validated on over 35,000 children. During the 30-month lab visit, children completed the assessment with a trained experimenter. It is necessary for a child to reach a ceiling on the task to generate a standardized score. Due to difficulties getting each child to fully complete the task, about half of the sample obtained a valid MEFS score (n = 31). For the present study, I rely only on these valid cases for exploratory analyses with children's early executive functions. MEFS scores were standardized to the sample.

Hair Cortisol Concentration

Children's hair cortisol concentration was collected and analyzed in alignment with the methodology described above in Study 1. Overall, 49 samples were collected from children at the 30-month timepoint. Samples were shipped to Yale Child Study Center for processing using the previously described methods. At this stage, 12 samples were destroyed as they were deemed too small to extract valid hormone levels. Mothers who reported using a corticosteroid on their child in the last 3 months (n = 3) or values deemed biologically implausible (≥ 750 pg/mg; Magnuson et al., 2024; n = 2) were set to missing. Outliers greater than 3 standard deviations from the mean were winsorized to match the next-highest value (n = 1). Finally, values were log-transformed (ln) to adjust for skewness then standardized for analyses.

Covariates

I replicated the covariates used in a previous publication (Sperber et al., 2022) that leverages this natural experiment design, which included the following characteristics: levels of

prenatal anxiety, depression, and perceived stress; maternal years of formal education; age at delivery; maternal race and ethnicity; and child age. One covariate used in the previous study (maternal receptive vocabulary scores) was excluded from the present analysis due to missing data. Epigenetic age models further adjust for whether the saliva sample was collected at the 30-month or 36-month appointment, and models examining the receptive vocabulary measure adjust for language of task administration.

To reduce the number of covariates included in linear analyses due to the decreased sample size, I utilized PCA to reduce the measures of prenatal mental health. Prenatal measures of anxiety (assessed via the Beck Anxiety Inventory; Beck et al., 1988), depression (Patient Health Questionnaire; Kroenke et al., 2009), and perceived stress (Perceived Stress Scale; Cohen & Williamson, 1988) were entered into PCA and a single component was extracted with an eigenvalue of 2.11 and factor loadings ranging from 0.52 – 0.61. This component explained 70% of the variance across the measures. The component was labeled "Prenatal Mental Health" and included as a covariate in analyses, in addition to those previously listed.

Attrition, Baseline Balance, and Weighting Procedures

A previous investigation found that families who completed follow-up visits during the pandemic tended to be more advantaged than the original sample (Sperber et al., 2022). Similar trends were observed in the present study (Table A2-1): families who completed at least one measure at the 30-month lab visit were more highly educated (OR = 2.26, SE = 0.66, $p < 0.01$), reported higher incomes (OR = 1.11, SE = 0.06, $p = 0.063$), were more likely to be White (OR = 2.86, SE = 1.59, $p = 0.059$), and less likely to be Hispanic than the full sample (OR = 0.29, SE = 0.15, $p = 0.017$). Importantly, families who returned for the 30-month visit did not differ from the original sample on their duration of pandemic exposure in the first year of life (OR = 1.38,

SE = 0.33, p = 0.181). Additionally, attrition did not vary based on any of the 1-month epigenetic measures (Table A2-1).

To ensure that the duration of pandemic exposure measure was exogenous to children's epigenetic profiles prior to the pandemic, I examined associations between this measure and children's epigenetic age at 1-month. All 1-month samples were collected prior to the pandemic, and should in theory be unassociated with the exogenous indicator (e.g., similar to 'baseline balance' in an experimental design). Surprisingly, there was variation in 1-month epigenetic scores based on duration of pandemic exposure (Table A2-2): greater pandemic exposure was associated with elevated Horvath (β = 1.27, SE = 0.16, p = 0.061), PedBE (β = 1.49, SE = 0.18, p < 0.001), and PhenoAge score (β = 1.33, SE = 0.16, p = 0.02) at 1-month, indicating some bias in pre-pandemic epigenetic profiles. Scores did not differ by pandemic exposure for DunedinPACE at 1-month, the primary outcome of interest.

To account for missing data and differential patterns of attrition, I implemented multiple imputation and inverse probability of attrition weighting (IPAW) procedures, which in combination have shown to be robust to missing panel data in cohort studies (Seaman et al., 2012). First, a variable reflecting whether a participant remained in the sample after the pandemic was regressed onto demographic characteristics to generate propensity scores. The inverse probability of each participants' odds of remaining in the sample were extracted and their distribution was examined. Next, I implemented multiple imputation with chained equations and 25 iterations to impute missing values using correlated auxiliary variables. These weights and imputed datasets were then used in longitudinal analyses of epigenetic aging.

Analytic Plan

Longitudinal Analyses: Epigenetic Aging

I utilized OLS with robust standard errors to examine the effect of the pandemic on epigenetic aging in toddlerhood. Covariates were steadily introduced in alignment with Study 1's approach. Model 1 presents the partial correlation between each pandemic exposure measure and epigenetic clock, residualized for technical artifacts and chronological age. This association is informative mainly for the duration of pandemic exposure measure as this variable is arguably exogenous from potential sources of bias. Next, Model 2 introduces the demographic covariates listed above, which are chiefly important for the disruptive life events measure since this is not an exogenous indicator. Finally, Model 3 further adjusts for 1-month epigenetic age. This final coefficient provides the best estimate of the effect of the pandemic for both measures as it adjusts for each child's pre-pandemic epigenetic 'baseline.' This is likely the best estimate of the pandemic's effect, given that there was unequal spread in pre-pandemic epigenetic scores by pandemic exposure. All models were weighted using the previously-generated IPAW. The final sample was restricted to participants who provided a valid epigenetic sample at the 30-month appointment and had non-missingness on baseline covariates; only missing values on the 1-month epigenetic age value and the disruptive life events measure were imputed. DNAm data processing and epigenetic clock calculation were conducted in R. Subsequent analyses occurred in Stata v.18.

Cross-Sectional Analyses: Developmental Outcomes

For developmental outcomes and hair cortisol concentration at 30-months, I implemented OLS regression with robust standard errors to estimate the effect of each pandemic exposure measure on each respective outcome. All measures were standardized to the sample. For these exploratory outcomes, the analytic sample was restricted to those who provided a valid score for

each respective outcome and the IPAW calculated above were applied to offset the effects of differential attrition.

4.2 Results

Descriptive statistics of the sample

Table 2-1 reports the demographics of the analytic sample, while Table A2-4 presents descriptives on the developmental measures. Mothers reported an average household income-to-needs ratio of 6.1 (SD = 7.5, range = 0 to 26.7) and 15.4 years of formal education (SD = 3.6). 59% of the children were female. Half of the mothers identified their ethnicity as Hispanic, while 20% identified as Black, 39% as White, and 41% as some other race. In the first year postpartum, infants experienced an average of 7.6 months of life during the pandemic (SD = 2.4) and mothers reported experiencing 11 pandemic-related disruptive life events (SD = 6.3).

Correlations

Table A2-3 reports the bivariate associations among the pandemic exposure measures, epigenetic clocks, and the exploratory developmental outcomes. Overall, there was no evidence of an association between either duration of pandemic exposure, or pandemic-related disruptive life events, with child hair cortisol concentration and developmental outcomes. Correlations ranged in magnitude from -0.20 to 0.26. Notably, greater behavior problems were associated with higher pandemic exposure ($r = 0.26$, $p = 0.04$). Additionally, greater disruptive life events were marginally associated with improved parent-reported language ability ($r = 0.22$, $p = 0.10$), which was the opposite of the hypothesized direction.

Pandemic Exposure Measures and DunedinPACE in Toddlers

Table 2-2 presents OLS models estimating the effect of duration of pandemic exposure and disruptive life events on DunedinPACE scores at 30-months, and Figures 2-2 through 2-4

presents these coefficients as forest plots. The results for all the epigenetic clocks are presented in Table A2-5 and as forest plots in Figures A2-1 through A2-3. Table A2-6 presents unimputed estimates of all the clocks.

I observed no difference in DunedinPACE scores by duration of pandemic exposure in the first year of life, regardless of adjustments for covariates or baseline epigenetic age. The same pattern was observed for disruptive life events: I observed no evidence of an association between disruptive life events and DunedinPACE scores, regardless of covariate adjustments. These null results were consistent across all other epigenetic clocks examined. Effect sizes ranged from -0.08 – 0.12 (SE's range 0.10 – 0.14) for duration of pandemic exposure, and from -0.00 – 0.29 (SE's range 0.12 – 0.28) for disruptive life events.

Pandemic Exposure Measures and Developmental Outcomes

Tables 2-3 and 2-4 report the linear association among the pandemic measures with child cognition, behavior, and stress physiology. These models adjust for child age at the time of the assessment. I observed no effect of duration of pandemic exposure on executive function, language ability, receptive vocabulary, and hair cortisol concentrations (Table 2-3). Effect sizes were small in magnitude (*ß's* range -0.05 – 0.10) and imprecise due to the reduced sample size for lab-based assessments (SE's range 0.15 – 0.20). In addition, the magnitude of the association between duration of pandemic exposure and behavior problems dropped substantially from bivariate estimates when adjusting for child age ($\beta = 0.10$, SE = 0.15, $p = 0.49$).

There was also little evidence of an association between disruptive life events and these outcomes (Table 2-4). Greater disruptive life events were marginally associated with lower hair cortisol concentrations ($\beta = -0.24$, SE = 0.15, $p = 0.12$). These effects were counter to the hypothesized direction. As an exploratory analysis, I examined socioeconomic factors as a

potential confound to explain these effects. When including maternal education as a covariate, the effect size reduced substantially (β = -0.04, SE = 0.22, p = 0.87). This is likely due to the confounding association between maternal education with disruptive life events (r = 0.37, p < 0.001) and maternal education with child hair cortisol concentration (r = -0.44, p < 0.001).

4.3 Discussion

Overall, there were no observed differences in the pace of biological aging among children born at the start of the COVID-19 lockdown compared to those born several months before the pandemic. This was further evidenced by a lack of significant differences across 5 additional epigenetic clocks investigated in the present study. Additionally, there was little evidence of associations among pandemic-related disruptive life events and epigenetic aging, and no robust differences in cognitive, behavioral, or HPA axis activation at 30-months of age based on these indicators. Collectively, these findings suggest cautious optimism for a generation of children who experienced large disruptions to typical contexts in the first year of life: we detected no robust effects of the pandemic on child development across multiple psychological and physiological domains. This interpretation is further supported by the null findings reported in a previous investigation of this sample in the first two years of life (Sperber et al., 2022).

One concern in the interpretation of the present findings is the exogeneity of the duration of pandemic exposure measure by infant epigenetics. An examination of baseline epigenetic scores collected before the pandemic found that out of the 6 clocks examined, 3 scores tended to be higher for infants with a greater duration of pandemic exposure. This indicates that there may have been baseline differences in epigenetic age at the start of the pandemic in our sample, which would violate a key assumption of the natural experiment design. Fortunately, I am able to adjust for epigenetic age at 1-month to remove this variance, but this artifact nonetheless raises

concerns for other unmeasured confounds that could challenge the integrity of our natural experiment design.

Assuming this external bias was removed via covariate adjustment, several explanations may account for the null effect of these pandemic exposures on young children's outcomes. First, it is critical to acknowledge the heterogeneity in the pandemic experience across families. While it is well-documented that low-income communities were more likely to experience stressors such as material hardship and unpredictability during the pandemic (Liu et al., 2022), some studies also reported unexpected improvements to aspects of family functioning. For example, there were documented improvements to low-income mother's mental health and sleep quality (Premo et al., 2023), the frequency of positive parent-child interactions (Kalil et al., 2020; Pitchik et al., 2021), and even children's vocabulary development (Kartushina et al., 2021) during the COVID-19 lockdown. In contrast, as observed in the present study, mothers with higher income – who likely had greater access to resources – simultaneously reported greater pandemic-related disruptive life events, and even greater depressive symptoms (Gustafsson et al., 2021). Collectively, these findings underscore the complex and nuanced experience of the pandemic across families, which likely mar any clear pattern of effects cross-sectionally.

Alternatively, it is possible that pandemic-related stressors do not impact the infant epigenome or cognitive development at this age. Previous work in this sample found no differences in parent-reported perceived stress or mental health symptoms due to exogenous pandemic exposure (Sperber et al., 2022). This suggests that possible mediating factors such as the home environment and the quality of parent-child interactions may not have changed drastically during the pandemic, buffering the impact of these uncertain times on infants. These null findings are further supported by longitudinal work in older children (deSteiguer et al.,

2025), though we cannot rule out the possibility of small effect sizes that we are underpowered to detect due to our small sample size.

Strengths & Limitations

A strength of this study is its natural experiment design, the use of repeated measures, and the longitudinal assessments of psychological and physiological outcomes. Furthermore, we contribute to a limited literature reporting longitudinal associations among epigenetic aging and developmental outcomes in the first few years of life. A weakness of this study is the small sample size, which limits our ability to rule out the existence of small effects that I am underpowered to detect. Additionally, we suffered from high levels of attrition following the COVID-19 pandemic. Future research should leverage longitudinal data using larger samples of children to examine these associations in detail. I situate these findings within the broader COVID-19 and development literature: overall, we find little evidence of robust effects of pandemic-related conditions on the development of very young children in the psychological or physiological domains examined here.

Table 2-1.
Descriptive Statistics of Study 2 Analytic Sample

	N	Mean (%)	SD	Min	Max	p-value
Pandemic Exposure Measures						
Duration of Pandemic Exposure *(months)*	66	7.61	2.40	2.53	12	
Disruptive Life Events	60	11.02	6.33	0	25	
Demographic Characteristics						
Child is female	66	59%				
Mother is Black	66	20%				
Mother is Other Race	66	41%				
Mother is White	66	39%				
Mother is Hispanic	66	50%				
Income-to-Needs *(winsorized)*	64	6.05	7.45	0	26.73	
Years of Maternal Education	66	15.41	3.63	8	22	
Epigenetic Clock Estimates (years)						
Horvath – 1 month	39	1.54	0.31	1.10	2.48	
Horvath – 30 months	52	4.74	1.06	2.11	7.10	**<0.001**
PedBE – 1 month	39	0.25	0.09	0.10	0.53	
PedBE – 30 months (*winsorized*)	52	4.14	0.65	2.62	6.14	**<0.001**
GrimAge – 1 month	39	42.47	1.08	40.11	44.35	
GrimAge – 30 months	52	41.72	2.75	34.72	46.61	**0.013**
PhenoAge – 1 month	39	48.69	2.40	43.13	53.59	
PhenoAge – 30 months	52	48.62	7.57	32.72	62.08	0.77
DunedinPACE[a] – 1 month	39	1.36	0.05	1.27	1.51	
DunedinPACE[a] – 30-months	52	1.42	0.08	1.24	1.58	**<0.001**
Epigenetic-g[b] – 1-month	39	-0.04	0.13	-0.45	0.22	
Epigenetic-g[b] – 30-months	52	0.00	0.18	-0.40	0.36	

Note. Epigenetic clocks reflect the raw, unadjusted estimate of biological age. P-value column reflects the results of a paired means test for each epigenetic clock.
[a]DunedinPACE scores reflect the estimated rate of aging per chronological year.
[b]Epigenetic-g scores were standardized within each timepoint.

Table 2-2.
Pandemic Exposure Measures and Epigenetic Aging in Toddlers

	DunedinPACE
Duration of Pandemic Exposure	
1. Partial Correlation	0.11
	(0.10)
2. Adjusts for Demographics	0.09
	(0.09)
3. Adjusts for 1-month Epigenetic Age	0.10
	(0.10)
Disruptive Life Events	
1. Partial Correlation	-0.00
	(0.19)
2. Adjusts for Demographics	0.28
	(0.24)
3. Adjusts for 1-month Epigenetic Age	0.29
	(0.24)
Observations	

Note. * $p < 0.05$. Model 1 reflects partial correlations between the outcome of interest and epigenetic age, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following demographic characteristics: maternal education, prenatal mental health, child sex, maternal race and ethnicity, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for 1-month epigenetic age. Sample restricted to participants with a valid epigenetic age estimate at 30-months. Coefficients have been weighted using IPAW procedures and reflect standardized betas. Robust standard errors in parentheses.

Table 2-3
Duration of Pandemic Exposure and Developmental Outcomes

	(1) Behavior Problems	(2) Language Ability	(3) Receptive Vocabulary[a]	(4) Executive Function	(5) Hair Cortisol Concentration
Duration of Pandemic Exposure	0.10	0.02	-0.02	-0.05	0.01
	(0.15)	(0.16)	(0.20)	(0.20)	(0.20)
Observations	62	61	30	31	32

Note. * $p < 0.05$. Models adjust for child age at the time of the assessment. Coefficients have been weighted using IPAW procedures and reflect standardized betas. Robust standard errors in parentheses.

[a] Model further adjusts for language of test administration (English or Spanish)

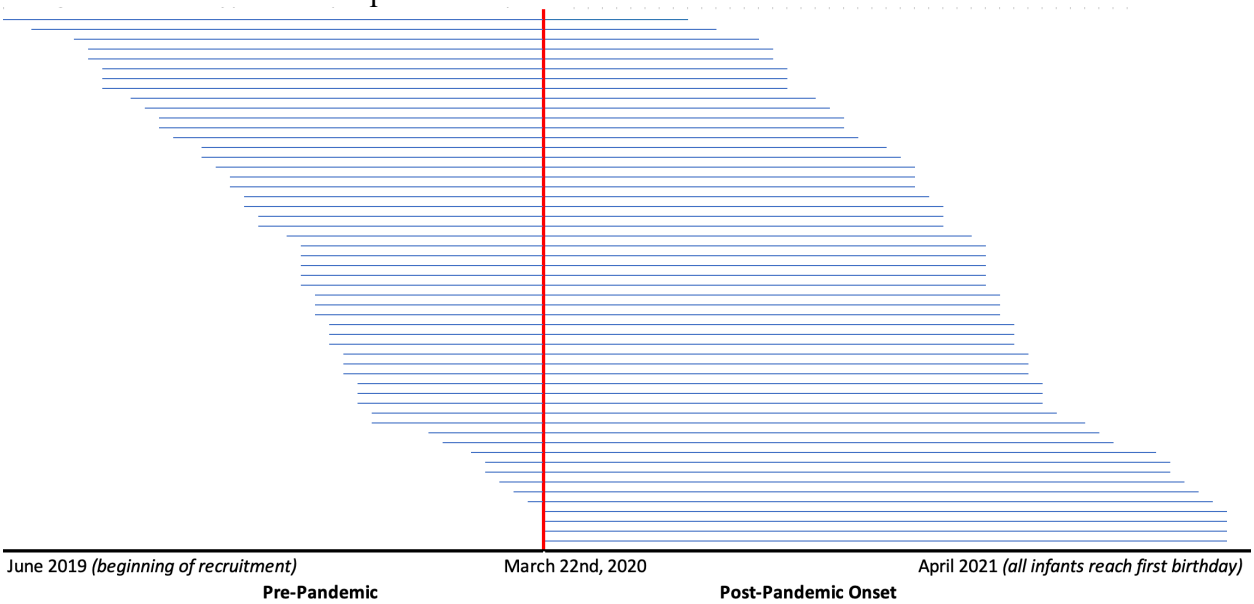
Table 2-4
Disruptive Life Events and Developmental Outcomes

	(1) Behavior Problems	(2) Language Ability	(3) Receptive Vocabulary[a]	(4) Executive Function	(5) Hair Cortisol Concentration
Disruptive Life Events	0.04	0.15	0.04	0.06	-0.24
	(0.18)	(0.15)	(0.26)	(0.14)	(0.15)
Observations	58	56	26	28	28

Note. * $p < 0.05$. Models adjust for child age at the time of the assessment. Coefficients have been weighted using IPAW procedures and reflect standardized betas. Robust standard errors in parentheses.

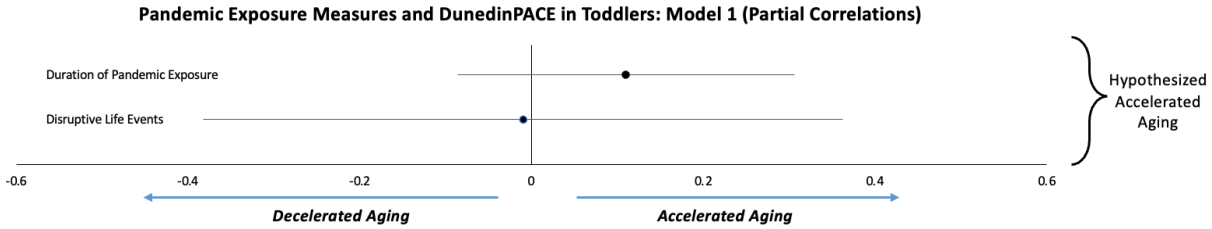
[a] Model further adjusts for language of test administration (English or Spanish)

Figure 2-1.
Distribution of Pandemic Exposure



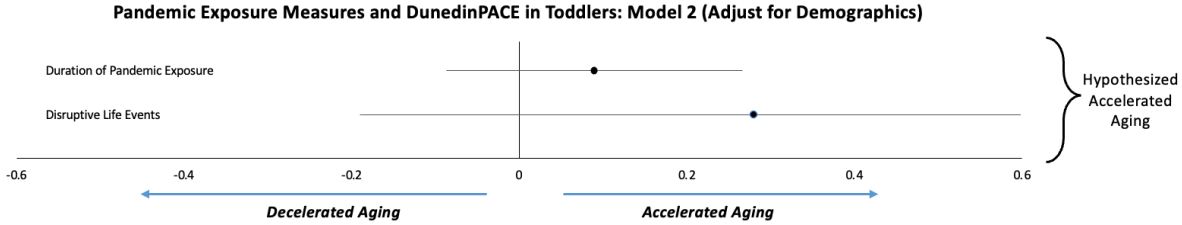
Note. $N = 52$. Each line reflects the pandemic exposure of a participant enrolled in the study that provided an epigenetics value at 30-months, sequential in age. The red line indicates the beginning of lockdown in NYC (March 22nd, 2020). The left side of the figure represents the amount of time each child spent alive prior to the pandemic in the first year of life. The right half of the figure represents the amount of pandemic exposure in the first year of life.

Figure 2-2.
Forest Plot: Pandemic Exposures and Epigenetic Aging in Toddlers (Model 1)



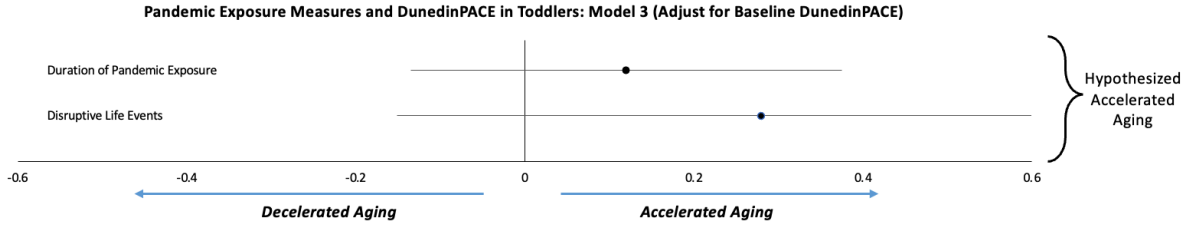
Note. N=52. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and DunedinPACE at 30-months, adjusted for technical factors and chronological age (i.e., Model 1 in Table 2-2).

Figure 2-3.
Forest Plot: Pandemic Exposures and Epigenetic Aging in Toddlers (Model 2)



Note. N=52. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and DunedinPACE at 30-months, adjusted for technical factors, chronological age, and demographic covariates (i.e., Model 2 in Table 2-2).

Figure 2-4.
Forest Plot: Pandemic Exposures and Epigenetic Aging in Toddlers (Model 3)



Note. N=52. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and DunedinPACE at 30-months, adjusted for technical factors, chronological age, demographic covariates, and epigenetic age at the 1-month timepoint (i.e., Model 3 in Table 2-2).

Chapter 5: Associations Among Epigenetic Aging and Developmental Outcomes in Toddlerhood

Research Question and Hypotheses

While the associations among accelerated aging, health, and cognitive functioning in adulthood are well-established, little research has examined these associations in early childhood. Study 3 examined the functional significance of epigenetic aging in childhood by estimating the concurrent associations among epigenetic age and developmental outcomes. In addition to DunedinPACE, I focus on Epigenetic-g as a main indicator of interest, as this measure was generated to be an epigenetic indicator of cognitive abilities. In alignment with Studies 1 and 2, I report results for all the clocks examined in the Appendix and describe them briefly below. I did not hypothesize a particular directionality of effects given that the association between cognition and biological maturation may not align with the patterns observed in previous studies of older children and adults.

Research Question 3: Do epigenetic profiles of biological aging and cognition at 30-months predict concurrent measures of psychological development? OLS regression with robust standard errors was used to examine the continuous association between epigenetic profiles at 30-months and concurrent assessments of behavior problems, language ability, receptive vocabulary, and executive functioning.

5.1 Methodology

Participants & Procedure

The sample described in Study 1 was used for the present analysis. Here, I focus on the DunedinPACE and Epigenetic-g estimates obtained in toddlerhood, in addition to the developmental assessments described in Study 2. At the 30-month timepoint, mothers completed

surveys of their child's behavioral and cognitive development. Children also completed a battery of lab-based cognitive tasks administered by a trained research assistant. All study procedures were approved by the Institutional Review Board of Teachers College, Columbia University.

Measures

Behavioral and Cognitive Development

The measures of behavioral problems, language abilities, receptive vocabulary, and executive functioning described in Study 2 were examined for the present analysis. I utilized results from the full sample of toddlers (i.e., Cohort 1 and 2) at 30-months. Descriptive statistics of the measures are reported in Table 3-1. All scores were standardized to the sample.

Cognitive Abilities Component

One potential concern with such an analysis is that cognitive measures such as these are prone to measurement error in young children. Thus, to further test proof of concept, I conducted PCA analysis to reduce the cognitive measures described above into a single "Cognitive Abilities" factor. Measures of language ability, receptive vocabulary, and executive function loaded onto a single component with an eigenvalue of 2.17 and factor loadings ranging from 0.55 – 0.60 (Table A3-1; $n = 65$). This component explained 72% of the variance and was extracted to predict 30-month epigenetic age as an exploratory outcome of interest.

Analytic Plan

After examining demographic characteristics and correlations amongst the variables of interest, I conducted OLS regression with robust standard errors to estimate the association between epigenetic profiles and developmental outcomes. The individual developmental measures, as well as the aggregate Cognitive Abilities Component, were regressed onto the concurrent measure of DunedinPACE and Epigenetic-g at 30-months; results from the other

epigenetic clocks are presented in Table A3-3. In alignment with the analytic approach of Study 1 and 2, I steadily introduced covariates to observe the changing association between epigenetic profiles and developmental outcomes.

Model 1 reflects the partial correlation between each developmental outcome and concurrent epigenetic profiles, residualized for technical factors and chronological age. One concern with these estimates is that any significant association observed may reflect the confounding influence of socioeconomic factors on both developmental outcomes and epigenetic age in toddlers, as was observed in Study 1. Therefore, Model 2 introduced controls for average family income-to-needs, years of maternal education, child age at the time of the developmental assessment, and the covariates used in Study 1b (cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month timepoint). This coefficient provides an improved estimate of whether epigenetic age can independently predict any signal in behavioral or cognitive development, above and beyond family SES. Finally, Model 3 adjusts for epigenetic age at 1-month in alignment with analyses from Study 1b. This final coefficient reflects the extent to which relative change in a child's epigenetic age from their baseline is associated with developmental outcomes.

Epigenetic variables were residualized for technical artifacts (slide and array), cell composition, and child chronological age. The analytic sample was restricted to participants with both a valid epigenetic age score at 30-months and each developmental outcome examined. Missing data on covariates was handled via multiple imputation with chained equations and 25 iterated datasets. Analyses were conducted in Stata v.18.

5.2 Results

Descriptive statistics

Table 3-1 presents descriptive statistics of the developmental outcomes assessed. Standardized assessments (M = 100, SD = 15) reflected the expected trends, with average scores ranging between 98.44 – 101.6 (SD range 9.7 – 26.2). Notably, the average bilingual standard score on the ROW-PVT was somewhat below the national average (M = 89.21, SD = 28.3).

Correlations among key variables

Table A3-2 presents the pairwise correlations among all epigenetic age variables at 30-months and the developmental outcomes of interest. Here, epigenetic age has been residualized for cell composition and batch effects (i.e., not adjusted for chronological age at the time of the lab visit). Notably, higher family income-to-needs was associated with improved developmental outcomes, including better language abilities ($r = 0.33$, $p < 0.001$), receptive vocabulary ($r = 0.56$, $p < 0.001$), and executive functioning ($r = 0.25$, $p = 0.02$). A similar pattern of associations was observed with maternal education. Additionally, the developmental outcome measures were moderately correlated with each other: higher executive function was associated with better receptive vocabulary scores ($r = 0.61$, $p < 0.001$) and language abilities ($r = 0.54$, $p < 0.001$). Similarly, reduced behavior problems was associated with better receptive vocabulary scores ($r = -0.22$, $p = 0.04$) and language abilities ($r = -0.44$, $p < 0.001$).

Associations with behavioral and cognitive development

Table 3-2 presents regression models estimating the association between the primary clocks of interest and child development with various model specifications. Figures 3-1 through 3-3 present these coefficients as forest plots to demonstrate the change in predictive power as controls for family SES, covariates, and baseline epigenetic age are introduced. Table A3-3 and Figures A3-1 through A3- presents the associations amongst all the epigenetic clocks examined, and Table A3-4 shows these results using non-imputed data.

As observed in partial correlations, there was no evidence of an association between Epigenetic-g and DunedinPACE with any of the developmental outcomes examined. Across 10 statistical tests, no coefficient was statistically significant. A handful of significant associations emerged amongst the other clocks examined, but there was large variation in the magnitude and directionality of coefficients. For example, greater behavioral problems were associated with a lower PedBE age (β = -0.17, SE = 0.07, p = 0.01), but higher GrimAge (β = 0.21, SE = 0.09, p = 0.02). Additionally, many estimates demonstrated large standard errors, suggesting a substantial amount of statistical noise.

After adjusting for SES, there remained no significant association among DunedinPACE or Epigenetic-g with any of the domains examined. For the other clocks, all of the previously significant effects reduced to non-significance, which indicates that confounding characteristics were likely accounting for the initial association. Once again, the directionality of coefficients varied greatly across all of the epigenetic clocks and measures tested. Indeed, when all of the cognitive outcomes were examined via the Cognitive Abilities Components, there was little evidence of a consistent association between epigenetic age and child cognition. After adjusting for baseline epigenetic age, the coefficients on the primary clocks of interest continued to reduce and were not statistically significant. For the other clocks, there also remained no significant association.

As a robustness check, I examined if Epigenetic-g was associated with a dummy indicator of whether a child completed any of the cognitive tasks in the lab. Completion of the ROW-PVT or the MEFS assessment was not associated with Epigenetic-g scores (OR = 1.20, SE = 0.26, p = 0.39).

5.3 Discussion

I find little evidence for the functional significance of DunedinPACE or Epigenetic-g for children's cognitive and behavioral development. No associations were significant, and effects varied widely across developmental domains. This pattern of effects was present in all of the other epigenetic clocks examined as well. Though a few significant associations emerged in partial correlations, these effects were inconsistent in their magnitude and directionality and varied for measures within the same domain (e.g., behavior problems). Overall, the present study finds no evidence that these epigenetic profiles are a reliable indicator for concurrent psychological development.

This study serves as a concurrent validity test for Epigenetic-g at this developmental stage. The measure was trained on numerous measures of general cognitive abilities in a sample of adults, and is subsequently associated with related health outcomes such as depression, social deprivation, body mass index, and alcohol consumption (McCartney et al., 2022). A recent investigation found that lower Epigenetic-g scores in middle childhood and adolescence was associated with both poorer cognition and socioeconomic disadvantage, indicating the measure may hold external validity in older children (Raffington et al., 2022). However, these results do not seem to persist into toddlerhood: I found no evidence of an association among Epigenetic-g, cognition, or socioeconomic disadvantage (Study 1b) in the first few years of life.

There may be several possible explanations for these null findings. First, it is possible that Epigenetic-g is not sensitive to socially-stratified cognitive profiles at this stage of life. Indeed, the sample examined in Raffington et al. (2022) consisted of children aged 8 to 19 years of age (M = 13.6 years, SD = 3.05). While I do observe evidence of socially-stratified cognition on behavioral assessments by 30-months of age, it is possible that Epigenetic-g is not sensitive to these disparities at this developmental stage. Alternatively, the small sample examined may have

limited our ability to detect true, small effects (Raffington et al.'s sample included 1,000+ participants). However, this seems less likely given emerging results from the Baby's First Years study, which found little to no association between cognitive abilities and Epigenetic-g in a large sample (n = 800+) of low-income four-year-olds (Raffington et al., under review). The findings of the present dissertation, combined with those from Baby's First Years, suggest this may not be an appropriate indicator of cognition in the first few years of life.

In addition, it is notable that no other epigenetic clock emerged as a predictor of behavior or cognition. Second- and third-generation clocks such as DunedinPACE, PhenoAge, and GrimAge, were trained to predict clinical markers of health and aging in adult samples. As such, the preferred outcomes of interest to rigorously assess such measures in childhood would include indicators of physical health and development, such as BMI or measures of maturation. Since these outcomes were not available in the present dataset, I chose to investigate psychological outcomes that theoretically align with maturation and well-being (e.g., behavior problems). Indeed, the adult literature provides some evidence for the presence of epigenetic age disparities by psychological constructs (e.g., Raffington et al., 2022). Nonetheless, it is surprising that none of these clocks demonstrated consistent signal with the outcomes tested here. Future research should examine health-related outcomes in young cohorts of children to examine if epigenetic clocks provide signal for physical health in real time.

Strengths and Limitations

A strength of this study is the examination of well-validated measures of development, including both parent-report and lab-based assessments. A limitation is the small sample size on and high measurement error on lab-based tasks, which likely contributed to noisy estimates.

Table 3-1
Descriptive Statistics of Study 3 Analytic Sample

	N	Mean	SD	Min	Max
Child Outcomes					
Behavior Problems	124	7.9	5.8	0	36
Language Ability	120	99.6	17.2	64	125.1
Executive Function	94	98.4	9.7	61	123
Receptive Vocabulary					
English Monolingual Standard Score	48	101.6	26.2	0	137
Spanish Bilingual Standard Score	28	89.21	28.3	0	131
Conceptual Score	76	27.58	14.4	0	64

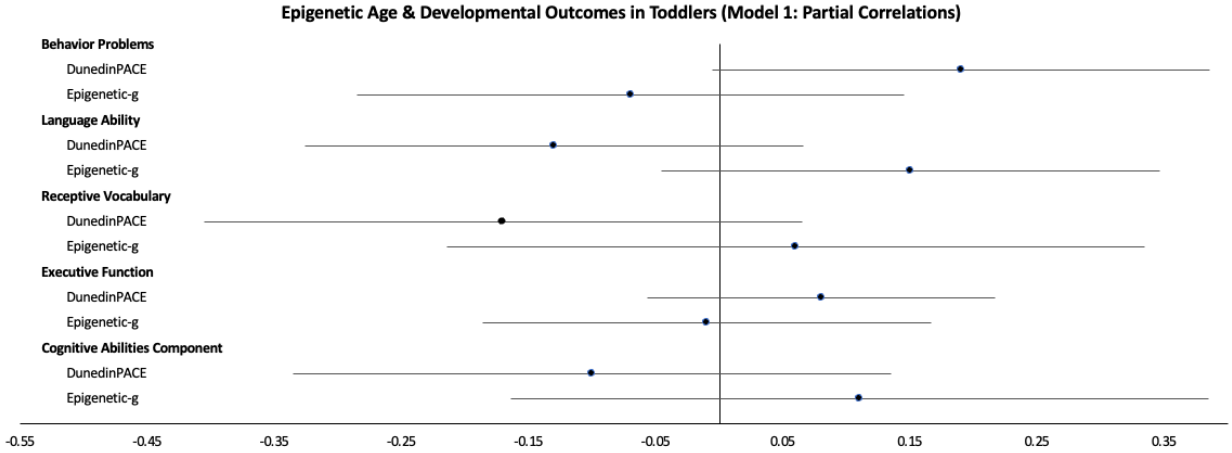
Note. Sample restricted to children with a valid epigenetic score at 30-months. Behavior Problems reflects a sum of parent-reported behaviors from the BITSEA Problems subscale. Language Ability, Executive Function, and Receptive Vocabulary reflect standardized scores where M=100, SD=15 for each measure. The Receptive Vocabulary Conceptual Score reflects overlapping items administered on both the monolingual and bilingual versions of the assessment.

Table 3-2
Epigenetic Age and Developmental Outcomes in Toddlers

	(1) Behavior Problems	(2) Language Ability	(3) Receptive Vocabulary	(4) Executive Function	(5) Cognitive Abilities Component [a]
DunedinPACE					
1. Partial Correlation	0.19	-0.13	-0.17	0.08	-0.10
	(0.10)	(0.10)	(0.12)	(0.07)	(0.12)
2. Adjusts for Demographics	0.13	-0.05	-0.06	0.11	0.00
	(0.09)	(0.09)	(0.10)	(0.08)	(0.10)
3. Adjusts for 1-month Epigenetic Age	0.13	-0.06	-0.06	0.10	-0.00
	(0.09)	(0.09)	(0.10)	(0.08)	(0.10)
Epigenetic-g					
1. Partial Correlation	-0.07	0.15	0.06	-0.01	0.11
	(0.11)	(0.10)	(0.14)	(0.09)	(0.14)
2. Adjusts for Demographics	-0.03	0.13	0.05	-0.08	0.08
	(0.12)	(0.10)	(0.10)	(0.09)	(0.12)
3. Adjusts for 1-month Epigenetic Age	-0.03	0.11	0.01	-0.11	0.08
	(0.12)	(0.10)	(0.09)	(0.09)	(0.12)
Observations	124	120	76	89	65

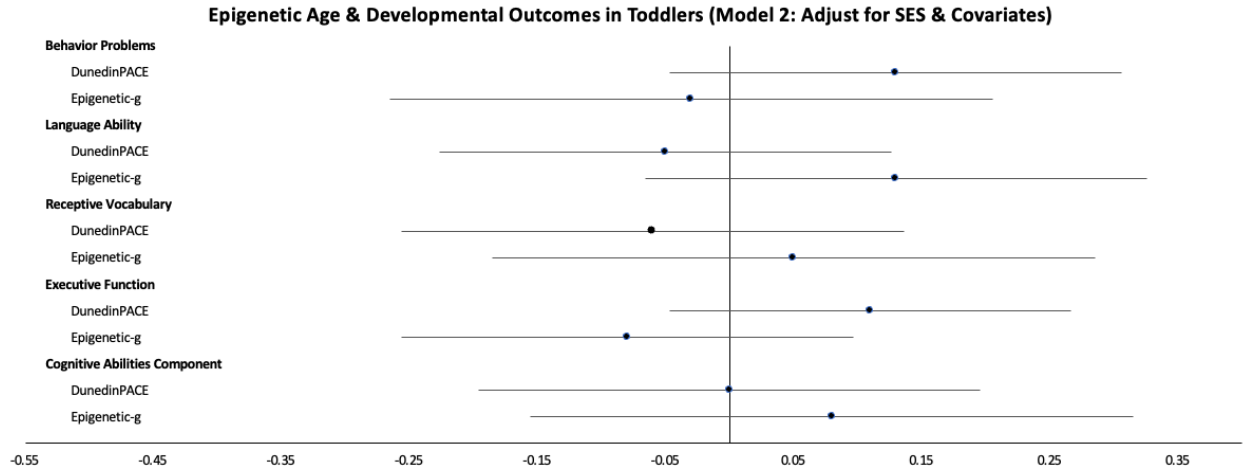
Note. *$p < 0.05$. Epigenetic variables have been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: family income-to-needs, maternal education, cohort, child sex, a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit, and child age at the time of the developmental assessment. Model 3 further adjusts for epigenetic age at 1-month. Sample restricted to participants with a valid epigenetic age at 30-months and each developmental outcome. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Figure 3-1
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 1)



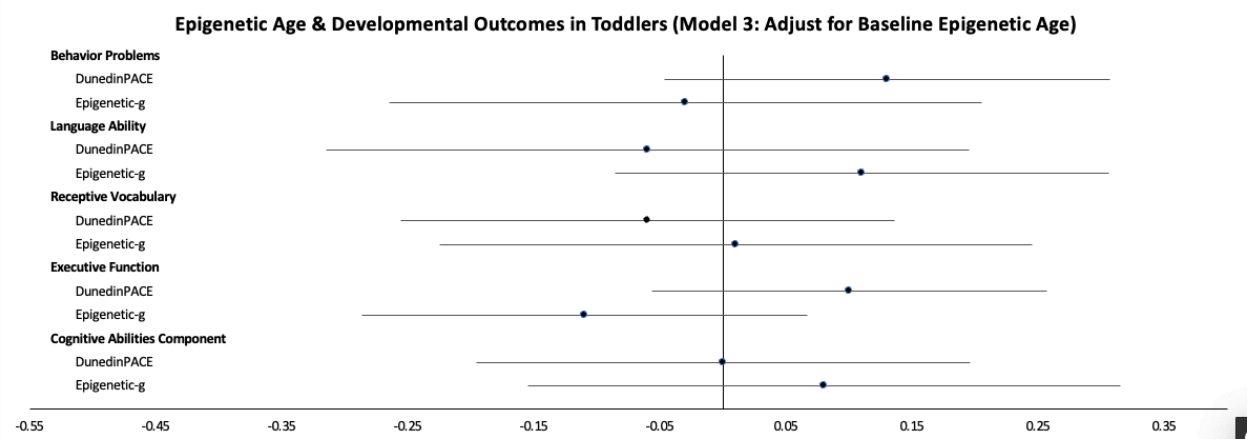
Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the partial correlation between each measure and Epigenetic-g, residualized for technical factors and chronological age (i.e., Model 1 in Table 3-2).

Figure 3-2
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 2)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each measure and Epigenetic-g, adjusted for technical factors, chronological age, family SES, and demographic covariates (i.e., Model 2 in Table 3-2).

Figure 3-3
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 3)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each measure and Epigenetic-g, adjusted for technical factors, chronological age, demographic covariates, and epigenetic age at 1-month (i.e., Model 3 in Table 3-2).

Conclusion

My dissertation extended the literature on biological aging, health, and development by investigating the associations among early exposure to stress and socioeconomic disadvantage, epigenetic aging, and developmental outcomes in children. This work sought to examine the '*when*, *what*, and *how'* aspects of epigenetic aging during early childhood. To answer these questions, I tracked the emergence of this biomarker across the first few years of life (Study 1), examined the effects of a global pandemic on children's biology and development (Study 2), and evaluated its associations with concurrent developmental outcomes (Study 3). Collectively, these findings inform the functional relevance of epigenetic aging for development in real-time and deepen our understanding of how early experiences may shape well-being.

Overview and Interpretation of Findings

Each study relied on the same sample of mothers and children followed from the end of pregnancy through the first 3 years of life. Study 1 sought to examine if adversity-related disparities in epigenetic aging are detectable within the first few years of life. To achieve this, I first examined epigenetic age in these children when they were newborns. This established whether perturbations in biological aging are present at the very beginning of postnatal life. I found little evidence of epigenetic disparities at 1-month of age, suggesting an equal biological 'baseline' at birth. This means that, above and beyond prenatal exposures such as chronic maternal HPA axis activation, higher perceived stress, or socioeconomic disadvantage, infants were generally born with similar epigenetic aging profiles. When these children returned to the lab at 2.5 years of age however, evidence of a diverging pattern emerged. First, there was a substantially greater spread of scores at 30-months across the sample compared to 1-month of

age, with aligns with previous longitudinal work (Simpkin et al., 2017). Furthermore, toddlers of mothers who reported higher perceived stress and lower family incomes tended to have an accelerated pace of biological aging. While interpretations of these findings are tempered by the tenuous application of these clocks in young children and strength of their effects, it does provide evidence for altered biological aging profiles after just a few years of postnatal experiences. On one hand, this finding could be interpreted as preliminary evidence for the weathering hypothesis *within* early childhood. Crucially, however, I cannot claim links to adverse health trajectories as the children were not observed beyond age 3. Nonetheless, this study is significant in that it is one of the first to examine such patterns at this early stage.

A second aim of this dissertation was to examine what *types* of adverse experiences lead to epigenetic signatures. Therefore, Study 2 examined the effect of the COVID-19 pandemic on children's epigenetic aging, stress physiology, and development. Due to the unpredictable nature of the pandemic's onset, I leveraged a natural experiment design using variation in children's age at the onset of the pandemic to predict subsequent outcomes. Overall, I did not detect evidence of differences in developmental trajectories based on the amount of time a child spent their first year of life during lockdown. Limited by a small sample and a restricted time range, I interpret these findings with cautious optimism: greater exposure to the socialized experience of the COVID-19 pandemic was not associated with robust differences in children's biological aging, chronic HPA axis activation, and developmental outcomes.

A question raised by the findings of Study 1 and Study 2 was the *functional significance* of biological aging for development in real time. Therefore, Study 3 examined the correlates of accelerated aging with children's concurrent behavior and cognition. Overall, I found no evidence of signal across multiple measures of epigenetic aging and child development.

Importantly, the outcomes examined were not the preferred indicators, as measures of physical health and/or maturation are better suited to detect signal with biological aging. Nonetheless, these findings indicate that accelerated epigenetic aging does not appear to hold functional relevance for children's psychological development during toddlerhood.

Collectively, these findings inform several important aspects of theory. First, it supports the first few years of life as a potential period by which adverse exposures may shape life-long health. While we cannot conclude from these early findings if these patterns will uphold across development or hold implications for later health – indeed, this and other studies report low stability in epigenetic profiles across childhood – it is preliminary evidence for the shaping of biological aging via early experience, possibly prior to the emergence of deleterious outcomes.

Second, these findings could counteract efforts to describe the COVID-19 pandemic as a ubiquitously 'adverse' experience with unavoidable long-term consequences. Though this is a small sample, these findings in conjunction with other work paint a heterogenous picture of families' lives during and after the pandemic. Indeed, it is likely the experience of the pandemic varied substantially by individual circumstances, and lingering effects may certainly exist in some domains. However, I detected little evidence of developmental or physiological disparities based on length of exposure to the incident alone – a positive sign for child development.

Third, the lack of concurrent association between accelerated aging and psychological development gives pause to the claim that accelerated aging in toddlerhood is evidence for the biological embedding of adversity. It is clear additional work is necessary to assess associations in the domains of physical health and development to fully flesh out the clinical relevance of this biomarker. Furthermore, longitudinal work to observe trajectories and patterns of epigenetic

profiles over time is essential to fully evaluating the validity and interpretation of accelerated aging in the first few years of life.

Measurement of Accelerated Aging in Childhood

To date, little research has critically examined epigenetic clocks in very young children. While it is common to evaluate epigenetic clock performance using mean age estimates and correlations with chronological age, there are several challenges to applying these standards in the present study. First, the restricted age range of the sample results in small to nonexistent correlations with epigenetic age. Second, the first-generation algorithms (i.e., Horvath and PedBE) were trained to predict chronological age, whereas the second- and third-generation clocks were trained to predict phenotypes of interest such as clinical markers of health or aging (PhenoAge and GrimAge) or the rate of biological aging (DunedinPACE). Consequently, the correlation between chronological age and epigenetic age is stronger in first-generation clocks than these later clocks (see Oblak et al., 2021 for an overview). Finally, blood-based clocks like DunedinPACE, PhenoAge, and GrimAge positively skew estimates of biological age when using pediatric saliva data (Apsley et al., 2025). Therefore, it is ineffective to evaluate clock performance based on correlations with chronological age or mean values alone in this study.

With these caveats in mind, different clocks must be evaluated using different methods. For the Horvath and PedBE clocks, the significant increase in biological age from 1-month to 30-months is noteworthy, as well as the accurate epigenetic age estimate of the sample at 30-months. These findings reaffirm the accuracy of these first-generation clocks during a period of rapid maturation. For second- and third-generation clocks, an important validity test is the examination of concurrent associations with observable characteristics. Indeed, the detection of associations between early adversity and these clocks, for example, is promising evidence for

both the weathering hypothesis and measurement within early childhood itself. In addition, the increased variance in these measures over time further suggests improving measurement properties over time. Epigenetic-g reflects a DNA methylation composite of cognitive abilities and is therefore theoretically distinct from the other biological age clocks examined here. To date, little work has attempted to validate this measure during early childhood. The lack of significant association with any of the cognitive measures assessed in this dissertation undermines support for the validity of this measure in very young samples. It is critical that future research with larger samples further interrogates the validity of this measure in childhood.

Nonetheless, across all of the studies, there was broadly low signal between these epigenetic clocks and the constructs examined. Table 4-1 presents the percentage of statistically significant tests for each clock out of all of the statistical tests conducted for each timepoint. The clocks that demonstrated the most signal with any construct across all timepoints were the PhenoAge (20%), GrimAge (20%), and DunedinPACE clocks (13%). This supports work which finds second- and third-generation clocks are more sensitive to SES constructs than first-generation clocks (e.g., Willems et al., 2025). However, this low percentage of positive tests may suggest that the first few years of life are still quite early in development for these clocks to robustly detect disparities in biological aging. Combined with the findings observed here that detectable signal increases from infancy to toddlerhood, it is possible that stronger effects may emerge over time with the accumulation of postnatal experiences. Given this low sensitivity and low inter-timepoint stability demonstrated in previous work, it is impossible to draw long-term inferences from these early correlations in childhood. Additional longitudinal research is necessary to determine when, and under what conditions, these clocks are best applied, and at what point in development can long-term inferences be made, if ever.

Stress, SES, and Mechanistic Pathways

A key question generated from this dissertation are the hypothesized mechanisms which explain how perceived stress and socioeconomic disadvantage impact children's biological aging in the first few years of life. This question is complicated by a few key observations; one, I found little to no evidence of a correlation between SES and maternal perceived stress across the early childhood; and two, there was a small association between SES and HPA axis activation in children, but this also did not correlate with children's biological aging or maternal perceived stress. Therefore, should a causal link between SES, stress, and biological aging exist, they likely operate via different pathways to impact children's epigenetic profiles.

A central proposition made by the poverty literature is that family stress is an implicit component of socioeconomic disadvantage. The Family Stress Model posits that economic strain causes psychological distress within the home, leading to greater interparental conflict and disrupted parenting practices which subsequently lead to child maladjustment (Masarik & Conger, 2017). Indeed, this was a theoretical driver of the present dissertation: if socioeconomic strain leads to greater stress, then stress may be a key mechanism to explain how socioeconomic disadvantage 'gets under the skin' to impact epigenetic aging. Nonetheless, accumulating evidence suggests a more complex story. For example, the Baby's First Years intervention led to unexpected *increases* in maternal psychological distress (Magnuson et al., 2024), yet also managed to slow the pace of children's biological aging (Raffington et al., under review).

Epel and colleagues (2018) argue that stress is a multi-dimensional construct, existing on psychological, physiological, and social planes. The predominant model by which stress impacts health is conceptualized via a linear pattern: a stressful event leads to an emotional and physiological response, with an ultimate end point and recovery period. Repeated activations of

this system are thought to contribute to physiological wear-and-tear on the body. However, this model may not be sufficient for chronic experiences like poverty. Indeed, these experiences often have no end period or recovery, and require constant adaptations. It is likely, therefore, that monthly retrospective measures of perceived stress are insufficient for capturing the complex, chronic nature of poverty.

Epel and colleague's (2018) explanation complicate the pathways by which socioeconomic disadvantage may impact biological aging. Factors beyond perceived and physiological stress – such as poor nutrition, exposure to environmental toxicants, or poor mental health – may be accountable for this association. Identifying these mechanisms is beyond the scope of the present dissertation; nonetheless, future work should attempt to disentangle possible pathways and complicate the definition of stress in the context of socioeconomic disadvantage. For example, emerging research suggests that the ratio between cortisol and dehydroepiandrosterone (DHEA) across development – rather than cortisol alone – may be an important area for future research (Kamin & Kertes, 2017)

Notably, the association between accelerated aging and perceived stress was more robust in this study than those reported with SES. There may be several possible explanations for this effect. First, it is possible that mothers who reported higher levels of stress parent in such a way that led to accelerated aging in their children. For example, psychobiological theory argues that maternal distress strains the integration of the physiological and behavioral systems necessary to provide care to offspring, resulting in children receiving fewer high-quality developmental inputs (Barrett & Fleming, 2011).

Alternatively, these mothers may have a predisposition towards greater stress and subsequently have children who appear biologically "older." Research by Luo and colleagues

support this narrative: meta-analytic work has found that nearly all measures of psychological stress are associated with personality traits like neuroticism (Luo et al., 2023), and monozygotic twin research implicates a strong genetic component to the continuity of perceived stress overtime (Luo et al., 2017). Additionally, emerging evidence suggests that genetic factors account for about half of the variation in change of DunedinPACE scores during the transition from adolescence to adulthood (Kuznetsov et al., 2025). While it is unclear the extent to which genetic influences impact DunedinPACE scores in early childhood, these findings give pause to the conclusion that environmental factors are entirely responsible for this association.

Implications

It is critical to contextualize these findings with a healthy amount of skepticism. The study of epigenetic profiles in childhood is a rapidly developing field, and this dissertation pushes on the boundaries of an already developing construct. It is possible these findings will be interpreted wildly different in 5 or 10 years. Nonetheless, at the present moment, this work makes an important and timely contribution to the field. With the caveat that there is still much to learn, I argue that epigenetic profiles are potentially informative to the study of social inequality and development. Epigenetic profiles are measurable across the life course; trainable on many different kinds of phenotypes; environmentally mediated; and possibly sensitive to early intervention (i.e., Baby's First Years). Greater research in early childhood using longitudinal, cross-sectional, and experimental designs will quickly contribute valuable information to the application and utility of these biomarkers across the lifespan.

Limitations

A limitation of this dissertation is the relatively small sample size. While I had access to rich, longitudinal data on children and families, previous studies of epigenetic patterns typically

involve sample sizes 10 times the size of the present work. Therefore, it is possible there are small effects that I am underpowered to detect. In addition, there are likely other factors beyond those assessed here that may moderate these effects – for example, it is plausible these effects may vary by child sex – but a small sample size makes these investigations tenuous. Similarly, future work would benefit from examining the ratio between cortisol and DHEA, rather than cortisol alone, in these association (e.g., see Kamin & Kertes, 2017). Finally, a critical limitation is the use of saliva tissue to assess epigenetic age. While it is convenient to collect in children, the clocks examined here were mostly developed using blood tissues, which have low-to-moderate cross-tissue correlations with saliva (Apsley et al., 2025; Raffington et al., 2023). While preliminary evidence suggests there is no moderating effect of tissue type on the correlation between socioeconomic disadvantage and accelerated aging (Willems et al., 2025), this remains an understudied yet rapidly developing question that should be further investigated.

Future Directions

As anticipated, this dissertation generated more questions than answers. A primary future direction for the field is to further evaluate the validity, stability, and functional significance of these measures within early childhood. Although the adult literature is decisive on these interpretations, these patterns remain inconclusive within the first few years of life. While an important long-term step is the creation of a large, longitudinal dataset from birth through late childhood to examine within-child stability, many of these other questions can be examined in the short-term using cohort studies. Examining epigenetic age as an outcome in targeted RCT's is also a useful area for future investigation to understand what experiences it is sensitive to. In addition, the creation of clocks using saliva tissue is an important step for generating greater confidence in the application of these measures to pediatric samples.

Table 4-1.
Overview of Statistical Tests by Epigenetic Clock

	(1) Study 1A	(2) Studies 1B and 2	(3) Study 3	(4) Overall hits
	Predicted at 1-month	Predicted at 30-months	Predicts outcomes at 30-months	Across all studies
Horvath	8%	0%	6%	4%
PedBE	8%	5%	6%	7%
PhenoAge	33%	28%	0%	20%
GrimAge	8%	28%	20%	20%
DunedinPACE	8%	28%	0%	13%
Epigenetic-g	0%	0%	0%	0%
Total Statistical Tests Per Epigenetic Clock	12	18	15	45

Note. Columns 1 – 3 reflect percent of significant tests out of total statistical tests conducted at each timepoint. Column 4 reflects total significant tests out of all statistical tests conducted for each epigenetic clock across the entire dissertation. The bottom row reflects the number of statistical tests conducted for each epigenetic clock at that timepoint.

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Table A1-1
Factor Loadings of Cumulative Perceived Stress Component

	Factor Loadings
Perceived Stress Scale	
Prenatal	0.38
6-months	0.39
12-months	0.42
24-months	0.38
Perceived Impact of Negative Life Events	
Prenatal	0.36
12-months	0.36
24-months	0.35

Table A1-2.
Mean Epigenetic Age by Child Sex

	Male	Female	Difference	p-value
1-month Raw Epigenetic Age				
Horvath	1.43	1.45	0.02	0.701
PedBE	0.28	0.29	0.01	0.832
PhenoAge	47.10	47.44	0.34	0.662
GrimAge	43.67	41.77	-1.90	<0.001
DunedinPACE	1.34	1.34	0.00	0.904
30-month Raw Epigenetic Age				
Horvath	3.14	3.35	0.21	0.42
PedBE	3.12	3.52	0.40	0.007
PhenoAge	46.31	46.78	0.47	0.761
GrimAge	53.85	50.33	-3.52	0.026
DunedinPACE	1.50	1.48	-0.02	0.229

Table A1-3
Correlation Matrix: Prenatal Adversity and Epigenetic Aging in Newborns

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
(1) Horvath 1-mo	1.00														
(2) PedBE 1-mo	0.52*	1.00													
(3) PhenoAge 1-mo	0.27*	0.31*	1.00												
(4) GrimAge 1-mo	0.10	0.10	0.34*	1.00											
(5) DunedinPACE 1-mo	0.05	0.06	0.20*	0.09	1.00										
(6) Epigenetic-g 1-mo	0.16*	0.16*	-0.06	-0.19*	0.15	1.00									
(7) Prenatal Perceived Stress	0.13	-0.05	0.09	0.15	0.08	0.00	1.00								
(8) Maternal hair cortisol	0.05	0.19*	0.22*	-0.05	0.20*	0.03	0.12	1.00							
(9) Prenatal income-to-needs	0.01	-0.10	-0.09	-0.09	-0.09	0.08	-0.17*	-0.08	1.00						
(10) Maternal education	0.02	-0.07	-0.17*	-0.15	-0.10	0.12	-0.01	-0.13	0.56*	1.00					
(11) Chronological age 1-mo	0.16*	0.42*	0.47*	0.16*	0.17*	-0.06	0.02	0.35*	0.10	-0.05	1.00				
(12) Infant is male	-0.02	0.01	-0.11	0.67*	0.00	-0.08	0.13	-0.02	-0.03	-0.06	-0.02	1.00			
(13) Gestational age	0.09	0.01	0.02	-0.08	0.08	0.15	-0.07	-0.09	0.16	0.20*	-0.24*	-0.08	1.00		
(14) Exclusively breastfed	0.07	0.00	-0.03	-0.13	-0.11	0.13	0.00	0.04	0.32*	0.41*	0.11	-0.04	0.22*	1.00	
(15) Alcohol use	0.11	0.13	0.00	0.02	0.06	0.06	-0.19*	-0.09	0.24*	0.25*	0.07	-0.04	0.18*	0.25*	1.00

Note. * $p<0.05$. Correlations reflect the association between the biological clocks after residualizing for cell composition and technical artifacts (slide, array, and plate).

Table A1-4
Correlation Matrix: Early Adversity and Epigenetic Aging Across Childhood

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
(1) Avg. income-to-needs	1.00																
(2) Maternal Education	0.67*	1.00															
(3) Chronological Age 30-mo	-0.03	0.01	1.00														
(4) Cumulative Perceived Stress	-0.09	-0.01	-0.16	1.00													
(5) Child Hair Cortisol	-0.19	-0.22*	-0.09	0.25*	1.00												
(6) Horvath 30-mo	-0.08	0.01	0.00	0.06	-0.02	1.00											
(7) PedBE 30-mo	-0.09	-0.04	0.25*	-0.12	0.05	0.39*	1.00										
(8) PhenoAge 30-mo	-0.19*	-0.15	0.10	-0.09	0.10	0.28*	0.40*	1.00									
(9) GrimAge 30-mo	-0.08	-0.16	0.38*	0.13	-0.12	0.04	0.14	0.16	1.00								
(10) DunedinPACE 30-mo	-0.19*	-0.17	-0.03	0.27*	0.13	-0.08	-0.11	0.31*	0.19*	1.00							
(11) Epigenetic-g 30-mo	-0.01	-0.06	-0.06	-0.02	-0.13	0.19*	0.13	0.18*	-0.04	0.16	1.00						
(12) Horvath 1-mo	0.05	0.02	-0.28*	0.04	0.12	0.13	-0.01	-0.03	-0.20*	-0.12	0.22*	1.00					
(13) PedBE 1-mo	-0.04	-0.06	-0.09	-0.05	-0.03	-0.01	0.15	-0.04	-0.05	-0.19*	0.17	0.48*	1.00				
(14) PhenoAge 1-mo	-0.12	-0.18*	-0.08	-0.09	0.09	-0.05	0.01	0.20*	-0.09	-0.01	0.04	0.25*	0.18*	1.00			
(15) GrimAge 1-mo	-0.19*	-0.15	-0.06	0.17	-0.18	-0.08	-0.10	0.06	0.39*	0.15	-0.13	0.10	0.14	0.32*	1.00		
(16) DunedinPACE 1-mo	-0.07	-0.10	-0.01	-0.02	0.02	-0.09	-0.23*	-0.02	0.01	0.16	0.08	0.05	0.04	0.22*	0.09	1.00	
(17) Epigenetic-g 1-mo	0.16*	0.12	-0.13	-0.05	-0.07	0.05	0.11	-0.12	-0.18	-0.20*	0.22*	0.16*	0.15	0.00	-0.19*	0.15	1.00

Note. * *p<0.05.* Epigenetic age values at 30-months have been residualized for cell composition and technical artifacts (slide, array, and plate). 1-month epigenetic age values were further residualized for chronological age at the time of data collection.

Table A1-5
Correlation Matrix: Early Adversity, Epigenetic Aging, and Demographics

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
(1) Avg. income-to-needs	1.00																
(2) Maternal Education	0.67*	1.00															
(3) Cumulative Perceived Stress	-0.09	0.01	1.00														
(4) Horvath 30-mo	-0.08	0.01	0.06	1.00													
(5) PedBE 30-mo	-0.14	-0.09	-0.08	0.42*	1.00												
(6) PhenoAge 30-mo	-0.19*	-0.17	-0.07	0.29*	0.39*	1.00											
(7) GrimAge 30-mo	-0.23*	-0.29*	0.22*	0.06	-0.08	0.18*	1.00										
(8) DunedinPACE 30-mo	-0.18*	-0.16	0.27*	-0.08	-0.10	0.31*	0.28*	1.00									
(9) Epigenetic-g 30-mo	0.00	-0.05	-0.03	0.19*	0.18*	0.20*	0.01	0.15	1.00								
(10) Cohort 1	-0.15*	-0.17*	-0.10	0.00	0.00	0.08	0.00	0.00	0.00	1.00							
(11) Child is Male	-0.19*	-0.08	0.20*	-0.10	-0.23*	-0.18*	0.58*	0.10	-0.23*	-0.08	1.00						
(12) Whether White	0.38*	0.47*	-0.06	0.03	-0.01	0.01	-0.15	-0.12	-0.02	-0.03	-0.05	1.00					
(13) Whether Hispanic	-0.43*	-0.56*	-0.10	-0.07	0.08	0.21*	0.21*	0.15	0.10	0.21*	-0.06	-0.43*	1.00				
(14) Whether Black	-0.28*	-0.24*	0.19*	0.12	0.06	0.02	0.12	0.00	-0.23*	-0.02	0.08	-0.46*	-0.06	1.00			
(15) Whether Asian	0.23*	0.26*	0.03	-0.08	-0.04	-0.22*	-0.14	0.08	-0.01	-0.16*	0.04	-0.24*	-0.22*	-0.16*	1.00		
(16) Whether Other Race	-0.29*	-0.44*	-0.14	-0.10	-0.01	0.10	0.14	0.08	0.24*	0.15*	-0.04	-0.52*	0.66*	-0.35*	-0.18*	1.00	
(17) Maternal Age	0.39*	0.35*	-0.10	-0.02	-0.03	-0.19*	-0.21*	-0.23*	0.01	-0.16*	-0.13	0.23*	-0.18*	-0.31*	0.15*	-0.06	
(18) Maternal ROWPVT	0.54*	0.49*	0.03	-0.04	-0.16	-0.17	-0.11	-0.12	0.03	-0.22*	-0.02	0.42*	-0.34*	-0.35*	0.14	-0.21*	1.00

*Note. * $p<0.05$.* Epigenetic age values at 30-months have been residualized for cell composition, technical artifacts (slide, array, and plate), and chronological age.

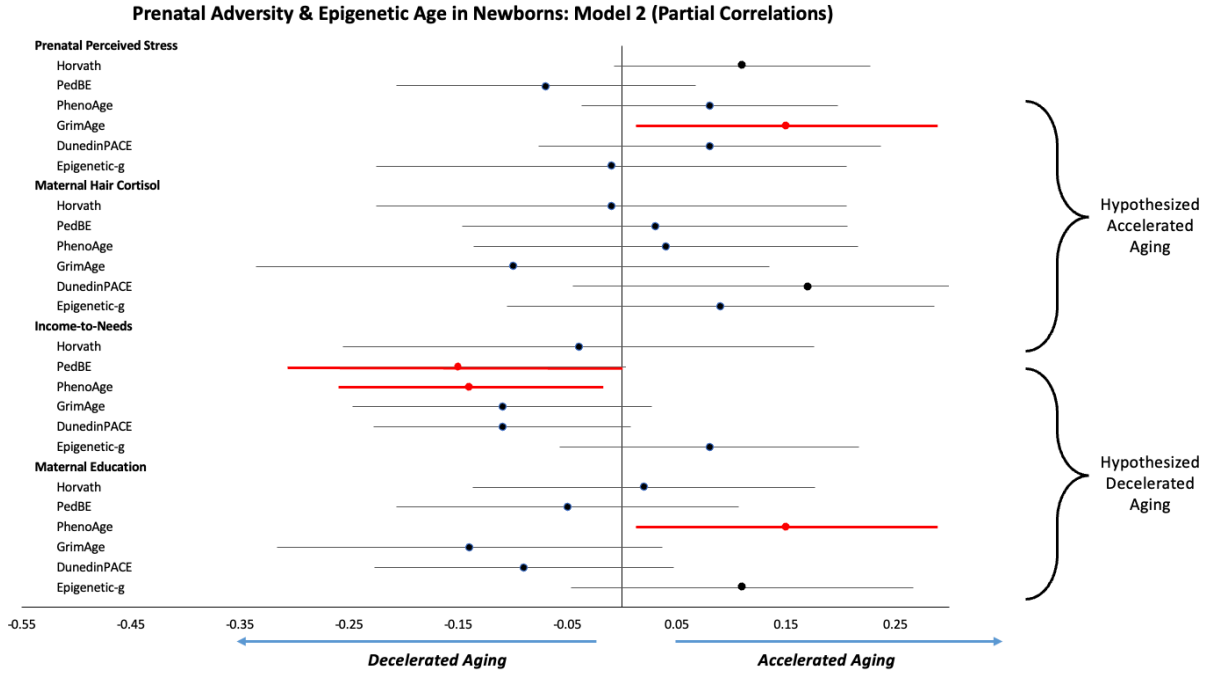
Table A1-6
Prenatal Adversity and Epigenetic Aging in Newborns (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Prenatal Perceived Stress Component						
1. Partial Correlation	0.11 (0.06)	-0.06 (0.07)	0.09 (0.06)	0.15 (0.08)	0.09 (0.08)	-0.01 (0.11)
2. Adjusts for Age	0.11 (0.06)	-0.07 (0.07)	0.08 (0.06)	0.15* (0.07)	0.08 (0.08)	-0.01 (0.11)
3. Adjusts for Demographics	0.15* (0.07)	-0.00 (0.07)	0.02 (0.06)	0.12 (0.08)	0.13 (0.09)	0.03 (0.11)
Maternal Hair Cortisol Concentration						
1. Partial Correlation	0.02 (0.09)	0.14 (0.08)	0.18* (0.09)	-0.05 (0.11)	0.20* (0.09)	0.05 (0.08)
2. Adjusts for Age [a]	-0.02 (0.11)	0.03 (0.09)	0.04 (0.09)	-0.11 (0.12)	0.17 (0.11)	0.09 (0.09)
3. Adjusts for Demographics [a]	-0.04 (0.11)	-0.01 (0.09)	0.02 (0.09)	-0.12 (0.13)	0.20 (0.12)	0.11 (0.10)
Income-to-Needs						
1. Partial Correlation	-0.02 (0.11)	-0.12 (0.08)	-0.09 (0.07)	-0.10 (0.07)	-0.09 (0.06)	0.08 (0.07)
2. Adjusts for Age	-0.04 (0.11)	-0.15 (0.08)	-0.14* (0.06)	-0.11 (0.07)	-0.11 (0.06)	0.08 (0.07)
3. Adjusts for Demographics	-0.06 (0.12)	-0.18* (0.08)	-0.09 (0.07)	-0.07 (0.08)	-0.13 (0.08)	0.00 (0.08)
Maternal Education						
1. Partial Correlation	0.02 (0.08)	-0.07 (0.08)	-0.17* (0.08)	-0.15 (0.09)	-0.10 (0.07)	0.12 (0.08)
2. Adjusts for Age	0.02 (0.08)	-0.05 (0.08)	-0.15* (0.07)	-0.14 (0.09)	-0.09 (0.07)	0.11 (0.08)
3. Adjusts for Demographics	0.06 (0.10)	-0.06 (0.09)	-0.07 (0.09)	-0.12 (0.12)	-0.17 (0.12)	-0.04 (0.11)
Observations	159					

Note. *$p<0.05$. Model 1 reflects partial correlations between the outcome of interest and each biological clock, residualized for technical artifacts (slide, array, and plate) and cell composition. Model 2 adjusts for infant chronological age at the time of sample collection. Model 3 adjusts for the following demographic characteristics: gestational age at birth, maternal race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standard betas. Robust standard errors are reported in parentheses.

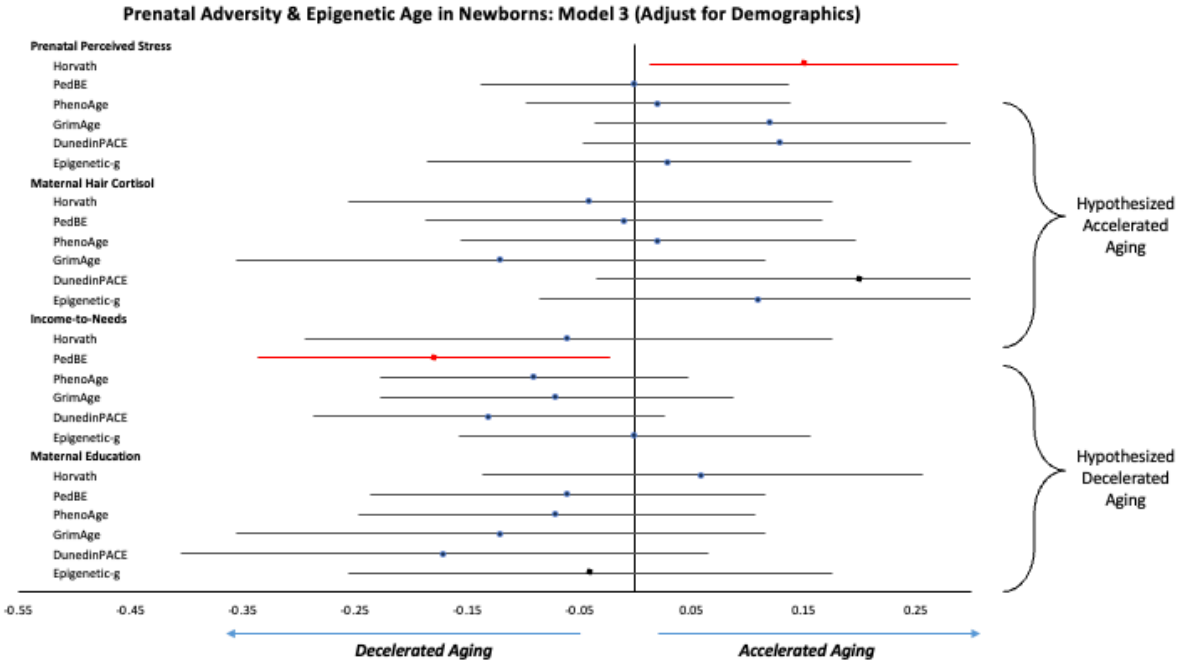
[a] Hair cortisol estimates in Models 2 and 3 further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Figure A1-1
Forest Plot: Prenatal Adversity and Epigenetic Aging in Newborns (Model 2)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the partial correlation between each construct and the epigenetic clock of interest, residualized for technical factors and chronological age (i.e., Model 2 in Table A1-5).

Figure A1-2
Forest Plot: Prenatal Adversity and Epigenetic Aging in Newborns (Model 3)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, and demographic covariates (i.e., Model 3 in Table A1-5).

Table A1-7
Non-imputed Estimates of Table A1-6
Prenatal Adversity and Epigenetic Aging in Newborns (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Perceived Stress Component						
1. Partial Correlation	0.13 (0.07)	-0.05 (0.07)	0.09 (0.06)	0.16 (0.08)	0.08 (0.08)	0.00 (0.11)
2. Adjusts for Age	0.12 (0.06)	-0.06 (0.06)	0.08 (0.06)	0.15 (0.08)	0.08 (0.08)	0.01 (0.11)
3. Adjusts for Demographics	0.16* (0.07)	0.00 (0.06)	0.02 (0.06)	0.13 (0.08)	0.14 (0.09)	0.04 (0.11)
Hair Cortisol Concentration						
1. Partial Correlation	0.04 (0.07)	0.19* (0.07)	0.22* (0.08)	-0.05 (0.10)	0.19* (0.08)	0.03 (0.07)
2. Adjusts for Age [a]	-0.01 (0.08)	0.02 (0.08)	0.04 (0.10)	-0.12 (0.11)	0.16 (0.10)	0.07 (0.09)
3. Adjusts for Demographics [a]	-0.08 (0.08)	-0.04 (0.09)	-0.01 (0.10)	-0.13 (0.12)	0.21 (0.12)	0.08 (0.10)
Income-to-Needs						
1. Partial Correlation	0.01 (0.11)	-0.10 (0.08)	-0.09 (0.07)	-0.09 (0.07)	-0.09 (0.06)	0.08 (0.06)
2. Adjusts for Age	-0.01 (0.11)	-0.14 (0.07)	-0.14* (0.06)	-0.10 (0.07)	-0.10 (0.06)	0.09 (0.06)
3. Adjusts for Demographics	-0.05 (0.12)	-0.18* (0.08)	-0.10 (0.07)	-0.07 (0.08)	-0.12 (0.08)	-0.00 (0.07)
Maternal Education						
1. Partial Correlation	0.02 (0.08)	-0.07 (0.08)	-0.17* (0.08)	-0.15 (0.09)	-0.10 (0.07)	0.12 (0.08)
2. Adjusts for Age	0.02 (0.08)	-0.05 (0.08)	-0.15* (0.07)	-0.14 (0.09)	-0.09 (0.07)	0.11 (0.08)
3. Adjusts for Demographics	0.06 (0.10)	-0.07 (0.09)	-0.07 (0.09)	-0.12 (0.12)	-0.17 (0.12)	-0.04 (0.11)
Observations (range)	110 – 159					

Note. *$p<0.05$. Model 1 reflects partial correlations between the outcome of interest and each biological clock, residualized for technical artifacts (slide, array, and plate) and cell composition. Model 2 adjusts for infant chronological age at the time of sample collection. Model 3 adjusts for the following demographic characteristics: gestational age at birth, mother race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standard betas. Robust standard errors are reported in parentheses.

[a] Hair cortisol estimates in Models 2 and 3 further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Table A1-8
Prenatal Adversity and Epigenetic Aging in Newborns: Joint Prediction (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Prenatal Perceived Stress Component	0.14 (0.08)	-0.03 (0.08)	-0.00 (0.06)	0.12 (0.08)	0.12 (0.09)	0.03 (0.12)
Maternal Hair Cortisol Concentration [a]	-0.04 (0.11)	-0.02 (0.09)	0.02 (0.09)	-0.13 (0.13)	0.20 (0.12)	0.11 (0.10)
Income-to-Needs	-0.07 (0.15)	-0.20* (0.09)	-0.07 (0.08)	-0.02 (0.09)	-0.07 (0.09)	0.02 (0.09)
Maternal Education	0.10 (0.12)	0.03 (0.11)	-0.03 (0.10)	-0.11 (0.12)	-0.14 (0.13)	-0.04 (0.13)
Observations	159					

Note. *$p<0.05$. All predictors were simultaneously entered into the model to predict biological age. Epigenetic clock variables were residualized for cell type composition and technical artifacts (slide, array, and plate). Model adjusts for infant chronological age at the time of sample collection and the following demographic characteristics: gestational age at birth, maternal race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standard betas. Robust standard errors are reported in parentheses.

[a] Hair cortisol estimates further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Table A1-9
Non-imputed Estimates of Table A1-8
(*Prenatal Adversity and Epigenetic Aging in Newborns: Joint Prediction*)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Perceived Stress Component	0.12 (0.08)	0.02 (0.09)	-0.04 (0.08)	0.11 (0.10)	0.15 (0.11)	0.03 (0.16)
Hair Cortisol Concentration [a]	-0.12 (0.09)	-0.05 (0.10)	-0.03 (.12)	-0.18 (.13)	0.18 (.12)	0.04 (.11)
Income-to-Needs	0.12 (.10)	-0.16 (.10)	-0.08 (.11)	0.01 (.10)	-0.05 (.10)	0.05 (.08)
Maternal Education	-0.03 (0.13)	-0.06 (0.13)	-0.08 (0.12)	-0.09 (0.14)	-0.17 (0.14)	-0.05 (0.17)
Observations				99		

*Note. *p<0.05*. All predictors were simultaneously entered into the model to predict epigenetic age. Epigenetic clock variables were residualized for cell type composition and technical artifacts (slide, array, and plate). Each model adjusts for infant chronological age at the time of sample collection and the following demographic characteristics: gestational age at birth, mother race and ethnicity, whether the infant was exclusively breastfed, and maternal report of alcohol consumption during pregnancy. Coefficients reflect standard betas. Robust standard errors are reported in parentheses.

[a] Hair cortisol estimates further adjusts for week of pregnancy when hair sample was collected from the parent and the lab that processed each hair sample.

Table A1-10
Early Adversity and Epigenetic Aging in Toddlers (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Cumulative Perceived Stress Component						
1. Partial Correlation	0.05 (0.09)	-0.11 (0.07)	-0.07 (0.09)	0.21* (0.09)	0.22* (0.09)	-0.01 (0.09)
2. Adjusts for Covariates	0.08 (0.10)	-0.06 (0.08)	-0.03 (0.09)	0.08 (0.08)	0.22* (0.09)	0.06 (0.09)
3. Adjusts for 1-month Epigenetic Age	0.07 (0.10)	-0.07 (0.08)	-0.01 (0.09)	0.04 (0.08)	0.22* (0.09)	0.07 (0.09)
Income-to-Needs						
1. Partial Correlation	-0.12 (0.16)	-0.18 (0.11)	-0.27* (0.12)	-0.31* (0.12)	-0.25* (0.12)	0.00 (0.12)
2. Adjusts for Covariates	-0.16 (0.16)	-0.26* (0.13)	-0.31* (0.12)	-0.18 (0.11)	-0.24* (0.12)	-0.05 (0.13)
3. Adjusts for 1-month Epigenetic Age	-0.15 (0.16)	-0.23 (0.13)	-0.27* (0.11)	-0.14 (0.10)	-0.24 (0.12)	-0.11 (0.13)
Maternal Education						
1. Partial Correlation	0.01 (0.10)	-0.09 (0.12)	-0.18* (0.08)	-0.30* (0.08)	-0.16 (0.09)	-0.05 (0.08)
2. Adjusts for Covariates	-0.01 (0.10)	-0.12 (0.12)	-0.19* (0.08)	-0.23* (0.07)	-0.15 (0.09)	-0.08 (0.09)
3. Adjusts for 1-month Epigenetic Age	-0.00 (0.10)	-0.09 (0.12)	-0.16 (0.08)	-0.19* (0.07)	-0.14 (0.09)	-0.10 (0.09)
Observations				128		

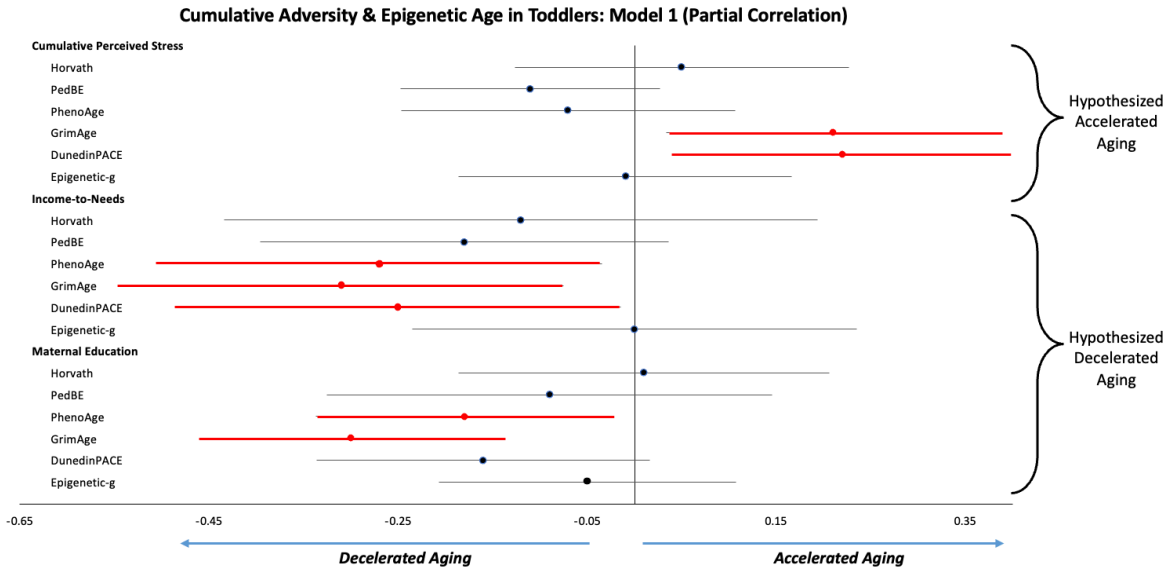
Note. *$p<0.05$. Model 1 reflects partial correlations between the variable of interest and each epigenetic clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month for each respective clock. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A1-11
Non-imputed Estimates of Table A1-10
Early Adversity and Epigenetic Aging in Toddlers (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Cumulative Perceived Stress Component						
1. Partial Correlation	0.06 (0.07)	-0.08 (0.07)	-0.07 (0.10)	0.22* (0.09)	0.27* (0.09)	-0.03 (0.08)
2. Adjusts for Covariates	0.08 (0.08)	-0.03 (0.07)	-0.03 (0.10)	0.10 (0.09)	0.25* (0.08)	0.03 (0.08)
3. Adjusts for 1-month Epigenetic Age	0.09 (0.08)	-0.04 (0.07)	0.02 (0.10)	0.07 (0.08)	0.21* (0.09)	0.02 (0.07)
Income-to-Needs						
1. Partial Correlation	-0.11 (0.16)	-0.19 (0.11)	-0.26* (0.12)	-0.31* (0.12)	-0.25* (0.12)	-0.00 (0.12)
2. Adjusts for Covariates	-0.16 (0.16)	-0.26* (0.13)	-0.31* (0.11)	-0.18 (0.11)	-0.24* (0.12)	-0.05 (0.13)
3. Adjusts for 1-month Epigenetic Age	-0.03 (0.17)	-0.14 (0.13)	-0.13 (0.11)	-0.09 (0.10)	-0.11 (0.12)	0.01 (0.13)
Maternal Education						
1. Partial Correlation	0.01 (0.10)	-0.09 (0.12)	-0.18* (0.08)	-0.30* (0.08)	-0.16 (0.09)	-0.05 (0.08)
2. Adjusts for Covariates	-0.01 (0.10)	-0.12 (0.12)	-0.19* (0.08)	-0.23* (0.07)	-0.15 (0.09)	-0.08 (0.09)
3. Adjusts for 1-month Epigenetic Age	0.09 (0.09)	-0.02 (0.11)	-0.07 (0.08)	-0.18* (0.08)	-0.10 (0.10)	-0.05 (0.09)
Observations (range)	92 – 128					

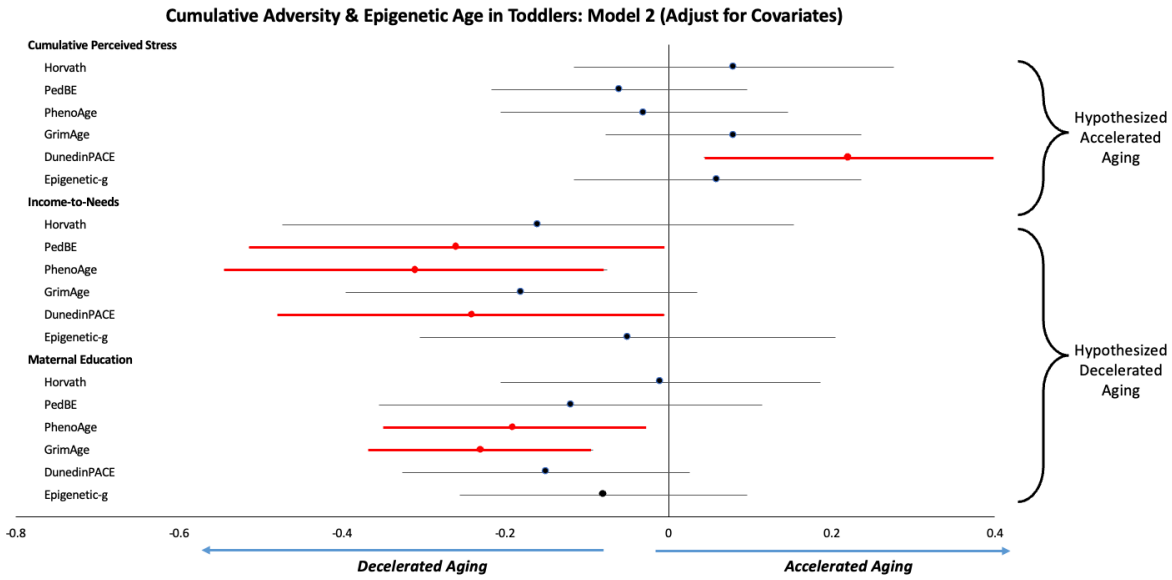
*Note. *p<0.05*. Model 1 reflects partial correlations between the outcome of interest and each epigenetic clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Figure A1-3
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 1)



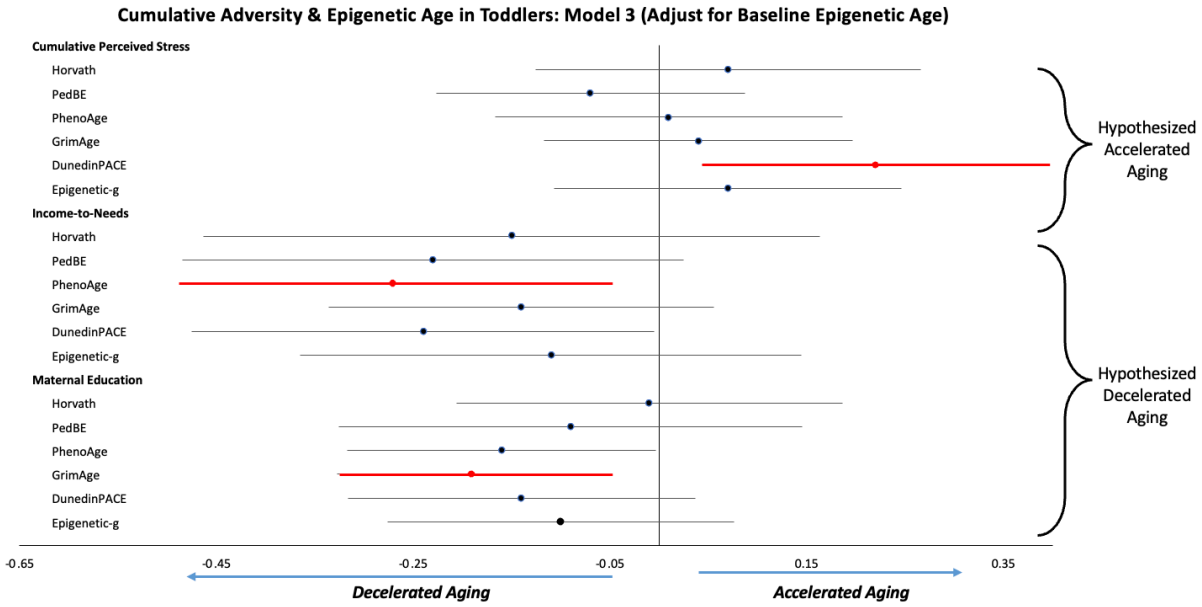
Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age (i.e., Model 1 in Table A1-10).

Figure A1-4
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 2)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, and covariates (i.e., Model 2 in Table A1-10).

Figure A1-5
Forest Plot: Early Adversity and Epigenetic Aging in Toddlers (Model 3)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age, covariates, and epigenetic age at 1-month of age (i.e., Model 3 in Table A1-10).

Table A1-12
Hair Cortisol and Epigenetic Aging in Toddlers

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Child Hair Cortisol Concentration						
1. Partial Correlation	-0.02 (0.12)	0.12 (0.12)	0.10 (0.10)	0.01 (0.09)	0.13 (0.11)	-0.13 (0.10)
2. Adjusts for Demographics	-0.03 (0.12)	0.08 (0.10)	0.12 (0.10)	0.06 (0.07)	0.10 (0.11)	-0.15 (0.09)
3. Adjusts for 1-month Epigenetic Age	-0.04 (0.12)	0.10 (0.10)	0.11 (0.10)	0.06 (0.08)	0.10 (0.11)	-0.14 (0.09)
Observations				77		

Note. *$p<0.05$. Sample restricted to participants with a valid hair cortisol measure at 30-months. Model 1 reflects partial correlations between child hair cortisol and each clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month for each respective clock. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A1-13
Non-imputed Estimates of Table A1-12
(Hair Cortisol and Epigenetic Aging in Toddlers)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Child Hair Cortisol Concentration						
1. Partial Correlation	-0.02	0.12	0.10	0.01	0.13	-0.13
	(0.12)	(0.12)	(0.10)	(0.09)	(0.11)	(0.10)
2. Adjusts for Covariates	-0.03	0.08	0.12	0.06	0.10	-0.15
	(0.12)	(0.10)	(0.10)	(0.07)	(0.11)	(0.09)
3. Adjusts for 1-month Epigenetic Age	0.04	0.13	0.14	0.10	0.05	-0.17
	(0.13)	(0.10)	(0.13)	(0.08)	(0.14)	(0.12)
Observations	63 – 77					

Note. *$p<0.05$. Sample restricted to participants with a valid hair cortisol measure at 30-months. Model 1 reflects partial correlations between child hair cortisol and each clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month for each respective clock. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A1-14
Early Adversity and Epigenetic Aging in Toddlers: Joint Prediction (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Cumulative Perceived Stress Component	0.07	-0.06	-0.01	0.05	0.22*	0.07
	(0.10)	(0.08)	(0.09)	(0.07)	(0.09)	(0.09)
Income-to-Needs	-0.31	-0.28	-0.21	0.10	-0.20	-0.00
	(0.26)	(0.15)	(0.17)	(0.12)	(0.16)	(0.17)
Maternal Education	0.16	0.05	-0.05	-0.24*	-0.04	-0.10
	(0.16)	(0.16)	(0.13)	(0.10)	(0.12)	(0.11)
Observations	128					

*Note. *p<0.05*. Epigenetic clocks have been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Models adjust for epigenetic age at 1-month for each respective clock and the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A1-15
Non-imputed Estimates of Table A1-14
Early Adversity and Epigenetic Aging in Toddlers: Joint Prediction (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Cumulative Perceived Stress Component	0.10	-0.05	-0.00	0.05	0.21*	0.02
	(0.08)	(0.07)	(0.10)	(0.09)	(0.09)	(0.08)
Income-to-Needs	-0.42	-0.37*	-0.20	0.25	0.03	0.05
	(0.26)	(0.17)	(0.17)	(0.15)	(0.14)	(0.18)
Maternal Education	0.27	0.21	0.05	-0.21*	-0.12	-0.11
	(0.15)	(0.15)	(0.11)	(0.10)	(0.13)	(0.13)
Observations	92					

*Note. *p<0.05*. Epigenetic clocks have been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Models adjust for epigenetic age at 1-month for each respective clock and the following covariates: cohort, child sex, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A2-1.
Participant Characteristics Predicting Post-Pandemic Attrition

	Odds Ratio (SE)	p-value
Demographics		
Duration of Pandemic Exposure	1.38 (0.33)	0.181
Maternal Education	2.26* (0.66)	0.006
Family Income	1.11 (0.06)	0.063
Maternal Age	1.30 (0.30)	0.260
Prenatal Mental Health	0.86 (0.20)	0.519
Whether mother is non-Hispanic Black	0.86 (0.48)	0.784
Whether mother is non-Hispanic White	2.86 (1.59)	0.059
Whether mother is Hispanic	0.29* (0.15)	0.017
Whether mother is non-Hispanic Other Race	0.48 (0.22)	0.110
Infant is Female	1.06 (0.54)	0.915
Epigenetic Age Value at 1-month		
Horvath	0.70 (0.20)	0.227
PedBE	1.10 (0.33)	0.751
PhenoAge	0.80 (0.24)	0.454
GrimAge	0.71 (0.22)	0.276
DunedinPACE	0.79 (0.23)	0.433
Epigenetic-g	1.24 (0.37)	0.480
N (range)	62 – 93	

Note. *$p < 0.05$. Logistic regression predicting the likelihood of returning to the lab at the 30-month visit. Higher values indicate greater likelihood of remaining in the analytic sample. Sample restricted to Cohort 1.

Table A2-2
Baseline Balance of 1-month Epigenetic Age by Pandemic Exposure

Epigenetic Age at 1-month	Beta (SE)	p-value
Horvath	1.27 (0.16)	0.061
PedBE	1.49* (0.18)	<0.001
PhenoAge	1.33* (0.16)	0.024
GrimAge	1.04 (0.13)	0.759
DunedinPACE	0.99 (0.13)	0.968
Epigenetic-g	1.10 (0.14)	0.469
N (range)	62	

Note. *$p < 0.05$. Duration of pandemic exposure predicting 1-month epigenetic age to examine 'baseline balance' of epigenetic age prior to the pandemic.

Table A2-3
Correlation Matrix: Pandemic Exposure Measures and Development

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
(1) Duration of Pandemic Exposure	1.00														
(2) Disruptive Life Events	0.15	1.00													
(3) Maternal Education	-0.01	0.37*	1.00												
(4) Chronological Age	-0.37*	-0.09	0.04	1.00											
(5) Horvath 30-mo	-0.09	0.12	-0.01	0.01	1.00										
(6) PedBE 30-mo	-0.22	0.08	0.02	0.21	0.50*	1.00									
(7) GrimAge 30-mo	-0.16	-0.04	-0.29*	0.14	0.12	0.14	1.00								
(8) PhenoAge 30-mo	-0.07	-0.15	-0.09	0.17	0.17	0.51*	0.23	1.00							
(9) DunedinPACE 30-mo	0.14	-0.04	-0.27	-0.02	-0.17	-0.09	0.28*	0.30*	1.00						
(10) Epigenetic-g 30-mo	0.08	0.01	-0.18	-0.07	0.10	-0.11	0.04	-0.04	0.33*	1.00					
(11) Behavior Problems	0.26*	0.01	-0.25*	-0.18	-0.09	-0.09	0.35*	0.05	0.29*	0.04	1.00				
(12) Language Ability	0.02	0.22	0.27*	-0.06	0.20	0.13	-0.06	0.10	-0.17	0.17	-0.21	1.00			
(13) Executive Function	-0.03	0.05	0.26	0.13	0.10	-0.10	-0.11	-0.02	0.09	0.25	0.01	0.50*	1.00		
(14) Receptive Vocab. Conceptual Score	-0.20	0.09	0.57*	0.20	-0.26	-0.01	-0.29	-0.06	-0.32	0.10	-0.15	0.53*	0.63*	1.00	
(15) Child Hair Cortisol	0.17	-0.18	-0.44*	-0.39*	-0.13	-0.01	0.16	0.25	0.23	-0.25	-0.08	-0.12	-0.28	-0.44*	1.00

Note. * $p<0.05$. Epigenetic variables have been residualized for technical factors (slide, plate, array) and cell composition.

Table A2-4
Descriptive Statistics of Study 2 Outcomes

	N	Mean	SD	Min	Max
Child Outcomes					
Behavior Problems	63	7.68	4.98	0	22
Language Ability	62	98.17	17.31	64	122.85
Executive Function	31	98.77	8.44	80	122
Hair Cortisol Concentration (*pg/mg; winsorized*)	32	20.04	24.10	2.53	96.42
Receptive Vocabulary					
English Monolingual Standard Score	16	99.31	30.56	0	137
Spanish Bilingual Standard Score	15	95.73	15.41	68	131
Conceptual Score	31	27.03	15.22	0	64

Note. Sample restricted to children in Cohort 1 with valid observations of each respective outcome in toddlerhood. Behavior Problems reflects a sum of parent-reported behaviors from the BITSEA Problems subscale. Language Ability, Executive Function, and Receptive Vocabulary reflect standardized scores where M=100, SD=15 for each measure. The Receptive Vocabulary Conceptual Score reflects overlapping items administered on both the monolingual and bilingual versions of the assessment.

Table A2-5
Pandemic Exposure Measures and Epigenetic Aging in Toddlers (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Duration of Pandemic Exposure						
1. Partial Correlation	-0.05	-0.01	0.12	0.07	0.11	0.03
	(0.13)	(0.10)	(0.13)	(0.14)	(0.10)	(0.10)
2. Adjusts for Demographics	-0.07	-0.06	0.09	0.04	0.09	0.03
	(0.14)	(0.13)	(0.14)	(0.10)	(0.09)	(0.11)
3. Adjusts for 1-month Epigenetic Age	-0.08	-0.07	0.10	0.03	0.10	0.03
	(0.14)	(0.12)	(0.14)	(0.10)	(0.10)	(0.11)
Disruptive Life Events						
1. Partial Correlation	0.12	0.05	-0.09	-0.12	-0.00	-0.00
	(0.20)	(0.12)	(0.19)	(0.19)	(0.19)	(0.16)
2. Adjusts for Demographics	0.11	-0.04	-0.03	0.00	0.28	0.11
	(0.27)	(0.19)	(0.26)	(0.20)	(0.24)	(0.23)
3. Adjusts for 1-month Epigenetic Age	0.13	-0.01	-0.00	-0.04	0.29	0.14
	(0.28)	(0.19)	(0.23)	(0.20)	(0.24)	(0.23)
Observations	52					

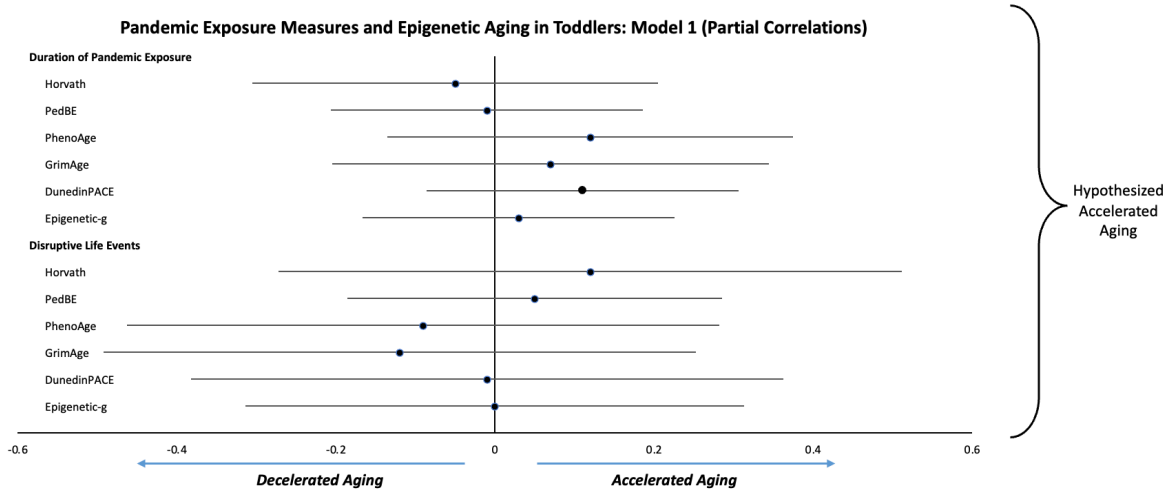
Note. * $p < 0.05$. Model 1 reflects partial correlations between the outcome of interest and each biological clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following demographic characteristics: maternal education, prenatal mental health, child sex, maternal race and ethnicity, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month for each respective clock. Sample restricted to participants with a valid epigenetic age estimate at 30-months. Coefficients have been weighted using IPAW procedures and reflect standardized betas. Robust standard errors in parentheses.

Table A2-6
Non-imputed Estimates of Table A2-5
Pandemic Exposure Measures and Epigenetic Aging in Toddlers (all clocks)

	(1) Horvath	(2) PedBE	(3) PhenoAge	(4) GrimAge	(5) DunedinPACE	(6) Epigenetic-g
Duration of Pandemic Exposure						
1. Partial Correlation	-0.05	-0.01	0.12	0.07	0.11	0.03
	(0.13)	(0.10)	(0.13)	(0.14)	(0.10)	(0.10)
2. Adjusts for Demographics	-0.07	-0.06	0.09	0.04	0.09	0.03
	(0.14)	(0.13)	(0.14)	(0.10)	(0.09)	(0.11)
3. Adjusts for 1-month Epigenetic Age	-0.10	-0.07	0.10	0.02	0.12	0.01
	(0.15)	(0.11)	(0.16)	(0.11)	(0.13)	(0.12)
Disruptive Life Events						
1. Partial Correlation	0.17	0.07	-0.16	-0.06	0.02	0.03
	(0.16)	(0.14)	(0.19)	(0.19)	(0.21)	(0.19)
2. Adjusts for Demographics	0.14	-0.05	-0.05	0.02	0.35	0.16
	(0.26)	(0.22)	(0.18)	(0.17)	(0.21)	(0.23)
3. Adjusts for 1-month Epigenetic Age	0.06	-0.09	-0.01	-0.16	0.28	0.29
	(0.30)	(0.20)	(0.17)	(0.22)	(0.22)	(0.28)
Observations	52 – 37					

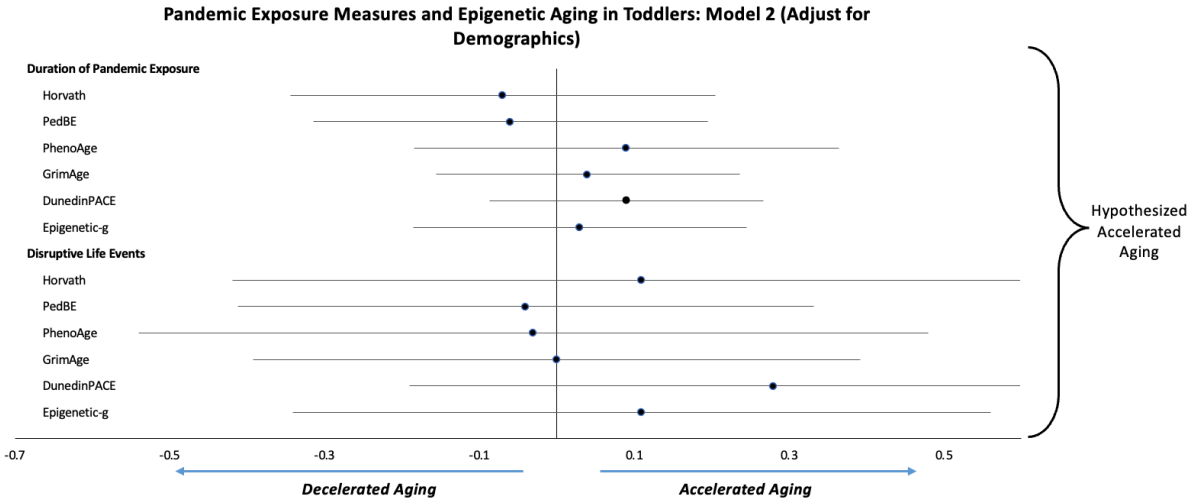
Note. * $p < 0.05$. Model 1 reflects partial correlations between the outcome of interest and each biological clock, residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following demographic characteristics: maternal education, prenatal mental health, child sex, maternal race and ethnicity, and a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit. Model 3 further adjusts for epigenetic age assessed at 1-month for each respective clock. Sample restricted to participants with a valid epigenetic age estimate at 30-months. Coefficients have been weighted using IPAW procedures and reflect standardized betas. Robust standard errors in parentheses.

Figure A2-1.
Forest Plot: Pandemic Exposures and Epigenetic Aging (Model 1)



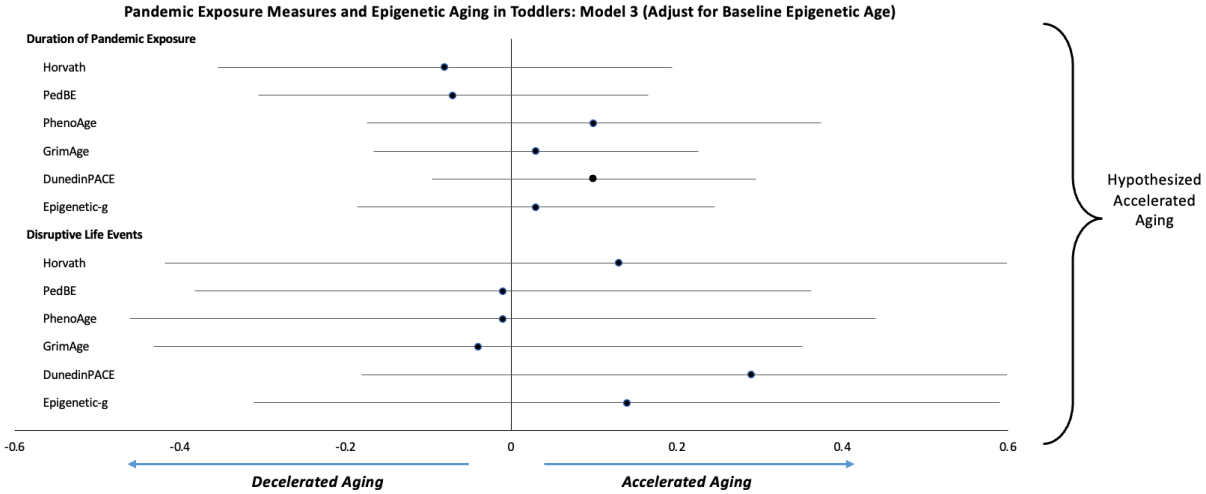
Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age (i.e., Model 1 in Table A2-4).

Figure A2-2.
Forest Plot: Pandemic Exposures and Epigenetic Aging (Model 2)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, and demographic covariates (i.e., Model 2 in Table A2-4).

Figure A2-3.
Forest Plot: Pandemic Exposures and Epigenetic Aging (Model 3)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors and chronological age, demographic covariates, and epigenetic age at 1-month of age (i.e., Model 3 in Table A2-4).

Table A3-1
Factor Loadings of Cognitive Abilities Composite

	Factor Loadings
Language Abilities	0.57
Receptive Vocabulary	0.55
Executive Function	0.60
Eigenvalue	2.17

Table A3-2
Correlation Matrix: Epigenetic Age and Developmental Outcomes

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
(1) Income-to-needs	1.00															
(2) Maternal Education	0.67*	1.00														
(3) Cumulative Perceived Stress	-0.09	0.01	1.00													
(4) Child Hair Cortisol	-0.19	-0.22*	0.25*	1.00												
(5) Chronological age	-0.03	0.01	-0.16	-0.09	1.00											
(6) Horvath 30-mo	-0.08	0.01	0.06	-0.02	0.00	1.00										
(7) PedBE 30-mo	-0.09	-0.04	-0.12	0.05	0.25*	0.39*	1.00									
(8) GrimAge 30-mo	-0.19*	-0.15	-0.09	0.10	0.10	0.28*	0.40*	1.00								
(9) PhenoAge 30-mo	-0.08	-0.16	0.13	-0.12	0.38*	0.04	0.14	0.16	1.00							
(10) DunedinPACE 30-mo	-0.19*	-0.17	0.27*	0.13	-0.03	-0.08	-0.11	0.31*	0.19*	1.00						
(11) Epigenetic-g 30-mo	-0.01	-0.06	-0.02	-0.13	-0.06	0.19*	0.13	0.18*	-0.04	0.16	1.00					
(12) Behavior Problems	-0.26*	-0.24*	0.29*	0.04	-0.07	-0.09	-0.16	-0.02	0.17	0.19*	-0.07	1.00				
(13) Language Ability	0.42*	0.38*	-0.12	-0.25*	0.02	0.18*	0.12	0.10	-0.07	-0.14	0.14	-0.44*	1.00			
(14) Receptive Vocab. Conceptual Score	0.61*	0.57*	0.00	-0.28*	0.33*	-0.13	-0.01	-0.06	-0.02	-0.17	0.04	-0.22*	0.62*	1.00		
(15) Executive Function	0.31*	0.34*	0.09	-0.07	0.14	-0.01	-0.03	-0.01	-0.14	0.09	-0.02	-0.08	0.54*	0.61*	1.00	
(16) Cognitive Component	0.61*	0.60*	0.04	-0.33*	0.27*	0.14	0.01	0.03	0.02	-0.12	0.10	-0.28*	0.85*	0.89*	0.81*	1.00

Note. * $p<0.05$. Clock values were residualized for cell type composition and technical factors (i.e., slide, and array)

Table A3-3
Epigenetic Age and Developmental Outcomes in Toddlers (all clocks)

	(1) Behavior Problems	(2) Language Ability	(3) Receptive Vocabulary	(4) Executive Function	(5) Cognitive Abilities Component [a]
Horvath					
1. Partial Correlation	-0.09	0.18	-0.14	-0.01	0.15
	(0.08)	(0.10)	(0.13)	(0.14)	(0.13)
2. Adjusts for Demographics	-0.05	0.20*	-0.08	-0.10	0.05
	(0.09)	(0.10)	(0.10)	(0.15)	(0.12)
3. Adjusts for 1-month Epigenetic Age	-0.04	0.19	-0.08	-0.11	0.05
	(0.09)	(0.10)	(0.10)	(0.15)	(0.12)
PedBE					
1. Partial Correlation	-0.17*	0.07	-0.13	-0.09	-0.08
	(0.07)	(0.07)	(0.12)	(0.10)	(0.11)
2. Adjusts for Demographics	-0.14	0.09	-0.06	-0.14	-0.10
	(0.07)	(0.09)	(0.10)	(0.11)	(0.11)
3. Adjusts for 1-month Epigenetic Age	-0.14	0.07	-0.06	-0.17	-0.11
	(0.07)	(0.09)	(0.10)	(0.10)	(0.11)
PhenoAge					
1. Partial Correlation	-0.02	0.09	-0.09	-0.02	0.01
	(0.08)	(0.10)	(0.12)	(0.11)	(0.12)
2. Adjusts for Demographics	-0.01	0.15	0.01	-0.03	0.07
	(0.08)	(0.09)	(0.10)	(0.11)	(0.10)
3. Adjusts for 1-month Epigenetic Age	0.00	0.15	0.00	-0.03	0.07
	(0.09)	(0.09)	(0.09)	(0.11)	(0.09)
GrimAge					
1. Partial Correlation	0.21*	-0.18	-0.33*	-0.30*	-0.23
	(0.09)	(0.10)	(0.13)	(0.12)	(0.14)
2. Adjusts for Demographics	-0.01	-0.03	-0.16	-0.21	0.05
	(0.13)	(0.13)	(0.16)	(0.12)	(0.16)
3. Adjusts for 1-month Epigenetic Age	0.03	-0.06	-0.16	-0.13	0.04
	(0.13)	(0.13)	(0.17)	(0.12)	(0.17)
DunedinPACE					
1. Partial Correlation	0.19	-0.13	-0.17	0.08	-0.10
	(0.10)	(0.10)	(0.12)	(0.07)	(0.12)
2. Adjusts for Demographics	0.13	-0.05	-0.06	0.11	0.00
	(0.09)	(0.09)	(0.10)	(0.08)	(0.10)
3. Adjusts for 1-month Epigenetic Age	0.13	-0.06	-0.06	0.10	-0.00
	(0.09)	(0.09)	(0.10)	(0.08)	(0.10)
Epigenetic-g					
1. Partial Correlation	-0.07	0.15	0.06	-0.01	0.11
	(0.11)	(0.10)	(0.14)	(0.09)	(0.14)
2. Adjusts for Demographics	-0.03	0.13	0.05	-0.08	0.08
	(0.12)	(0.10)	(0.10)	(0.09)	(0.12)
3. Adjusts for 1-month Epigenetic Age	-0.03	0.11	0.01	-0.11	0.08
	(0.12)	(0.10)	(0.09)	(0.09)	(0.12)
Observations	124	120	76	89	65

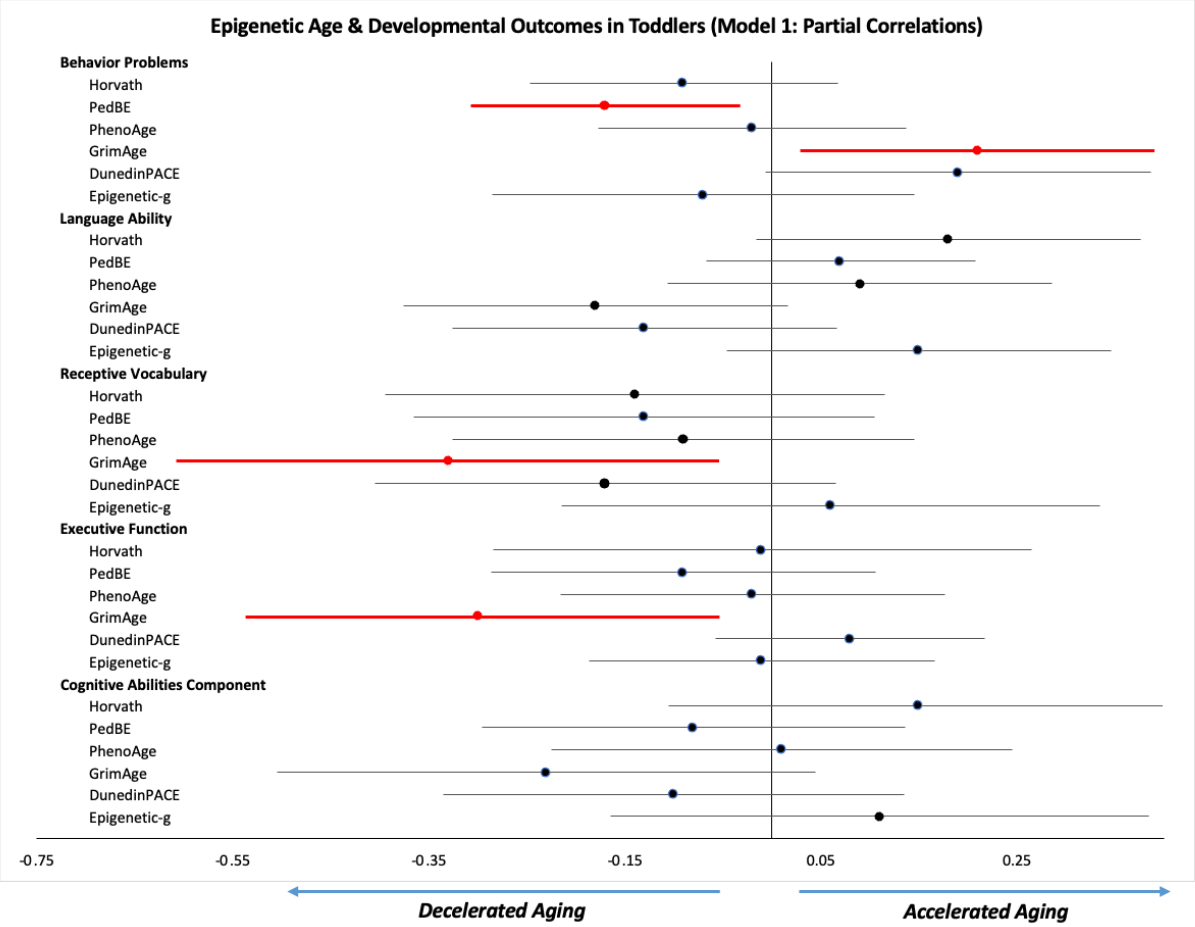
Note. *$p < 0.05$. Epigenetic variables have been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: family income-to-needs, maternal education, cohort, child sex, a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit, and child age at the time of the developmental assessment. Model 3 further adjusts for epigenetic age at 1-month. Sample restricted to participants with a valid epigenetic age at 30-months and each developmental outcome. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Table A3-4
Non-imputed Estimates of Table A3-3
Epigenetic Age and Developmental Outcomes in Toddlers (all clocks)

	(1) Behavior Problems	(2) Language Ability	(3) Receptive Vocabulary	(4) Executive Function	(5) Cognitive Abilities Component [a]
Horvath					
1. Partial Correlation	-0.09	0.18	-0.14	-0.01	0.15
	(0.08)	(0.10)	(0.13)	(0.14)	(0.13)
2. Adjusts for Demographics	-0.06	0.20	-0.08	-0.10	0.04
	(0.09)	(0.10)	(0.10)	(0.15)	(0.12)
3. Adjusts for 1-month Epigenetic Age	-0.04	0.13	0.06	-0.16	0.19
	(0.10)	(0.13)	(0.11)	(0.17)	(0.14)
PedBE					
1. Partial Correlation	-0.17*	0.07	-0.13	-0.09	-0.08
	(0.07)	(0.07)	(0.12)	(0.10)	(0.11)
2. Adjusts for Demographics	-0.13	0.10	-0.06	-0.14	-0.10
	(0.07)	(0.09)	(0.10)	(0.11)	(0.11)
3. Adjusts for 1-month Epigenetic Age	-0.08	-0.01	0.08	-0.13	-0.04
	(0.09)	(0.11)	(0.10)	(0.11)	(0.12)
PhenoAge					
1. Partial Correlation	-0.02	0.09	-0.09	-0.02	0.01
	(0.08)	(0.10)	(0.12)	(0.11)	(0.12)
2. Adjusts for Demographics	-0.03	0.15	-0.00	-0.04	0.05
	(0.08)	(0.10)	(0.11)	(0.12)	(0.11)
3. Adjusts for 1-month Epigenetic Age	-0.01	0.14	0.11	-0.03	0.12
	(0.09)	(0.10)	(0.09)	(0.13)	(0.10)
GrimAge					
1. Partial Correlation	0.21*	-0.18	-0.33*	-0.30*	-0.23
	(0.09)	(0.10)	(0.13)	(0.12)	(0.14)
2. Adjusts for Demographics	0.00	-0.02	-0.16	-0.21	0.07
	(0.13)	(0.13)	(0.17)	(0.12)	(0.16)
3. Adjusts for 1-month Epigenetic Age	0.03	-0.10	-0.05	-0.14	0.17
	(0.14)	(0.14)	(0.18)	(0.13)	(0.17)
DunedinPACE					
1. Partial Correlation	0.19*	-0.13	-0.17	0.08	-0.10
	(0.10)	(0.10)	(0.12)	(0.07)	(0.12)
2. Adjusts for Demographics	0.12	-0.06	-0.06	0.10	-0.01
	(0.10)	(0.09)	(0.10)	(0.08)	(0.10)
3. Adjusts for 1-month Epigenetic Age	0.11	-0.04	-0.02	0.11	0.04
	(0.10)	(0.11)	(0.11)	(0.10)	(0.11)
Epigenetic-g					
1. Partial Correlation	-0.07	0.15	0.06	-0.01	0.11
	(0.11)	(0.10)	(0.14)	(0.09)	(0.14)
2. Adjusts for Demographics	-0.03	0.13	0.05	-0.08	0.09
	(0.12)	(0.10)	(0.10)	(0.09)	(0.12)
3. Adjusts for 1-month Epigenetic Age	-0.10	0.18*	0.10	-0.12	0.24*
	(0.10)	(0.09)	(0.07)	(0.12)	(0.10)
Observations	105 - 124	102 - 120	63 - 76	76 - 89	54 - 65

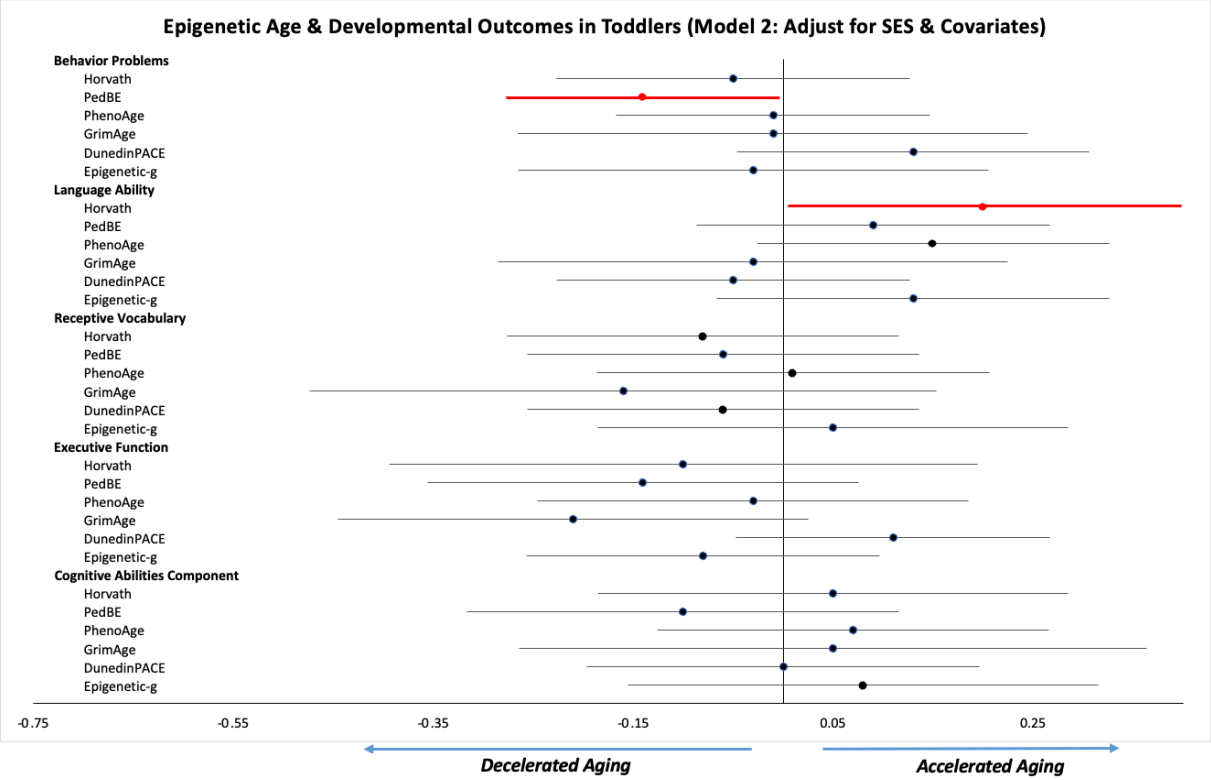
Note. *$p < 0.05$. Epigenetic variables have been residualized for technical artifacts (slide & array), cell composition, and child chronological age. Model 2 adjusts for the following covariates: family income-to-needs, maternal education, cohort, child sex, a dummy indicator of whether the saliva sample was collected at the 30-month or 36-month visit, and child age. Model 3 further adjusts for epigenetic age at 1-month. Sample restricted to participants with a valid epigenetic age at 30-months and each developmental outcome. Coefficients reflect standardized betas. Robust standard errors in parentheses.

Figure A3-1
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 1)



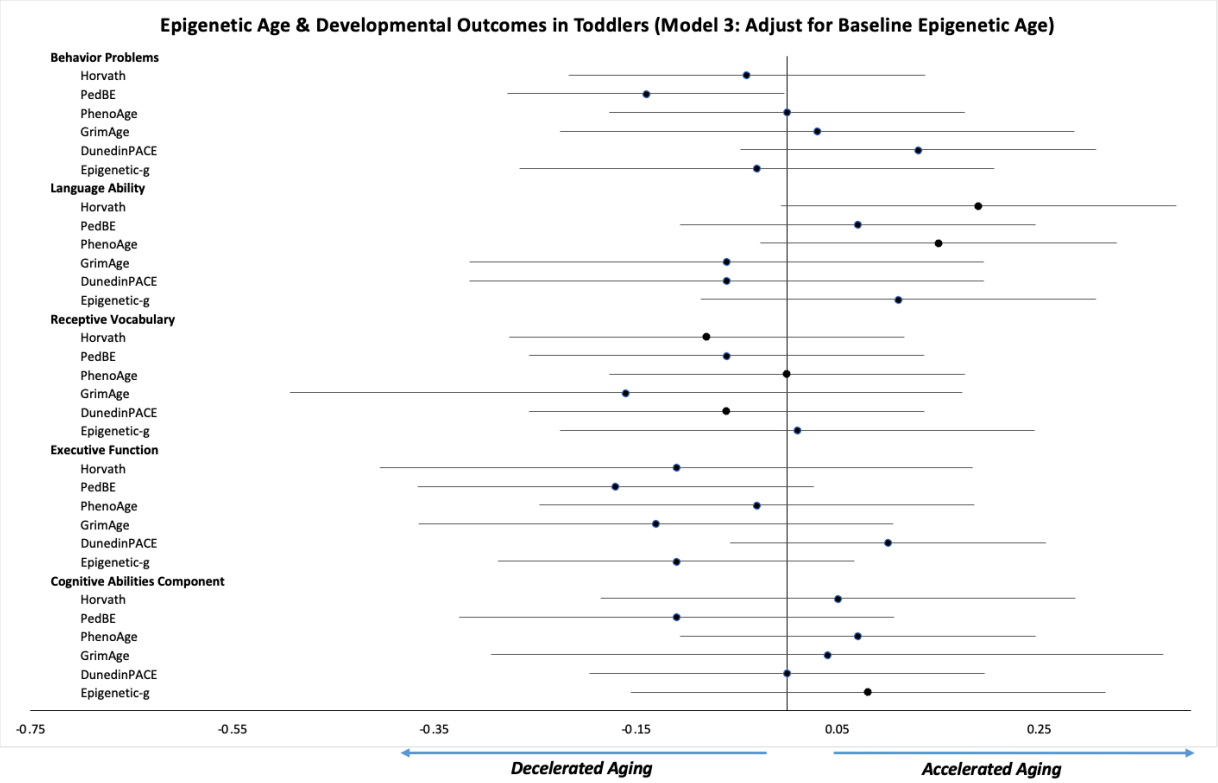
Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the partial correlation between each measure and the epigenetic clock of interest, residualized for technical factors and chronological age (i.e., Model 1 in Table A3-3).

Figure 3-2
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 2)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each measure and the epigenetic clock of interest, adjusted for technical factors, chronological age, family SES, and demographic covariates (i.e., Model 2 in Table A3-3).

Figure 3-3
Forest Plot: Epigenetic Age and Developmental Outcomes (Model 3)



Note. Significant effects indicated by red line ($p < 0.05$). Effect sizes reflect the association between each construct and the epigenetic clock of interest, adjusted for technical factors, chronological age, demographic covariates, and epigenetic age at 1-month (i.e., Model 3 in Table A3-3).