1-1-2017

Points of Leverage: Interrupting the Intergenerational Transmission of Adversity

Lisa Schlueter
University of Denver

Recommended Citation

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Abstract

Early life stressors, such as abuse and neglect, have been associated with poor physical and mental health outcomes in adulthood. Moreover, animal models suggest that caregivers' early life stress can have intergenerational effects that then impact the health and well-being of their offspring. Although animal models are compelling, and inter-generationally transmitted and co-occurring risks are well-documented, proximal mechanistic explanations for how caregiver's history of childhood adversity can result in changes to their child's stress physiology and outcomes have not yet been systematically tested in humans. Thus, among a sample of low-income, predominantly Latino families participating in Early Head Start (EHS), the current study explored whether caregiver history of adversity predicted infant and toddler physiology, and if three pathways, one psychological (caregiver mental health), one physical/environmental (environmental instability), and one biological (epigenetic), mediated the effects of caregiver history of adversity on infant and toddler dysregulated stress physiology. I also explored whether caregiver warmth and responsivity either mediated or moderated the direct relationship between caregiver history of adversity and infant and toddler stress physiology. Results showed that after controlling for important covariates (income-to-needs, caregiver race and ethnicity, and child early life stress), higher caregiver history of adversity predicted infant and toddler diurnal cortisol (e.g., higher noon and bedtime values), but no relationship was found for infant and toddler stress reactivity cortisol. Mediation analyses demonstrated that current caregiver mental health symptoms partially mediated the relationship between caregiver history of adversity and infant and toddler noon and bedtime cortisol values. Further, environmental instability fully mediated the relationship between caregiver history of adversity and infant and toddler noon cortisol, but was non-significant for bedtime cortisol values. Caregiver adversity was not related to infant and toddler methylation rates of the human glucocorticoid receptor gene NR3C1, nor caregiver warmth and responsivity. However, caregiver warmth and responsivity moderated the effects of caregiver history of adversity on infant and toddler noon and bedtime cortisol such that when infants and toddlers experienced lower warmth and responsivity (both chronically and acutely) and high caregiver history of adversity they experienced particularly high noon and bedtime cortisol values. Results suggest proximal processes account for many of the effects of caregiver history of adversity on diurnal, but not stress reactive, cortisol in infants and toddlers in a sample of families experiencing significant current economic and psychosocial adversity.

Document Type
Dissertation

Degree Name
Ph.D.

Department
Psychology

First Advisor
Sarah Enos Watamura, Ph.D.

Second Advisor
Julia Dmitrieva

Third Advisor
Pilyoung Kim

Keywords

Adverse childhood experiences, Cortisol, Early life stress, Environmental instability, Maternal depression, Warm and responsive caregiving

Subject Categories

Psychology | Social and Behavioral Sciences

Publication Statement

POINTS OF LEVERAGE: INTERRUPTING THE INTERGENERATIONAL

TRANSMISSION OF ADVERSITY

_____

A Dissertation

Presented to

the Faculty of Social Sciences

University of Denver

_____

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

_____

by

Lisa J. Schlueter

August 2017

Advisor: Sarah Enos Watamura, Ph.D.

Author: Lisa J. Schlueter
Title: POINTS OF LEVERAGE: INTERRUPTING THE INTERGENERATIONAL
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Acknowledgements

This dissertation would not have been possible without the support of many people. I would like to thank my advisor Dr. Sarah Enos Watamura for her unyielding support and guidance throughout graduate school. I would also like to thank the entire University of Denver Child Health and Development Lab, in particular, Elanah Barrow, Amy Dominguez, Eliana Hurwich-Reiss, Ariel Julian, Natasha Link, Marina Mendoza and Allison Stiles for their commitment to collecting and preparing this data, and their continued support and friendships. I would also like to thank Dr. Julia Dmitrieva for her compassion, mentorship, and guidance throughout graduate school. I would also like to thank my invaluable colleagues for their support and comradery throughout graduate school, and a special thank you to Samantha Brown, Emma Espel, Eliana Hurwich-Reiss, Daniel Lumian, and Aleja Parsons, for their support during the dissertation process. Thank you for reminding me what is truly important in life and helping me maintain balance and laughter amidst graduate school. Furthermore, I want to give a tremendous thank you to our community partners, child, and caregiver participants, and funders, who have made this work possible.

Lastly, I want to thank my entire family, in particular my parents. I could not have made it through the dissertation and doctoral process without your continued love, support, and understanding. I am forever grateful.

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Chapter One: General Background

The current study explored the risk and protective factors that predict a key component of later psychological and physical health outcomes – child stress physiology. These questions were explored in a population of infants and toddlers and their families enrolled in Early Head Start as part of a larger ongoing Buffering Toxic Stress Consortium study funded by the Administration for Children and Families. The target population consisted largely of Latino immigrant families, living at or near the poverty line, with over half of the current sample endorsing at least moderate caregiver mental health symptoms and substantial caregiver history of childhood adversity. Within this low income, high adversity context we were able to evaluate several theoretically relevant mechanistic links between caregiver history of childhood adversity and infant and toddler stress physiology in an effort to identify and better understand how stress gets "under the skin" and persists across generations. The aims of the current study included the examination of psychological, physical/environmental, and biological pathways, as well as, the exploration of the effects of caregiver adversity on infant and toddler stress physiology above and beyond the influence of the child's own early life experience. The findings from this study have the potential to identify constellations of family-level strengths (e.g., positive caregiver mental health, caregiver warmth and responsivity) that promote resilience within this low income, high adversity sample.

Literature Review

Dysregulated stress physiology. Research with human and non-human animals emphasizes the hypothalamic-pituitary-adrenal (HPA) axis as a common pathway through which toxic stress (or chronic/severe stress experienced in childhood in the absence of protective adult relationships) gets "under the skin" to compromise well-being (Gunnar & Vazquez, 2006). In particular cortisol has been implicated as the primary physiologic biomarker for poor psychological and physical health outcomes. Cortisol is the primary downstream hormonal product of the HPA axis which plays an important role in a range of metabolic, stress-management, immunologic and restoration processes. Under normative conditions, the HPA axis response and cortisol levels specifically are best understood as adaptive regulatory pathways through which acute threats or challenges are managed (Sapolsky, Romero, & Munck, 2000). However, with chronic activation, systems for managing stress can become overused and this overuse can directly lead to increasing sensitivity to stress (Schuder, 2005), dampened immune system (Fan et al., 2009), and dysregulated cardiovascular function (Weber-Hamann et al., 2002). Ultimately chronic activation of the stress system can reduce both health-related quality of life and life expectancy (Brown, Varghese, & McEwen, 2004).

Economic disadvantage has been shown to place children at greater risk for chronic stress system activation, and ultimately greater dysregulation of this stress system, in the form of both heightened (Lupien, King, Meaney, & McEwen, 2001) and dampened (Badanes, Watamura, & Hankin, 2011; Lumeng et al., 2014) cortisol relative to their more

economically advantaged peers (see also Gunnar & Vazquez, 2001, for a review). Stress is thought to be most adaptively managed when the physiologic response is quick, efficient, appropriate in degree, and terminated as soon as the threat is contained. Therefore, both over-reactive and under-reactive profiles are thought to reflect a dysregulated system, to be less effective at handling challenges and to result in long term negative health consequences (McEwen & Stellar, 1993). Dysregulated cortisol response profiles have been observed for both diurnal and stress reactive values (Heim, Ehlert, & Hellhammer, 2000; De Kloet, Joëls, & Holsboer, 2005). For example, a study of homeless children's cortisol demonstrated that children with high levels of negative life events had higher levels of morning cortisol than those with fewer negative life events (Cutuli, Wiik, Herbers, Gunnar, and Masten, 2010). Similarly, in a Head Start sample a lower income-to-needs ratio was associated with higher stress reactive cortisol across a structured stress paradigm (Blair et al., 2005).

In the absence of acute stress, cortisol follows a circadian rhythm. Typical diurnal patterns are established in infancy and show a morning peak and evening nadir (Larson, White, Cochran, Donzella, & Gunnar, 1998; Price, Close, & Fielding, 1983; Watamura, Donzella, Kertes & Gunnar, 2004). However, chronic stress can disrupt the basal function of this system resulting in two types of dysregulated circadian profiles across the day: blunted awakening response with lower levels across the day or higher awakening response without typical decline (Cohen et al., 2006; Gunnar & Vasquez, 2001, Stone et al., 2001). Indeed, an attenuated pattern (flat awakening or very low across the day) has

been reported in 15-20% of children living in very high-risk conditions (Hankin et al., 2010, Badanes et al., 2011). However, these findings taken together with substantial individual differences in child stress physiology among children living at or near the poverty line suggest that economic disadvantage alone is not sufficient to index exposure to chronic stress and similarly, restricted economic resources alone do not explain how stress gets "under the skin". Therefore, the current project aimed to further elucidate factors involved in the mechanistic pathways leading to dysregulated stress physiology, by examining the influence of caregiver mental health, environmental instability, epigenetic modifications, and caregiver warmth and responsivity within a low-income, stress exposed sample.

This exploration is particularly relevant as extant literature suggests that early stress system dysregulation, if unmitigated, may set children on a trajectory for poor outcomes. Specifically, children with dysregulated physiology are at greater risk for poor physical, psychological, social, and academic outcomes than their peers with typical physiologic functioning (Alink, Lenneke, Cicchetti, Kim, & Rogosch, 2012; Hankin, Badanes, Abuela, & Watamura, 2010; Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010; Shirtcliff, Granger, Booth, & Johnson, 2005; Van Goozen, Matthys, Cohen–Kettenis, Buitelaar, & Van Engeland, 2000).

Caregiver history of childhood adversity. Findings from the Adverse Childhood Experiences (ACE) Study suggest that several adverse experiences in the first 18 years of life can have effects on health and physiology that can persist into adulthood. According

to this study and subsequent work using this instrument, physical or emotional abuse by a parent, sexual abuse, substance abuse, incarceration or mental illness of a household member, exposure to domestic violence, and emotional or physical neglect, are major risk factors for lifetime illness and early death (Felitti et al., 1998). In regard to physical health and health behaviors, higher ACE scores were associated with increased incidence of smoking (Anda et al., 1999), alcohol abuse (Anda et al., 2014), illicit drug use (Dube et al., 2003), and heart (Dong et al., 2004), liver (Dong, Dube, Felitti, Giles, & Anda, 2003) and lung disease (Anda et al., 2008). Moreover, adverse childhood experiences were also associated with lower rates of psychological well-being such as greater incidence of suicide attempts and developing affective disorders such as depression (Chapman et al., 2004; Edwards, Holden, Anda, & Felitti, 2003; Anda et al., 2014). This is particularly concerning as affective disorders themselves are associated with poorer interpersonal relationships, poorer job performance, and greater substance use (Broadhead, Blazer, George, & Tse, 1990; Kessler, et al. 2006). The ACE findings are particularly compelling as the study also identified links between history of adversity and disease in a low-risk sample receiving regular health care, indicating the effects of childhood adversity on lifelong health do not require exposure to risk that persists beyond early development. It is expected that this risk will be disproportionately higher for individuals facing additional stressors such as those living in poverty, with less education, and/or who are single parents (ACF, 2002; ACF, 2006). Indeed, the ACE study revealed that not only was child maltreatment indicative of lifelong health and well-being, but it

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conferred economic risk as well, with individuals experiencing more adversity in childhood reporting more absences from work, as well as serious financial and job problems (Anda et al., 2004). Taken together, there is strong evidence to suggest that adverse experiences early in life can disrupt positive, healthy developmental trajectories resulting in poor adult outcomes.

Intergenerational transmission of risk. The ACEs work highlights the persisting effects of early adversity on health, behavior, and well-being, factors which very likely provide a mechanistic link between caregiver early adversity and child development. Accordingly, there is a wealth of evidence supporting risk for the continuity of maltreatment across generations (Caspi & Elder, 1988; Neppl, Conger, Scaramella, & Ontai, 2009). For example, children exposed to harsh, rejecting, or aggressive parenting are more likely to exhibit a broad range of developmental problems including aggressive, antisocial, or delinquent behaviors (Conger, Neppl, Kim, & Scaramella, 2003; Dogan, Conger, Kim, & Masyn, 2007; Hops, Davis, Leve, & Sheeber, 2003; Norman et al., 2012). Moreover, these children are more likely to later adopt a similarly harsh parenting style toward their own children (Caspi & Elder, 1988; Neppl, Conger, Scaramella, & Ontai, 2009). This intergenerational transmission of risk, or 'cycle of abuse,' is largely assumed to be transmitted via psychosocial transmission such as modeling, impaired social skills, and limited social resources/support. Indeed, extant evidence indicates that the presence of a safe, stable, and supportive relationship during childhood (Herrenkohl, Klika, Brown, Herrenkohl, & Leeb, 2013) or later with an intimate partner (Conger,

Schofield, Neppl, & Merrick, 2013; Jaffee et al., 2013; Thornberry et al., 2013) serves a protective role effectively disrupting the continuity of maltreatment across generations. In particular, higher levels of maternal warmth and lower levels of hostility and intrusiveness were observed among mothers with a history of maltreatment who also reported higher levels of social support (either from an adult figure in childhood or a spouse in adulthood) (Jaffee et al., 2013). Despite these findings, mothers with a history of maltreatment who were high on maternal warmth were still more likely to report a lifetime history of depression and lower levels of social support overall as compared to mothers with no history of maltreatment (Jaffee et al., 2013). These findings suggest that maltreatment is a powerful predictor of child and adult outcomes; however, with the support of a caring adult, individuals with a history of maltreatment or adversity can disrupt intergenerational continuity in maltreatment.

Despite the robust literature on maltreatment, little research has specifically investigated the intergenerational impact of other adverse childhood experiences in the context of child stress physiology with regard to proximal mechanisms for transmission of risk. Therefore, it remains unclear what role caregiver history of adversity plays in shaping the developmental context experienced by the child. Assessing the contribution of caregiver history of adversity to child stress physiology, as examined in the current study, may be particularly informative as it has the potential to reveal hidden vulnerabilities in the child's environment as early as before birth, well before more traditional measures of risk can be administered. Although it may not be possible to

reverse the damaging effects of early life stress for the primary caregiver, if the primary caregiver's history is shown to negatively influence children's stress physiology, this information will be crucial for targeting interventions to be effective in disrupting intergenerational transmissions of risk that perpetuate poverty and poor physical and mental health.

Caregiver mental health. An estimated 17% of women with young children have elevated levels of depressive symptoms (McCue Horwitz, Briggs-Gowan, Storfer-Isser, & Carter, 2007). For low-income mothers these estimates are disproportionately higher, (Lanzi, Pascoe, Keltner, & Ramey, 1999) with over half (52%) of low-income mothers whose children are eligible to attend Early Head Start reporting clinical levels of depressive symptoms (ACF, 2006). Moreover, current and lifetime rates of depression are higher for individuals who report higher rates of adverse childhood experiences (for a review see Chapman, Dube, & Anda, 2007).

The relationship between maternal depression and problematic child emotional and behavioral outcomes has been widely established (Goodman et al., 2011). Indeed, infants of mother's exhibiting higher depressive symptoms display impaired/delayed cognitive and motor development, indicating the effects of maternal psychosocial well-being on child development are apparent very early in her child's life (Cogill, Caplan, Alexandra, Robson, & Kumar, 1986; Petterson & Albers, 2001). Further, maternal depressive symptoms are likely to persist into the preschool period and children who have been exposed to chronic maternal depression are more likely to exhibit poorer school performance (Elgar, McGrath,

Waschbusch, Stewart, & Curtis, 2004) and more internalizing and externalizing symptoms in early childhood (Goodman et al., 2011; Horwitz, Briggs-Gowan, Storfer-Isser, & Carter, 2009; Trapolini, McMahon, & Ungerer, 2007).

The impact of maternal depression on child outcomes is theorized to operate in part through disrupted caregiving behaviors, wherein the mother may physically withdraw from the caregiving context resulting in lower rates of engagement and interaction with their child and/or display low or flat affect and increased hostility and negativity (Strand & Wahler, 1996; Downey & Coyne, 1990). However, other processes are also plausible – for example, maternal dysregulated stress physiology is the most reliable biological marker of current depression (Burke, Davis, Otte, & Mohr, 2005; Parker, Schatzberg, & Lyons, 2003). This dysregulation resolves with appropriate treatment (Nylen, Moran, Franklin, & O'hara, 2006), suggesting an important protective role of stress management in reducing the occurrence and transmission of risk factors.

Compromised mental health, economic strain, and higher perceived life stress have all been associated with lower quality parenting behaviors (Newland, Crnic, Cox, & Mills-Koonce, 2013; Webster-Stratton & Hammond, 1988). This is concerning as lower rates of maternal sensitivity and responsivity have also been associated with greater HPA-axis reactivity in infants, young children, and in adulthood (Dawson & Ashman, 2000; Kalinichev et al., 2002). For example, elevations in cortisol were found for infants who were cared for by less interactive and responsive caregivers (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992). Evidence from the animal literature also highlights changes to

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the HPA-axis for offspring who experienced less attentive caregiving or frequent maternal separation (Daniels, Pieterson, Carstens, & Stein, 2004; Sanchez, 2006). Together these findings suggest that exposure to maternal depression, separately or in combination with other risk factors, particularly early in life may place young children at greater physiologic risk and warrants investigation of maternal depression as a factor linking maternal history of childhood adversity to dysregulated child stress physiology and furthermore whether this relationship is further explained by the impact of caregiver mental health on caregiver warmth and responsivity.

Environmental instability. In addition to higher rates of maternal depression, low-income families are also more likely to experience household instability (e.g. poor housing quality, overcrowding, unstable neighborhoods, or frequent relocations) (U.S. Census Bureau, 2009; Joint Center on Housing, 2009). This type of environmental instability may exacerbate behavioral problems in children. Indeed, in previous analyses with data from children included here, I found that household instability moderated the association between maternal depressive symptoms and child internalizing behaviors, such that children exhibited increased internalizing symptoms as maternal depressive symptoms increased in families with high (but not low) household instability (McFadyen-Ketchum, Mendoza, & Watamura, 2015). Additionally, household instability has been associated with greater socio-emotional, physical and academic problems in children (Rafferty, 1991).

The impact of environmental instability on child outcomes is theorized to operate in part through disruptions in the family's social and support networks and through changes

in children's routines leading to greater emotional distress for parents and children (Suglia, Duarte, & Sandel, 2011). As novel and uncontrollable situations have been consistently demonstrated as robust activators of the stress response system (Dickerson & Kemeney, 2004; Larson, Gunnar, & Hertsgaard, 1991), this may offer one explanation for elevated cortisol levels found in families experiencing crowding, noise, and low-housing quality (Evans, 2003). Importantly, chronic exposure to these environmental risk factors, particularly in the absence of a supportive caregiver, may have a cumulative effect on HPA-axis functioning (Evans & Kim, 2007), further highlighting the need to understand how environmental stability contributes to intergenerational transmission of risk.

Epigenetic modification. Recent advances in molecular biology have identified processes through which social experience can introduce environmentally responsive yet stable alterations in gene activity. The processes of altering gene activity without changing the underlying DNA sequence are collectively known as epigenetics. In particular, these processes can serve to either enhance or reduce accessibility to the DNA, and ultimately transcriptional processes, and occur via histone protein modification or DNA methylation (Feng et al, 2007; Razin, 1998). Of the two processes, DNA methylation is considered to be the more stable and enduring modification because DNA methylation patterns are inherited by daughter cells during cell division (Fukuda & Taga, 2005). It is through this process that early methylation patterns are able to persist across development and can be transmitted from mother to offspring.

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Variations in early maternal care have been shown to alter DNA methylation. Pioneering work from Meaney and colleagues (1993), established links in postnatal maternal care (i.e., high licking and grooming dams), altered DNA methylation, and long-term changes in behavior in rodent offspring. In particular, methylation of the glucocorticoid receptor (GR) gene, the gene responsible for production and maintenance of receptors responsive to cortisol, was implicated in high levels of HPA axis stress reactivity observed among offspring who received lower levels of maternal care both naturally (Francis, Champagne, & Meaney, 2000) and through experimental manipulations designed to elicit high levels of maternal stress (Plotsky & Meaney, 1993). Importantly, follow-up studies utilizing cross-fostering (i.e., placing offspring of low licking and grooming dams in the care of high licking and grooming dams and vice versa) to distinguish the influence of behavioral exposure from genetic influence suggest a direct relationship between maternal care and subsequent development of individual differences in behavioral and HPA axis responses to stress (Francis, Diorio, Liu, & Meaney, 1999; Weaver et al., 2004). Further analysis and replication with this same cross-fostering model have demonstrated that HPA-axis changes and associated epigenetic profiles that emerge early in the life of the offspring are sustained into adulthood (Weaver et al., 2004).

Recent work has found similar epigenetic effects of early maternal care and social experience on the *human* glucocorticoid receptor gene (NR3C1 1F promotor region) (human homologue to rat GR 1_7 promoter region). For example, increased methylation of

the NR3C1 promoter region was observed in the postmortem hippocampus of suicide victims with a history of childhood abuse (McGowan et al., 2009), adolescents with a substantiated case of physical maltreatment (Romens, McDonald, Svaren, & Pollack, 2014), adolescents of mothers with a history of intimate partner violence (Radtke et al., 2011), and in newborns exposed to elevated levels of prenatal stress (Mulligan, D'Errico, Stees, & Hughes, 2012; Ostlund et al., 2016) and depression (Oberlander et al., 2008). Importantly, these epigenetic effects were observed for the infants only (not mothers) and were associated with higher cortisol reactivity at 3 months (Oberlander et al., 2008). Taken together these findings suggest that methylation of the GR gene may represent a key mechanism through which early experience alters the HPA-axis and disrupts stress physiology. As this is an important emerging area of research, the current study examined all 13 possible methylation regions (i.e., CpG sites) of the 1F exon and promoter of the human GR gene (NR3C1) previously identified by several relevant studies (Mulligan et al., 2012; Oberlander, 2008; Romens et al., 2014).

Caregiver warmth and sensitivity. As described above, extant literature suggests maternal behaviors may play an important role in determining child outcomes. In particular, warm and sensitive caregiving were found to disrupt the intergenerational continuity of maltreatment (Caspi & Elder, 1988; Neppl, Conger, Scaramella, & Ontai, 2009) and epigenetically program stress systems to effectively manage threat with fewer consequences to long term health and well-being (Francis, Diorio, Liu, & Meaney, 1999; Weaver et al., 2004). Moreover, parenting behaviors in the context of poverty, including

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maternal sensitivity (Farrell, Simpson, Carlson, Englund, & Sung, 2017; Van der Voort, Juffer, & Bakermans-Kranenburg, 2014) and intrusiveness (Cooper-Vince, Pincus, & Comer, 2014) are highly predictive of child outcomes (Conger et al., 1992). As parenting behaviors are heavily influenced by depression and environmental risk (Lovejoy, Graczyk, O'Hare, & Neuman, G, 2000; McLoyd & Wilson, 1991), the examination of multiple methods of transmission is critical in understanding the complex relationships among proximal risk factors commonly experienced by low-income families.

Maternal sensitivity has been shown to support the child's health, well-being, growth and learning, in part by signaling to the child that an adult is available and dedicated to buffering them from external threats. Evidence from the animal literature suggests that this caregiving role is particularly relevant early in life when the stress physiology of the offspring is developing (Liu et al., 1997) and that in the absence of regular, high quality caregiving pervasive changes in the HPA-axis can be observed that persist into adulthood (Kalinichev et al., 2002). These findings highlight a potentially promising point for intervention; in that warm and responsive caregiving may moderate the relationship between caregiver history of adversity and child outcomes, wherein poor outcomes are not observed for children of caregivers with a high history of adversity who also demonstrate high levels of warm and responsive caregiving (e.g., buffering).

As such, recent animal and human research on caregiver warmth and sensitivity have motivated a wave of recent interventions targeting parenting behaviors in low income families (i.e., children broadly at risk). In particular, these interventions assume a

mediation pathway through which parents' history of maltreatment is transmitted to the child via parenting behaviors. However well intended, these intervention studies fail to acknowledge that though statistically more likely to demonstrate 'negative' parenting behaviors, many low income parents and/or parents with a history of maltreatment do indeed demonstrate warm and responsive parenting behaviors (without/before intervention). Therefore, a thorough exploration of transmission of risk warrants an examination of the potential interaction of caregiver history of adversity and caregiver warmth and responsivity on child outcomes. In particular, an in-depth investigation of parenting behaviors as both a potential mediator as well as a moderator of the relationship between caregiver history of childhood adversity and dysregulated child stress physiology is warranted.

Promisingly, abundant research has also shown that parenting behaviors are modifiable (for a review see Bakermans-Kranenburg, van IJzendoorn, & Juffer, 2003) and that increases in maternal sensitivity have been shown to normalize children's physiological stress functioning suggesting flexibility in the system (Fisher & Stoolmiller, 2008; Martin, Kim, & Fisher, 2016). Taken together, this suggests that high rates of responsiveness and sensitivity from caregivers, even in families facing multiple adversities, may buffer the influence of environmental stressors on child stress physiology, ultimately promoting a normative (adaptive) stress physiology.

Chapter Two: Current Study

The current study tested four potential proximal and modifiable pathways through which caregiver history of childhood adversity could lead to current dysregulated infant and toddler stress physiology. This study is an important first step toward understanding the best factors to target in order to prevent caregiver history of adversity from limiting children's optimal health and wellbeing (see Figure 1). To reflect current theoretical perspectives and empirical data, I explored one psychological (maternal mental health), one physical/environmental (household instability), and one biological (epigenetic) pathway that may mediate the effects of caregiver history of childhood adversity on infant and toddler stress physiology. Current working models also suggest that the critical buffer against long term maladaptive outcomes in the face of early life stress is effective buffering by adult caregivers. Therefore, I also tested the role of caregiver warmth and responsivity as both a potential mediator and moderator of the relationship between caregiver history of childhood adversity and infant and toddler stress physiology.

Aim 1. Examine whether caregiver history of childhood adversity predicts infant and toddler stress physiology. Dysregulated stress physiology is predictive of poor current and later psychological and physical health. For Aim 1, I assessed whether caregiver's history of childhood adversity predicted infant and toddler stress physiology above and beyond the child's own lifetime experience of early life stress. I hypothesized

dysregulated stress hormone profiles for infants and toddlers of caregivers with greater history of adversity early in life.

Aim 2. Determine whether current caregiver mental health and/or environmental instability mediate the relationship between caregiver history of childhood adversity and infant and toddler stress physiology. Within a sample of low-income families, I assessed the influence of caregiver history of childhood adversity in predicting infant and toddler stress physiology via current caregiver mental health and/or household instability. Extant animal and human literature has demonstrated that early disruptions in caregiving can result in greater internalizing symptoms and more dysregulated stress physiology in children. Therefore, I expected caregivers who endorse higher levels of anxious and depressive symptoms and report more household instability would be less resourced and less available. These proximal mechanisms were explored as pathways through which the effects of caregiver history of childhood adversity persist into the next generation to affect child health and well-being.

Aim 3. Explore epigenetic modifications as a potential mechanism of biological embedding of stress across generations and assess whether caregiver warmth and responsivity moderates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology to further explain the mediation of epigenetic modifications. A wealth of animal studies and a few recent human studies demonstrate that epigenetic modifications, such as glucocorticoid receptor gene (NR3C1) methylation, are responsive to environmental stimuli. Furthermore, they suggest that early experience may 'set' the system and determine how an individual's physiology will

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operate across their lifetime. DNA was collected from a subsample of infants and toddlers (n=59), these data were used to explore whether caregiver history of childhood adversity operates in part via NR3C1 methylation profiles to predict infant and toddler physiology. I predicted higher glucocorticoid receptor methylation and more dysregulated stress physiology for infants and toddlers of caregivers with greater history of childhood adversity after controlling for the infant and toddlers' own lifetime experience of early life stress.

Aim 4. Investigate whether caregiver warmth and responsivity moderates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology. Extant literature demonstrates that consistent and sensitive care from a supportive adult can buffer children from the deleterious effects of early life stress. In the current study, I examined whether high caregiver warmth and responsivity changed the relationship between caregiver history of adversity and infant and toddler outcomes.

Aim 5. Explore a full model in which caregiver warmth and responsivity mediates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology, and furthermore, if the relationship between caregiver history of childhood adversity and caregiver warmth and responsivity is further explained by caregiver mental health and/or environmental instability. This aim seeks to understand whether caregiver warmth and responsivity is best understood as a mediator (or moderator; see Aim 4), as well as fully incorporate the variables of interest presented above in a full statistical model to further elucidate intergenerational transmission of adversity and highlight points of intervention.

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Chapter Three: Method

Participants

      Participants included the first 3 cohorts (n=167 children and their families) of a larger longitudinal intervention study. However, the current analyses include only data from the first two research visits before families were randomized to an intervention or control group. Of the 167 recruited in the first 3 cohorts, 10 families were lost before the first screening visit, and another 23 families were unable or unwilling to provide saliva samples. The resulting sample included 134 children attending programs receiving Early Head Start funding, 24 of whom were part of sibling pairs. Children ranged in age from 5-44 months (*M*=24.29, *SD*= 9.89, Median= 25.5) and 42% were female. Primary caregivers (*N*=125) ranged in age from 18-49 years (*M*=31.04; *SD*=6.50). The majority of primary caregivers were mothers (98%) and of Latino origin (65%). Of Latino caregivers, 52% chose to be interviewed in Spanish and 52% were immigrants who identified Mexico as their country of origin. Approximately half of caregivers who did not identify as Latino identified as minority race (17.1%): African American/Black (13.7%), Asian (1.8%), American/Native Indian/Alaska Native (0.8%), and Other Pacific Islander (0.8%), and the remaining 17.9% identified as White/Caucasian and not Latino. The mean after tax income for all families was $22,481 (*SD*=$15,613) and mean income-to-needs ratio was 0.89. Table 1 includes demographic information for the overall sample of caregivers and children. The current sample of families who completed the screening visit

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and provided saliva samples did not differ from the larger recruited sample on age, sex, or caregiver history of adversity, but did have a higher income than families who were not retained through saliva sampling. Part way through the larger study, the decision was made to collect DNA samples to assess telomere length and DNA methylation. DNA was successfully collected from a subsample of 59 children. This subsample did not differ from the current (or larger) sample on child age, sex, household income, or caregiver history of adversity, however, children with DNA samples had older caregivers on average (*M*=32.6, *SD*=6.1) as compared to caregivers who we were unable to collect samples (*M*=29.5, *SD*=6.4); *t*(132)=-2.84, *p*<.01.

Measures

Caregiver history of childhood adversity. Primary caregivers completed the Adverse Childhood Experiences (ACE) questionnaire, which covers a broad range of possible adverse childhood experiences including psychological, physical, and sexual abuse, and household dysfunction in a variety of categories including mental illness, substance abuse, domestic violence, and criminal involvement (Felitti et al., 1998). The ACE questionnaire was developed and utilized in a large-scale health assessment conducted at Kaiser Permanente in collaboration with the Center for Disease Control (Center for Disease Control, 2013; Felitti et al., 1998). The ACE questionnaire has been successfully administered among large ethnically diverse samples (Schilling, Aseltine, & Gore, 2007). Two summary variables, a total ACE score (range: 0-10, 1 point for each of 10 categories of adverse childhood experiences) and a dichotomous ACE score

of 6 or more vs. 5 or fewer ACES (because 6 or more was associated with 20-year reduction in lifespan in the original work) were used for the current analyses.

Infant and toddler physiology. *Diurnal Cortisol Profiles.* Caregivers were asked to collect salivary cortisol ten times across two days (wake, wake +15m, wake +60m, noon, and bedtime) and record the sample times in a sampling diary. All salivary samples were collected using synthetic Salivettes (Sarsdedt, Nümbrecht, Germany) or infant synthetic saliva swabs (Salimetrics, State College, PA) and extracted by centrifuging for 4 min at 2,500 rpm. The vials were frozen at $-20^{o}C$ until data collection was complete. The samples were then sent to the Biochemical Laboratory, Psychobiology, University of Trier, Germany to be assayed. Samples were assayed in duplicate using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection and then averaged (Höferl, Krist, & Buchbauer, 2005).

Cortisol values were averaged within each time point. Variables explored in relationship to caregiver history of adversity were each individual diurnal sample point (e.g., wake, wake +15, wake +60, noon, and bedtime), the resulting diurnal slope, area under the curve with respect to ground (AUC_G) for the cortisol awakening response, and area under the curve with respect to ground (AUC_G) as an average of two days (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Dysregulated diurnal values (very low or heightened relative to the sample) were used to reflect disruption of the HPA-axis.

Stress Reactive Cortisol. Trained research assistants collected salivary cortisol eight times across a structured stress paradigm presented to the infant and toddler and his

or her caregiver. Samples were collected at arrival to the home (0 min), just before a 7-min challenge (12min baseline), at the conclusion of the challenge (18min), 26-min following arrival (26min reactivity to arrival), 20-min following challenge baseline (32min reactivity 1), 20-min following conclusion of challenge (39min reactivity 2), 22-min after reactivity 2 (61min recovery 1), and 19-min after recovery (75min recovery 2). Trained research staff collected the samples and recorded the times and dates of the stress reactivity samples. Variables explored for stress reactive cortisol include the maximum value, minimum value, and difference between those values for each saliva sample collected for the child across the stress paradigm. Additionally, the area under the curve with respect to ground (AUC_G) was calculated to determine stress reactivity across the home visit (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). AUC_G values that diverge from the average and/or that represent a maladaptive response pattern (e.g., failure to mount a response or failure to recover from a response) were used to reflect disruption of the HPA-axis.

Using methods developed in previous studies we asked parents to report any current medications or ongoing illnesses their infant and toddler had, and their infant and toddler's use of steroid medication or chronic conditions that impact (or are impacted by) cortisol levels (e.g., asthma; Moore et al., 1985). Additionally, on sampling days, parents were asked to report on the presence of other factors that influence cortisol values (e.g., food/caffeine intake, wake/sleep times, use of medication and presence of illness symptoms) and this information was recorded on the sampling sheet. This allowed for exclusion of individual samples or sampling days where samples may be contaminated.

In the final sample of 2,546 total saliva samples, 7 samples were deleted or adjusted. Three individual samples were deleted for contamination and 4 individual samples considered reliable and physiologically possible were winsorized to 3 standard deviations above the mean to avoid skew but retain rank-ordered level. Neither diurnal nor stress reactive salivary samples were collected if the infant and toddler had a fever or presented with other health conditions, medication, or illness symptoms. If needed, sampling was rescheduled to accommodate these constraints to a time when the child was not ill or family schedules were not atypical.

   Stress paradigm. Because the theoretically most concerning stress profile based on work with adults is one in which the infant and toddler is physiologically unresponsive to stress, it was important to provide a sufficient challenge to elicit behavioral distress. Eliciting distress in the current sample is crucial to identifying children who are in need of intervention because of an unresponsive stress system. Note that in previous work, as many as 18% of four year olds did not mount an appropriate physiologic response to a similar but shorter and milder task, and this was connected to current infant and toddler well-being (Badanes et al., 2011; Hankin et al., 2010). Therefore, in the current study, we added several additional challenges to our previously successful protocol, adapting established procedures such as the NICHD Early Child Care Research Network's (1998) three bags task and Lab-TAB (Gagne, Van Hulle, Aksan, Essex, & Goldsmith, 2011) to design a structured stress paradigm that would mimic normative challenges and more reliably elicit a moderate stress response from most children in the relatively large target age range (6 months through 3 years). Children were asked to participate in this seven

minute semi-structured moderate stress paradigm with the support of their primary caregiver at home. During the interaction, the dyad was presented with arm restraint, restricted access to an attractive toy, face washing, a scary mask, a scary robot, a loud alarm, and finally the caregiver waving goodbye and exiting the infant and toddler's field of view. Caregivers were asked to maintain neutral affect while children were presented with these challenges. Stressors continued in a prespecified order, increasing in intensity until or unless the infant and toddler became distressed for 30 seconds. If the infant and toddler reached distress levels, the researchers discontinued the protocol. Caregivers were carefully prepared for the experience, provided visual prompts to remind them of the next stressor, and encouraged to discontinue at any point if they felt they or their child was too distressed.

Caregiver mental health. The *Center for Epidemiologic Studies-Depression Scale* (CES-D; Radloff, 1977) was used to assess current depressive symptomology in the caregiver. The CES-D consists of 20 common affective symptoms and asks individuals to self-report on his or her experience with the listed symptoms over the past week ranging from 0 (*rarely*) to 3 (*most or all the time*). Higher scores reflect the presence of more depressive symptoms. A score higher than 15 is expected to reflect clinical levels of depression. A total symptom score was used in the current study analyses. The CES-D has proved reliable in community samples (Radloff, 1977) and with Mexican American mothers of young children (α = .90-.93; Beeber et al., 2010). The Spanish translation of the CES-D showed adequate reliability when used with a similar Head Start sample of families by our team (α = .91) and within the current sample (α=.89). The *Generalized Anxiety*

Disorder Scale (GAD-7; Löwe et al., 2008) was used to assess generalized anxiety symptomology in the primary caregiver. The GAD-7 consists of seven items that reflect core symptoms of generalized anxiety disorder and asks individuals to self-report on his or her experience with the listed symptoms over the past two weeks ranging from 0 (*not at all*) to 3 (*nearly every day*). Higher scores reflect the presence of more anxiety-related symptoms. Scores of 5, 10, and 15, represent mild, moderate, and severe anxiety symptom levels, respectively. The GAD-7 has demonstrated good reliability and validity among ethnically diverse adults (Spitzer, Kroenke, Williams & Lowe, 2006). The GAD-7 had good reliability within the current study (α=.88). As the measures of mental health symptoms were highly correlated (r =.51, p<.001) and extant literature demonstrates that depression and anxiety are highly comorbid conditions (Cummings, Caporino, & Kendall, 2014) and perhaps especially so when measured with distress symptoms scales like the CES-D and the GAD-7, a total symptom score of anxious and depressive symptoms was used here to examine caregiver mental health.

Environmental instability. To assess the stability of families' physical environment, a broad array of potential indicators of environmental instability were indexed from three different parent-report questionnaires encompassing crowding, perceived crowding, frequent relocations, and changes in household membership, loss/gain of guardian, stability of income, emergency repairs/heating or plumbing issues, food insecurity, transportation, and family conflict were recoded into 0 (*no risk*) or 1 (*risk*) then summed (see Measures: Figure A). Total scores for environmental instability ranged from 0 to 20 with higher scores reflecting greater environmental instability.

Epigenetic modifications. Epigenetic modifications of the HPA-axis were assessed via methylation status of the human glucocorticoid receptor gene (NR3C1, Exon 1F). During a home visit, trained research assistants collected a single, 2 mL saliva sample from each infant and toddler using Oragene kits (DNAgenotek). Samples were collected 30-60 minutes after the research team arrived at the home to ensure that infant and toddler had not consumed food or drink prior to sampling. After collection, samples were immediately mixed 1:1 with the stabilizing solution provided in the Oragene kit and stored at room temperature until data collection was complete. DNA from the samples was extracted and purified according to the manufacturer's instructions using prepIT® MAX (DNAgenotek, Ottawa, ON, Canada). Use of these DNA collection, extraction, and purification steps have been shown to yield high quantity (median= 17.3μg) and quality genomic DNA (Niles, Rabuka, & Iwasiow, 2010). Extracted, purified samples were diluted to 20ng/μl and 50μl of DNA per participant (1μg total) were loaded onto a 96-well plate. Plates were shipped to EpigenDx, Inc. (Hopkinton, MA) who then performed bisulfite modification, PCR amplification, and targeted bisulfite pyrosequencing across 15 CpG sites (Assay ID: ADS749-FS, Human NR3C1 (GCR) Exon 1F, Location -3260 to -3201 from ATG, 7 CpG sites; ADS2386-FS, Human NR3C1 (GCR) Promoter, Location -3181 to -3125 from ATG, 8 CpG sites). The total percent methylation and percent methylation at each CpG sites were used in analyses (Oberlander et al., 2008).

Chronic caregiver warmth and sensitivity. A composite of chronic, or ongoing, warmth and sensitivity was constructed using two questions from the Windshield Survey (Biederman and Cole, 1992) *family's preparations for session and organization of session*

ranging from 0 (*surprise/difficulty*) to 3 (*good hosts*) and *primary respondent's receptivity towards visitors* ranging from 0 (*very uncomfortable*) to 3 (*very warm*). Additionally, two subscales of the *Home Observation for Measurement of the Environment Infant/Toddler Version* (HOME-IT; Caldwell & Bradley, 2003) were used. The Parental Responsivity subscale, which examines a caregiver's verbal and emotional responsiveness to his or her infant and toddler, was used as a measure of a caregiver sensitivity. This 11-item subscale is comprised of 10 observations and 1 interview question. Example items include: *parent caresses or kisses infant and toddler at least once* and *parent spontaneously vocalizes to infant and toddler at least twice.* The Acceptance of Child subscale examines caregiver's tolerance of suboptimal behavior and restraint from employing restriction or punishment. This 8-item subscale is comprised of 5 observations, 2 environmental assessments, and 1 interview question. Example items include: *parent does not express overt annoyance with or hostility to infant and toddler* and *parent does not interfere with or restrict infant and toddler more than 3 times during visit.* The Acceptance of Child subscale demonstrated good reliability overall in the current study ($\alpha = .75$). Finally, the 12-item Parent-Child Dysfunctional Interaction subscale of the *Parenting Stress Index- Short Form* (PSI-SF; Abidin, 1995), a self-report that assesses the caregiver's perceptions of their relationship quality often in comparison to their relationship expectations was reverse coded and summed. Example items include: *this child is not able to do as much as I expected* and *I expected to have closer and warmer feelings for this child than I do and this bothers me* with the scale ranging from 1 (*strongly agree*) to 5 (*strongly disagree*). Higher scores indicated a better caregiver-child relationship. The English PSI-SF parent-child

dysfunctional interaction subscale has demonstrated adequate internal consistency among low-income samples (α = .76; Whiteside-Mansell et al., 2007). Prior research with Latino immigrant mothers demonstrated the Spanish-PSI to have strong internal consistency and discriminant validity (α =.88 - .94; Solis & Abidin, 1991). In the current study, the parent-child dysfunctional interaction subscale demonstrated good reliability overall (α = .77). The composite was then formed by summing the *z*-scores of each subscale. Higher overall scores indicate greater chronic caregiver warmth and responsivity.

Acute caregiver warmth and sensitivity. A composite of acute warmth and sensitivity was created by summing scores for caregiver sensitivity, positivity, animation, and stimulation during a 10 minute, filmed caregiver-child semi-structured interaction which occurred prior to the structured stress paradigm. The "3-bag" task and scoring of the filmed interactions have been previously established and the coded task was available for a subsample of our participants (n=76) (Mills-Koonce, 2013, NICHD SECCYD, 1999, Gagne, 2011). This measure demonstrated good reliability overall in the current study (α = .86). Higher overall scores indicate greater acute caregiver warmth and responsivity. Chronic and acute variables were highly correlated (r= .51, *p*<.001), but were examined separately in analyses.

Control variables. Caregivers were asked to report on his or her infant and toddler's demographic characteristics. In all models, the necessity to include child's sex, age in months and the caregiver's age in years, race using NIH categories, ethnicity (Latino or non-Latino), and nativity (US or foreign born) as covariates was examined. Finally, the Life Events and Circumstances Checklist (LEC; Work, Cowen, Parker, &

Wyman, 1990), a measure that asks caregivers to report on a range of stressful experiences that commonly occur among low income populations, was used to capture any stressful experiences in the lifetime of the target child.

Procedure

Participants were recruited by a bilingual research team in person during EHS drop off and pick up, at family events, and via the child's family educator for families in home-based programs. All data used in the current study was collected in the family's home by a team of at least two bilingual data collectors across two 2-hour home visits that occurred within 2-6 weeks of each other (M= 7.56, SD= 8.08, Median = 4.86). At the first home visit, caregivers reported on their history of adversity, current mental health and psychosocial characteristics and the physical environment by completing in-home interviews with research staff. Interview format was employed due to varying literacy and education levels of our participants and conducted in either Spanish or English depending on the caregiver's preference and with pictorial aids. Most Spanish questionnaires were taken from previously validated translations, however a few study specific measures were translated and back-translated by bicultural/bilingual members of our team using standardized procedures. Responses were collected in a secure computerized program and periodically downloaded and checked. Caregivers were taught to collect saliva samples and left with supplies and instructions to collect diurnal salivary samples from both themselves and their child on scheduled typical days at home. At the second visit, the caregiver and child participated in a semi-structured play interaction followed by the structured stress paradigm. During the visit, stress reactive salivary

samples were collected from caregiver and child by research staff. For the subset of

families with DNA samples, one additional saliva sample was collected for DNA

analysis. Following these two home visits, trained research staff rated caregiver's warmth

and responsivity toward the child (based on ~4hr in-home observation). Participants were

given $150 for completion of the first two home visits of the study.

Chapter Four: Results

Analytic Approach

The current study assessed whether caregiver's history of adversity predicted infant and toddler's diurnal and stress reactive physiology, and if so, whether this potential relationship (if any) was mediated by more proximal factors such as caregiver mental health and/or current environmental instability, and epigenetic modifications of the human glucocorticoid receptor gene. In addition, this study examined whether caregiver warmth and responsivity moderated the relationship between caregiver history of adversity and infant and toddler diurnal and stress reactive physiology (Figure 1).

Preliminary analyses. *Individual factors.* Independent sample t-tests and correlations were used to assess whether child sex, age, and early life stress were related to any of the key variables. Infant and toddler sex was not related to any key variables. Infant and toddler age was negatively related to infant and toddler diurnal wake value, $r= -.26$, $p< .01$. Child early life stress was positively related to infant and toddler total percent methylation of NR3C1, $r= .41$, $p<.01$, and to infant and toddler diurnal bedtime cortisol values, $r= .25$, $p<.05$. Child early life stress was controlled for in all analyses including infant and toddler outcomes.

Demographic factors. At the family level, income-to-needs ratio, as well as caregiver race, ethnicity, and nativity were examined for relationships to the key outcome

variables. Income-to-needs ratio was negatively related to environmental instability, $r = -.27$, $p<.01$, and positively related to chronic warmth and responsivity, $r = .35$, $p<.001$, and to acute warmth and responsivity, $r = .23$, $p=.05$, and was subsequently controlled for in all analyses. There was an effect of caregiver race (White/Caucasian race, minority race, biracial/multiracial race) on caregiver history of adversity, $F(2, 122) = 4.86$, $p = .01$. Post hoc comparisons using the Tukey HSD test indicated that the mean ACE score for the White/Caucasian group ($M= 2.19$, $SD =2.00$) was lower than for the minority race group ($M=3.29$, $SD=2.52$). However, the smaller biracial or multiracial group ($M= 4.30$, $SD=2.87$) did not significantly differ from the White/Caucasian or minority race group. Significant group differences were found for researcher-rated chronic caregiver warmth and responsivity according to race, $F(2, 122) = 5.34$, $p = .01$. Post hoc comparisons using the Tukey HSD test indicated that the mean chronic warmth and responsivity score for the White/Caucasian group ($M= 0.39$, $SD =2.73$) was higher than for the minority race group ($M= -1.77$, $SD=3.82$). However, the biracial or multiracial group ($M= 0.72$, $SD=2.71$) did not significantly differ from the White/ Caucasian or the minority race group. There was a difference in caregiver history of adversity by ethnicity, $t(123)=2.51$, $p=.01$, such that Latino participants reported lower ACE scores ($M= 2.24$, $SD= 2.07$) than did non-Latino participants ($M= 3.35$, $SD= 2.55$). Finally, there was an effect of caregiver ethnicity/nativity (Latino foreign born, Latino U.S. born, non-Latino U.S. born) on caregiver history of adversity, $F(2, 122) = 10.58$, $p = .00$. Post hoc comparisons using the Tukey HSD test indicated that the mean score for Latino immigrant group ($M= 1.75$, $SD =1.63$) was lower than the US born Latino group ($M=3.29$, $SD=2.45$) and the US born

non-Latino group (M= 3.57, SD= 2.59). Identified relationships were controlled for in subsequent analyses.

Descriptive statistics for key study variables for both infants and toddlers and caregivers are displayed in Tables 1 and 2. Additionally, bivariate correlations between key study variables can be found in Table 3.

Aim 1. Examine whether caregiver history of childhood adversity predicts infant and toddler stress physiology. Linear regressions were run to explore whether caregiver history of adversity assessed as a continuous linear measure (e.g., total ACE score) predicted infant and toddler diurnal and stress reactive cortisol controlling for income-to-needs ratio, caregiver race, ethnicity, nativity, and child early life stress. No relationships were found. Next, a one-way ANOVA was used, and estimated marginal means were examined, to assess whether caregiver history of adversity differed by the number of adversities experienced 0-1 (*low adversity*), 2-3 (*mid-low adversity*), 4-5 (*mid-high adversity*) and 6 or more (*high adversity*) on key study variables (Table 4). Caregivers who experienced high adversity (ACE score of 6 or more) significantly differed from the other three groups on both mental health symptoms and environmental instability (see Table 4). These differences are depicted in Figures 2-4. As a result, caregiver history of adversity was dichotomized as high vs. lower adversity (ACE score 6 or more =1 and 0-5 = 0) and this variable was used in subsequently analyses. Linear regressions were then rerun to explore whether caregiver history of adversity (e.g., ACE 6 or more) predicted infant and toddler diurnal and stress reactive physiology controlling for income-to-needs ratio, caregiver race, ethnicity, nativity, and child early life stress.

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Higher caregiver history of adversity (e.g., ACE 6 or more) was related to higher infant and toddler noon, $\beta = .28$, $t(77) = 2.45$, $p < .05$, and bedtime cortisol values, $\beta = .34$, $t(80) = 2.81$, $p < .01$, explaining a significant proportion of the variance in infant and toddler noon, $R^2 = .19$, $F(5, 77) = 4.27$, $p < .01$ and bedtime cortisol values, $R^2 = .11$, $F(5, 80) = 2.73$, $p < .05$ (see Figure 5). No relationship was found between caregiver history of adversity and infant and toddler stress reactive cortisol (see Table 5 and Figure 6). Higher caregiver history of adversity was also related to higher caregiver mental health symptoms, $\beta = .48$, $t(119) = 5.50$, $p < .001$, and greater environmental instability, $\beta = .32$, $t(119) = 4.49$, $p < .01$. Caregiver history of adversity was not related to caregiver warmth and responsivity (chronic or acute measures).

Aim 2. Determine whether current caregiver mental health and/or environmental instability mediate the relationship between caregiver history of childhood adversity and infant and toddler stress physiology. *Caregiver Mental Health.* Regression analyses were used to investigate the hypothesis that caregiver mental health mediates the relationship between caregiver history of adversity and infant and toddler diurnal and stress reactive cortisol. Based on the results from Aim 1, only noon and bedtime cortisol were modeled, because all other infant and toddler cortisol indices were not related to caregiver history of adversity. Results indicated that caregiver history of adversity was a significant predictor of caregiver mental health as noted above and caregiver mental health was a significant predictor of infant and toddler noon, $\beta = .30$, $t(83) = 2.70$, $p < .01$ and bedtime cortisol values, $\beta = .38$, $t(86) = 3.31$, $p < .01$. Caregiver history of adversity remained a significant predictor of infant and toddler noon and

bedtime cortisol values after controlling for the mediator, caregiver mental health, consistent with partial mediation (see Figures 7-8). *Environmental Instability.* Regression analyses were used to investigate the hypothesis that environmental instability mediates the relationship between caregiver history of adversity and infant and toddler diurnal and stress reactive cortisol. Results indicated that caregiver history of childhood adversity was a significant predictor of environmental instability as noted above and of noon cortisol values, $\beta = .35$, $t(83) = 2.86$, $p < .01$; however, environmental instability was a not significant predictor of infant and toddler bedtime cortisol values. Mediation analyses for noon values revealed that environmental instability reduced the previously significant effect of caregiver history of childhood adversity to a non-significant effect, suggesting full mediation (see Figures 9-10).

The PROCESS macro (Hayes, 2012) was used to explore the direct and indirect effects of caregiver history of adversity on infant and toddler diurnal cortisol while modeling a process in which higher caregiver history of childhood adversity predicts greater caregiver mental health, which in turn predicts greater environmental instability, which in turn would translate into higher infant and toddler diurnal cortisol values. This multiple mediation model was not supported for the noon or bedtime values in the current sample.

Aim 3a. Explore glucocorticoid receptor methylation as a potential mechanism mediating the relationship between caregiver history of childhood adversity and infant and toddler stress physiology. In a subsample of infants and toddlers from whom DNA was collected, the potential contribution of NR3C1

methylation (e.g., epigenetic modifications) to the relationship between caregiver history of childhood adversity and infant and toddler diurnal and stress reactive cortisol was assessed. Linear regressions were conducted for total percent NR3C1 CpG site methylation. Methylation for NR3C1 was below 20% for all infants and toddlers in this current sample (Table 2, Figure 11). Caregiver history of childhood adversity was not related to percent total NR3C1 CpG site methylation nor individual methylation across 15 CpG sites after controlling for income-to-needs ratio, race, and child early life stress. Therefore, the model did not meet criteria to test mediation.

Aim 3b. Assess whether caregiver warmth and responsivity moderates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology to further explain the mediation of epigenetic modifications. The PROCESS macro (Hayes, 2012) was used to explore the mediation of the effect of caregiver history of childhood adversity on infant and toddler noon and bedtime cortisol values by infant and toddler NR3C1 methylation, with both direct and indirect effects of caregiver history of childhood adversity moderated by caregiver chronic (and acute) warmth and responsivity. This moderated mediation was not supported in the current sample.

Aim 4. Investigate whether caregiver warmth and responsivity moderates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology. *Chronic caregiver warmth and responsivity.* The potential buffering role of caregiver warmth and responsivity (e.g., chronic and acute) on the relationship between caregiver history of childhood adversity and infant and toddler

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diurnal and stress reactive cortisol was explored. Hierarchical multiple regression

analyses controlled for income-to-needs ratio, caregiver race, caregiver nativity, and child

early life stress. In the main-effects-only model, caregiver history of childhood adversity

and chronic caregiver warmth and responsivity predicted infant and toddler noon and

bedtime values, such that exposure to 6 or more ACEs was associated with higher infant

and toddler noon and bedtime cortisol values while higher chronic caregiver warmth and

responsivity was related to lower infant and toddler noon and bedtime cortisol values

(e.g., healthier diurnal values). The final multiple regression models that tested main-

effects and the interaction between caregiver history of childhood adversity and chronic

caregiver warmth and responsivity on noon and bedtime cortisol were also statistically

significant (Table 6). Given this significant interaction effect, simple effects analyses

were conducted at lower and higher levels of chronic caregiver warmth and responsivity

to determine the nature of the interaction on infant and toddler noon and bedtime cortisol

values. Tests of simple slopes showed that chronic caregiver warmth and responsivity

moderated the relationship between caregiver history of childhood adversity and infant

and toddler noon and bedtime cortisol such that caregiver history of adversity were more

strongly associated with higher noon and bedtime cortisol values at lower levels of

chronic caregiver warmth and responsivity (Figures 12-13).

     Acute caregiver warmth and responsivity. Regarding acute caregiver warmth and

responsivity, in the main-effects-only model, caregiver history of childhood adversity and

acute caregiver warmth and responsivity significantly predicted infant and toddler noon

and bedtime values. In this model, exposure to 6 or more ACEs was associated with

higher infant and toddler noon and bedtime cortisol values, and higher acute caregiver warmth and responsivity was marginally related to lower infant and toddler noon and bedtime cortisol values (e.g., healthier diurnal values). The final multiple regression models that tested main-effects and the interaction between caregiver history of childhood adversity and acute caregiver warmth and responsivity was statistically significant and accounted for significant variance in both infant and toddler noon and bedtime cortisol values (Table 7). Simple effects analyses were also conducted at lower and higher levels of acute caregiver warmth and responsivity. Tests of simple slopes showed that acute caregiver warmth and responsivity moderated the relationship between caregiver history of childhood adversity and infant and toddler noon and bedtime cortisol such that caregiver history of childhood adversity was more strongly associated with higher noon and bedtime cortisol values at lower levels of acute caregiver warmth and responsivity (Figures 14-15).

Aim 5. Explore a full model in which caregiver warmth and responsivity mediates the relationship between caregiver history of childhood adversity and infant and toddler stress physiology, and furthermore, if the relationship between caregiver history of childhood adversity and caregiver warmth and responsivity is further explained by caregiver mental health and/or environmental instability. Mplus FMIL with bootstrapping was used to explore the full model (Muthen & Muthen, 2007). Caregiver warmth and responsivity was not found to mediate the relationship between caregiver history of childhood adversity and infant and toddler cortisol values. Furthermore, neither caregiver history of childhood adversity nor environmental

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instability predicted caregiver warmth and responsivity. Moreover, neither caregiver

mental health nor environmental instability were found to mediate the relationship

between caregiver history of childhood adversity and caregiver warmth and responsivity.

The full model was not supported in the current sample.

Chapter Five: Discussion

The current study examined how caregiver's history of adversity may impact the next generation via more proximal environmental factors or caregiver characteristics and behaviors. In particular, the contributions of environmental instability, caregiver mental health and caregiver warmth and responsivity, on infant and toddler diurnal and stress reactive cortisol in the face of high caregiver history of adversity were explored. Analyses revealed that higher rates of caregiver history of adversity indeed has a negative impact on infant and toddler diurnal cortisol, specifically on higher noon and bedtime values, resulting in a flattened diurnal profile. By itself this finding is noteworthy, considering how young the children were and the fact that caregiver history of adversity was predictive of infant and toddler cortisol even when controlling for stressful family events in the life of the child.

Important proximal mediation and moderation effects were also identified. Specifically, both caregiver mental health and environmental instability helped to explain some of the effects of caregiver history of adversity on infant and toddler noon and bedtime cortisol values, offering insight and targets for intervention. Perhaps most importantly, given the evidence for continuity of risk, low caregiver warmth and responsivity moderated the effect of caregiver history of adversity on infant and toddler diurnal cortisol. Specifically, when observers rated low levels of caregiver warmth and responsivity, for both chronic and acute measures, higher caregiver adversity was

associated with a substantial increase in both noon and bedtime cortisol, with values approaching those similar to mid-morning levels for infants and toddlers experiencing less adversity. These findings align with the animal literature and current theoretical perspectives on parental buffering. Furthermore, the current data highlight the powerful potential for parenting interventions which target warm and responsive caregiving behaviors to offset negative outcomes for low-income infants and toddlers.

No relationship was found between caregiver history of adversity and infant and toddler stress reactive cortisol. It is important to note, not only did the sample size drop considerably from the first to the second data collection visit, attrition was much higher in families with a higher history of adversity (36%) than in families with a lower history (17%). Therefore, the most parsimonious explanation for the lack of significant findings is low power and restricted variance. This potential explanation is supported by the data (Figure 6), which suggests a similar overall attenuated pattern in the small sample of infants and toddlers with stress reactivity data and caregiver history of adversity at 6 or above. It is also possible, however, that caregiver history of adversity, caregiver mental health and global environmental instability impact basal cortisol more strongly than acute cortisol, particularly when acute cortisol is assessed in the presence of a caregiver. Future studies should consider including a second acute stress challenge with a trained research assistant-infant dyad. Inclusion of a second challenge would not only provide additional data regarding infant and toddler stress reactivity in the form of multiple measurement, but also allow a direct comparison between challenge conditions (i.e., caregiver-child vs.

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research assistant-child dyads) and a comprehensive assessment of caregiver history of adversity on child stress reactive cortisol.

No relationship was found between infant and toddler total glucocorticoid receptor methylation (or individual CpG methylation) and infant and toddler noon or bedtime cortisol values. Further, a moderated mediation, examining the mediation of the effect of caregiver history of adversity on infant and toddler noon and bedtime cortisol values by infant and toddler NR3C1 methylation, with both direct and indirect effects of caregiver history of adversity moderated by caregiver warmth and responsivity was not supported. Given the strong theoretical framework and previous data in humans suggesting an important role for NRC31 methylation, as well as the observation of the moderation (without the mediation of NR3C1) of caregiver warmth and responsivity on the relationship between caregiver history of adversity and infant and toddler diurnal cortisol), and the small and restricted sample of infants and toddlers with available DNA in the current study (n=59; high adversity sample n=6) I did not attempt to interpret these null results. Data collection is ongoing and data will be reexamined when an appropriate sample size has been collected. This is an important first step in replicating the animal literature, however, future studies should consider including more regions of interest.

Overall, the current study's sample of low-income families with high rates of caregiver anxious and depressive symptoms had high variability across assayed infant and toddler cortisol values. Although differences were not observed for infant and toddler diurnal cortisol when examining caregiver history of adversity continuously, clear patterns emerge when the groups are broken out by level of adversity experienced (see

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Figure 17). Children of caregivers with the highest experienced adversity have what appear to be blunted or attenuated diurnal profiles across the day. More specifically, they begin the day with lower values on average and, rather than demonstrating the typical diurnal decrease, remain at this level across the day. Indeed, higher noon and bedtime cortisol values were consistently observed for and related to risk factors within this sample. In contrast, infants and toddlers of caregivers with the next highest experienced adversity (ACE= 4-5), visually display a heightened diurnal profile across the day. More specifically, they begin with higher wake values on average, and maintain these higher values across the morning (see Figure 16). However, no significant group differences were observed. This observation is particularly noteworthy as this pattern is generally recognized as the other form of dysregulated stress physiology. These observed patterns support the current working theory of how stress 'gets under the skin.' Specifically, when faced with multiple stressors the HPA-axis is over activated resulting in higher cortisol levels until the system hits a threshold (chronic and pervasive stressors) and stops responding (conceptualized as burn out and/or conservation of resources). Furthermore, dysregulated child stress physiology, particularly in the form of higher basal noon and bedtime values, as observed here is particularly concerning as this early dysregulation could have implications for other circadian patterns, such as sleep quality and duration. This is particularly relevant as sleep is necessary for growth and restorative processes and disruptions to sleep have been associated with poor physical and mental health outcomes. Taken together, higher diurnal values later in the day may place children at greater risk

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than is currently understood. As such, future studies should examine whether attenuated patterns are associated with poorer sleep quality and duration in young children.

Finally, when examining all collected infant and toddler cortisol values (e.g., diurnal and stress reactive), children of caregivers with the highest history of adversity exhibit a marginally constrained physiologic range as compared to children of caregivers with a lower history of adversity (see Figure 18). Taken together, these findings suggest not only a trend of attenuated HPA-axis activity, but also less physiologic variability for children of caregivers with the highest history of adversity. These findings contribute to the growing evidence that hypocortisolism early in life may indeed be a risk factor (Badanes, Watamura, & Hankin, 2011). These interesting patterns warrant follow-up with a larger sample for further validation.

In line with previous literature (e.g., Essex, Klein, Cho, & Kalin, 2002), caregiver anxious and depressive symptoms were related to infant and toddler diurnal cortisol. Caregiver mental health was also positively related to environmental instability such that caregivers who endorsed more anxious and depressive symptoms also reported more environmental instability risk factors. However, it should be noted that a multiple mediation model was not supported which suggests that caregiver mental health and environmental instability are independently contributing to infant and toddler diurnal cortisol and thus should be consider separately when developing and administering interventions (e.g., targeting caregiver mental health alone is not likely to reduce environmental instability and in turn infant and toddler diurnal cortisol). Further, in contrast to previous literature, caregiver mental health was not related to chronic

caregiver warmth and responsivity (Conger et al., 2002). There are several potential

explanations for why this relationship was not supported in the current sample. First, the

construct of caregiver mental health within this sample included both anxious and

depressive symptoms. There is evidence to suggest that these two highly comorbid

mental health symptoms may impact caregiving behaviors differently. Therefore,

collapsing measures of anxiety and depression into one larger/global construct of mental

health may have impacted analyses in the current study. Future work should consider

analyzing the impact of these distinct mental health symptoms on caregiving behaviors

independently. Second, all family-level factors controlled for income-to-needs, race, and

nativity. This stringent level of analyses may have concealed potentially interesting

relationships. For example, controlling for income-to-needs, when income and mental

health are known to be highly correlated, may have restricted the range of mental health

symptoms thus reducing our ability to detect this previously established relationship.

Moreover, there is evidence that race and/or nativity may differential impact caregiving

behaviors (Lansford, Deater- Deckard, Dodge, Bates, & Pettit, 2004). In particular,

future work should consider examining the moderation of nativity on the relationship

between caregiver mental health and caregiver warmth and responsivity.

Taken together, the current study's finding suggest several pathways through

which a caregiver's history of adversity may be transmitted to the next generation

impacting child health and well-being. Observations of dysregulated cortisol as early as

infant and toddlerhood are particularly concerning as these profiles place children at

greater risk for poor psychological, social, and academic outcomes and can result in

lifelong negative consequences for physical health and well-being (Alink, Lenneke, Cicchetti, Kim, & Rogosch, 2012; Hankin, Badanes, Abuela, & Watamura, 2010; Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010; Shirtcliff, Granger, Booth, & Johnson, 2005; Van Goozen, Matthys, Cohen–Kettenis, Buitelaar, & Van Engeland, 2000). Promisingly, the current study's findings suggest that targeting caregiver mental health through intervention and environmental instability through policy has the potential to improve both caregiver psychological and child physical well-being. Additionally, the findings presented here support the potential buffering role of warm and responsive caregiving in which stress experienced by the child becomes manageable and does not result in dysregulated physiology.

Although the current study intentionally examined proximal and modifiable factors individually to best understand how caregiver history of adversity impacts current child health and well-being, it would advantageous to consider how these variables operate collectively utilizing a cumulative risk model (Evans & Kim, 2007).

Limitations

Despite its numerous strengths and contributions to the early risk and adversity literature, as well as literature on ethnically diverse families with young children, this study is not without limitations. Attrition occurred across visits for the whole sample, however, the greatest percentage of participant loss from first to second visit was for families with the highest caregiver history of adversity. Restricted samples sizes and the decision to analyze paths separately may have interfered with my ability to detect some relationships. Future studies that seek to answer similar questions would benefit, when possible, from

collecting all measures of interest at the same visit. Although this would prohibit longitudinal analyses it should protect against the issues that arise from small sample sizes and missing data. Furthermore, the current analyses included race, ethnicity and nativity as covariates, however, closer examination of these individual factors may be warranted. Future studies should consider including these variables as moderators within their transmission of risk models to best understand how race, ethnicity, and nativity may interact with history of adversity to impact mental health and caregiving behavior. This is particularly relevant as Latino families are a rapidly expanding segment of the population and few studies have integrated the nativity (e.g., acculturation/generational) and child stress physiology literature. A comprehensive understanding of this how these factors may interact to result in child dysregulated physiology is crucial to shaping policy and understanding how to best support the health and development of all children (and their families). Moreover, future studies would benefit from longitudinal designs able to detect other proximal risk and protective factors which may unfold over the course of development to impact infant and toddler stress physiology.

Conclusion

This research has made significant gains in our understanding of how environmental, psychosocial and physiological stressors can get "under the skin" and contribute to intergenerational risk. However, future studies would benefit by combining these multiple pathways into a single model. Future work should also consider additional physiological measures (e.g., inclusion of more methylation sites). Understanding the

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mechanistic pathways in which risk is conferred is crucial to supporting the development

of high risk children.

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Appendix A: Tables

Table 1

Participant Demographics (Child n=134; Caregiver n=125)

	M(SD)
Child Age (months)	24.30(9.89)
Caregiver Age (years)	31.04(6.50)
Income-to-Needs Ratio	.89(.73)

	n (%)
Child Female	56 (41.8%)
Caregiver Female	121(97.6%)
Caregiver Race	
Caucasian/White	92(73.6%)
Minority Race	24(19.2%)
Biracial or Multiracial	9(7.2%)
Caregiver Ethnicity	
Latino	91(72.8%)
Caregiver Nativity	
Latino Immigrant	65(52.0%)
Caregiver History of Adversity (6+)	15(12%)

Note. Minority race includes African American, American/Native Indian/Alaska Native, Asian, and other Pacific Islander.

Table 2

Descriptives of Independent and Dependent Variables

	n	*M* (*SD*)	Range
Child			
Diurnal Cortisol (μg/dL)			
Wake Value	127	.30 (.18)	.00-1.11
Wake +15 Value	123	.33 (.22)	.01-1.60
Wake + 60 Value	120	.21(.14)	.01-.85
Noon Value	113	.13(.11)	.01-.71
Bedtime Value	114	.07 (.08)	.00-.46
Diurnal Slope	116	-.03 (.03)	-.29-.02
Diurnal CAR AUC_G	121	1276.00 (1111.73)	38.51-7680.55
Diurnal AUC_G	128	4974.07 (3510.79)	49.17-17139.24
Stress Reactive Cortisol (μg/dL)			
Max Value	98	.26 (.23)	.06-1.74
Min Value	98	.08 (.06)	.02-.41
MaxMin Diff	98	.18 (.21)	.00-1.65
AUC_G	94	7.26 (5.45)	.77-31.17
All Cortisol (μg/dL)			
Min Value	133	.04 (.05)	.00-.44
Max Value	133	.52 (.29)	.01-1.74
Range	133	.48 (.27)	.01-1.65
NR3C1 Methylation (%)	59	3.80 (3.85)	.00 - 15.10
Caregiver			
ACE Score	125	2.54 (2.25)	.00-9.00
Anxious & Depressive Symptoms	125	17.38 (14.06)	.00-66.00
Warmth & Responsivity, Chronic	125	.00 (3.06)	-7.84-5.45
Warmth & Responsivity, Acute	76	11.08 (3.38)	5.00-17.00
Family			
Environmental Instability	125	3.25 (2.15)	0.00-11.00

Note. Wake Value= First child diurnal cortisol sample value, Wake+ 15= Second child diurnal cortisol sample value (collected 15 minutes after the wake sample), Wake+ 60= Third child diurnal cortisol sample value (collected 60 minutes after the wake sample), Noon= Fourth child diurnal cortisol sample value (collected at noon), Bedtime= Fifth child diurnal cortisol sample value (collected prior to child's bedtime), CAR AUC_G= Area under the curve with respect to ground for the child cortisol awakening response, Diurnal AUC_G= Area under the curve with respect to ground for all five child diurnal cortisol sample values, SR Max= Highest child stress reactive cortisol sample value collected during the stress paradigm, SR Min= Lowest child stress reactive cortisol sample value collected during the stress paradigm, SR MaxMin= The difference between the highest and lowest child stress reactive cortisol sample values collected during the stress paradigm, SR AUC_G= Area under the curve with respect to ground for child stress reactive cortisol sample values, Range= The difference between the highest and lowest samples values for all collected child cortisol (diurnal and stress reactive), NR3C1 Methylation = Child total glucocorticoid receptor methylation, ACE Score= Caregiver total score on the Adverse Childhood Experience questionnaire.

Table 3

Inter- Correlations of Key Variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. ACE 6[+]	--	.48***	.40***	-.11	.06	-.13	-.17	-.12	.22*	.21*	.16	-.12	.07	-.07	-.13	-.04	-.11	-.16	.06
2. Men Health		--	.24**	-.18*	-.17	-.15	-.05	.04	.18	.23*	.21*	-.01	-.02	-.06	-.10	-.04	.06	-.10	-.18
3. Env Instab			--	-.12	-.06	-.02	-.10	-.02	.28**	.34***	.12	-.05	.24***	-.07	-.13	-.04	-.07	-.03	.17
4. C WarmRsp				--	.51***	-.02	.10	-.17	-.25**	-.20*	-.00	-.07	-.11	-.09	-.11	-.07	-.27*	.05	.23
5. A WarmRsp					--	-.08	.01	-.09	-.23*	-.15	-.05	-.02	.01	-.28*	-.40***	-.19	-.46**	-.15	-.08
6. Wake						--	.31***	.20*	.41***	.24*	-.27**	.46***	.15	-.03	.08	-.05	-.12	.58***	.11
7. Wake+15							--	.38***	.27**	.04	-.50***	.68***	.41***	.24*	.12	.24*	.19	.63***	-.08
8. Wake+ 60								--	.37***	.36***	-.21*	.42***	.54***	.20	.07	.20	.24	.44***	-.08
9. Noon									--	.57***	-.02	.34***	.73***	.13	.08	.12	.13	.38***	-.08
10. Bedtime										--	.04	.19	.55***	-.02	-.05	-.01	.22	.21*	-.05
11. Diurnal Slope											--	-.36***	-.02	-.48**	-.12	-.49***	-.52***	-.48***	.22
12. CAR AUC_G												--	.53***	.03	.07	.02	-.06	.56***	-.00
13. Diurnal AUC_G													--	-.00	-.01	.00	-.11	.31***	-.07
14. SR Max														--	.42***	.97***	.87***	.54***	-.26*
15. SR Min															--	.19	.68***	.12	-.03
16. SR MaxMin																--	.80***	.55***	-.27*
17. SR AUC_G																	--	.54***	-.29
18. Range																		--	-.14
19. NR3C1																			--

Note. ACE 6[+]= Caregiver history of adversity, Men Health= Caregiver total anxious and depressive symptoms, Env Instab= Environmental instability, C WarmRsp= Chronic caregiver warmth and responsivity, A WarmRsp= Acute caregiver warmth and responsivity, Wake= First child diurnal cortisol sample value, Wake+ 15= Second child diurnal cortisol sample value (collected 15 minutes after the wake sample), Wake+ 60= Third child diurnal cortisol sample value (collected 60 minutes after the wake sample), Noon= Fourth child diurnal cortisol sample value (collected at noon), Bedtime= Fifth child diurnal cortisol sample value (collected prior to child's bedtime), CAR AUC_G= Area under the curve with respect to ground for the child cortisol awakening response, Diurnal AUC_G= Area under the curve with respect to ground for all five child diurnal cortisol sample values, SR Max= Highest child stress reactive cortisol sample value collected during the stress paradigm, SR Min= Lowest child stress reactive cortisol sample value collected during the stress paradigm, SR MaxMin= The difference between the highest and lowest child stress reactive cortisol sample values collected during the stress paradigm, SR AUC_G= Area under the curve with respect to ground for child stress reactive cortisol sample values, Range= The difference between the highest and lowest samples values for all collected child cortisol (diurnal and stress reactive), NR3C1= Child total glucocorticoid receptor methylation.
*p<.05, **p<.01, ***p<.001

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Table 4

Key Variable Scores for Low Adversity, Mid-Low Adversity, Mid-High Adversity, and High Adversity Caregiver Groups

	Low		Low-Mid		Mid-High		High		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA
N	48		40		22		15		
Mental Health	11.25***	9.20	18.73***	12.49	16.59***	12.52	34.53	18.64	*P < .001*
Environmental Instability	2.60***	2.06	3.03***	1.31	3.32**	1.96	5.80	2.78	*P < .001*
Chronic Warmth & Responsivity	0.49	3.37	-0.60	2.91	0.54	2.81	-0.75	2.57	N.S.
Acute Warmth & Responsivity	11.03	3.58	10.60	3.35	12.11	3.33	11.71	2.75	N.S.
Child Noon Cortisol	.11*	.08	.13†	.10	.15	.10	.20	.19	N.S.
Child Bedtime Cortisol	0.06†	0.05	0.07	0.08	0.08	0.10	0.12	0.14	*P < .10*
Child SR AUC_G	9.79	8.66	6.90	4.34	8.93	5.36	5.96	3.68	N.S.
NR3C1 Methylation	3.50	3.48	2.98	3.34	5.27	4.41	4.48	5.52	N.S.

Note. The ANOVA column refers to the statistical significance of a one-way ANOVA across each row. Asterisks (*) indicate statistically significant differences between each risk group and high adversity (ACE score 6 or more) on Tukey HSD post hoc tests. SR AUC_G= Area under the curve with respect to ground for child stress reactive cortisol sample values.
$^{\dagger}p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$.

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Table 5

Regression Coefficients Predicting Child Physiology

	Noon Cortisol			Bedtime Cortisol			Stress Reactive AUC_G		
	B	SE *B*	β	*B*	SE *B*	β	*B*	SE *B*	β
Step 1									
Constant	.12	.01		.07	.01		9.46	3.51	
Caregiver History of Adversity	.14	.04	.38***	.10	.03	.39***	-1.16	2.69	-.06
Step 2									
Constant	.25	.05		.09	.04		9.46	3.51	
Caregiver History of Adversity	.11	.04	.28*	.09	.03	.34**	-1.16	2.69	-.06
Income-to-needs ratio	-.03	.02	-.20	-.01	.01	-.05	.58	1.07	.06
Race	-.01	.02	-.10	-.01	.01	-.07	-.78	1.04	-.11
Ethnicity	-.08	.05	-.30	-.01	.04	-.03	2.78	3.19	.18
Nativity	-.01	.03	-.09	-.01	.02	-.07	-1.48	1.7	-.20
Child Early Life Stress	-01	.01	-.08	.01	.01	.09	-.60	.59	-.12

Note. AUC_G= Area under the curve with respect to ground for child stress reactive cortisol sample values.
*p < .05, **p < .01, ***p < .001.

Table 6

Caregiver History of Adversity and Chronic Caregiver Warmth and Responsivity with Child Noon Cortisol

Diurnal Cortisol			
Correlates	***B(SE)***	***t***	***CI***
Income-to-needs ratio	-.01(.02)	-.69	[-.04, .02]
Caregiver Race	-.01(.02)	-.66	[-.05, .03]
Caregiver Ethnicity	-.03(.01)	-2.26*	[-.06, -.00]
Child Early Life Stress	.00(.01)	.03	[-.02, .02]
Caregiver History of Adversity	.10(.04)	2.50*	[.02, .17]
Chronic Caregiver Warmth & Responsivity	-.03(.01)	-2.26*	[-.05, -.00]
History of Adversity x Warmth & Responsivity	-.14(.04)	-3.80***	[-.21, -.06]
Constant	.20(.04)	4.54***	[.11, .28]
R^2 Change	.12		
Total *R^2*	.38***		
n	84		

Note. B= Beta, *(SE)*, Standard Error, *CI*= 95% Confidence Interval
*p < .05, ***p < .001.*

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Table 7

Caregiver History of Adversity and Chronic Caregiver Warmth and Responsivity with Child Bedtime Cortisol

Diurnal Cortisol			
Correlates	**B(SE)**	**t**	**CI**
Income-to-needs ratio	.01(.01)	.89	[-.01, .03]
Caregiver Race	-.00(.02)	-.19	[-.03, .03]
Caregiver Ethnicity	.00(.01)	.19	[-.02, .02]
Child Early Life Stress	.01(.01)	1.87†	[-.00, .02]
Caregiver History of Adversity	.09(.03)	2.99**	[.03, .14]
Chronic Caregiver Warmth & Responsivity	-.03(.01)	-3.05**	[-.04, -.01]
History of Adversity x Warmth & Responsivity	-.06(.03)	-2.02*	[-.11, -.00]
Constant	.05(.03)	1.50	[-.02, .12]
R^2 Change	.04		
Total R^2	.32*		
n	82		

Note. B= Beta, (SE), Standard Error, CI= 95% Confidence Interval
$\dagger p < .10$, *p < .05, **p < .01.

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Table 8

Caregiver History of Adversity and Acute Caregiver Warmth and Responsivity with Child Noon Cortisol

Diurnal Cortisol

Correlates	B(SE)	t	CI
Income-to-needs ratio	-.02(.02)	-.99	[-.06, .02]
Caregiver Race	-.03(.03)	-.98	[-.08, .03]
Caregiver Ethnicity	-.04(.02)	-2.42*	[-.08, -.01]
Child Early Life Stress	-.01(.01)	-1.09	[-.04, .01]
Caregiver History of Adversity	.26(.06)	4.36***	[.14, .38]
Chronic Caregiver Warmth & Responsivity	-.04(.01)	-2.87**	[-.07, -.01]
History of Adversity x Warmth & Responsivity	-.18(.08)	-2.37*	[-.33, -.03]
Constant	.24(.06)	4.09***	[.12, .36]
R^2 Change	.05		
Total R^2	.40*		
n	65		

Note. B= Beta, *(SE)*, Standard Error, *CI*= 95% Confidence Interval
*p < .05, **p < .01, ***p < .001.

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Table 9

Caregiver History of Adversity and Acute Caregiver Warmth and Responsivity with Child Bedtime Cortisol

Diurnal Cortisol

Correlates	B(SE)	t	CI
Income-to-needs ratio	.01(.01)	.33	[-.02, .03]
Caregiver Race	-.01(.02)	-.54	[-.05, .03]
Caregiver Ethnicity	-.01(.01)	-.60	[-.04, .02]
Child Early Life Stress	.01(.01)	.82	[-.01, .03]
Caregiver History of Adversity	.16(.05)	3.48**	[.07, .25]
Acute Caregiver Warmth & Responsivity	-.01(.01)	-1.39	[-.03, .01]
History of Adversity x Warmth & Responsivity	-.12(.06)	-2.06*	[-.24, -.00]
Constant	.08(.05)	1.67	[-.02, .17]
R^2 Change	.05		
Total R^2	.28*		
n	66		

Note. B= Beta, *(SE)*, Standard Error, *CI*= 95% Confidence Interval
*p < .05, **p < .01.*

Appendix B: Figures

Figure 1. Conceptual Model
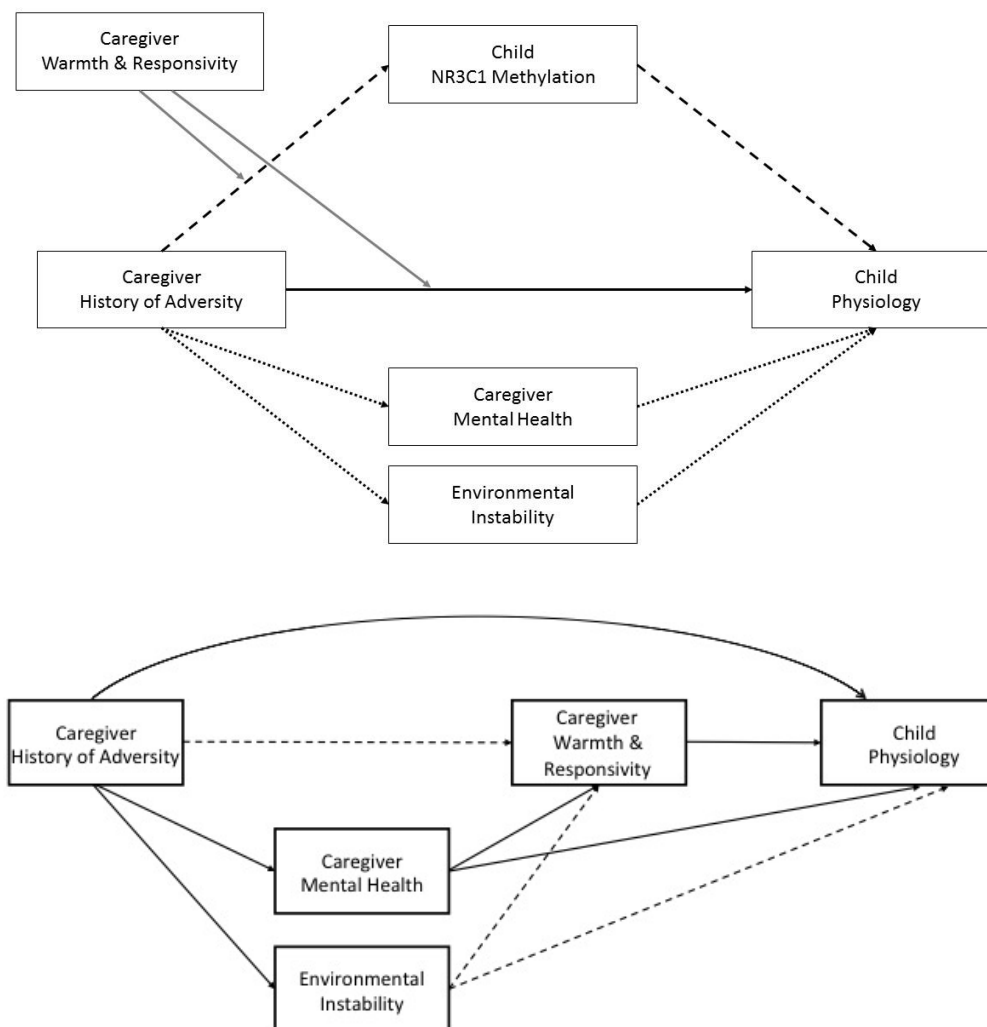
Figure 1. Aim 1 assesses the relationship between caregiver history of adversity and child physiology. Aim 2 investigates whether caregiver mental health or environmental instability mediate the relationship between caregiver history of adversity and child physiology. Aim 3 explores whether methylation of the glucocorticoid receptor (NR3C1) mediates the relationship between caregiver history of adversity and child physiology. Aim 4 assesses whether caregiver warmth and responsivity changed the relationship between caregiver history of adversity and child physiology. Aim 5 assesses whether caregiver warmth and responsivity mediates the relationship between caregiver history of adversity and child physiology.

Figure 2. Caregiver Mental Health by Caregiver Adverse Childhood Experiences Score
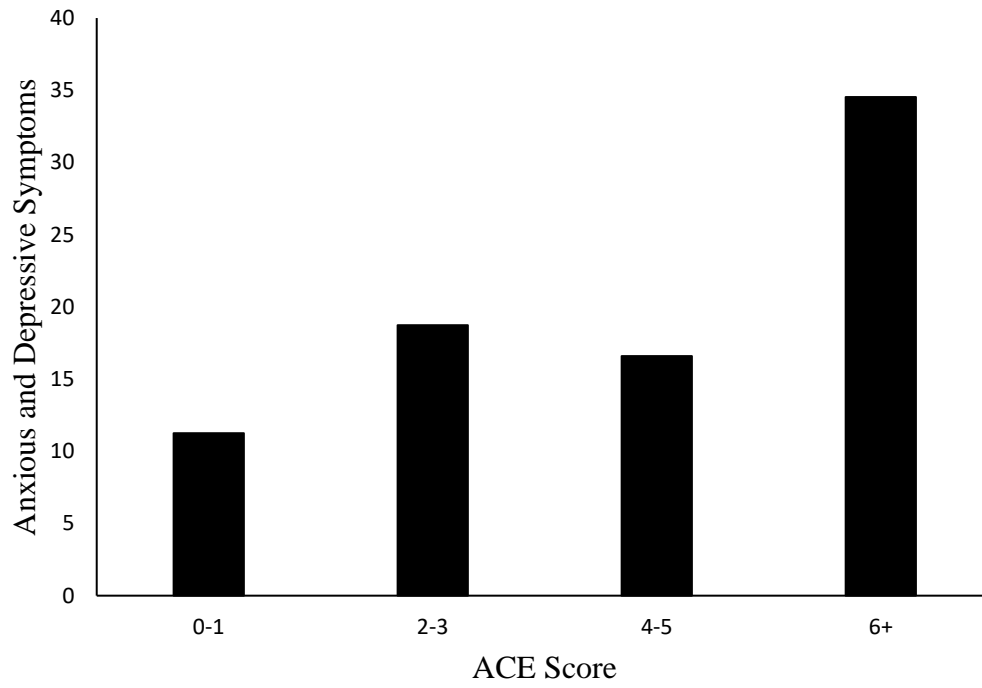
Figure 3. Caregiver Mental Health by Caregiver Adverse Childhood Experiences Score
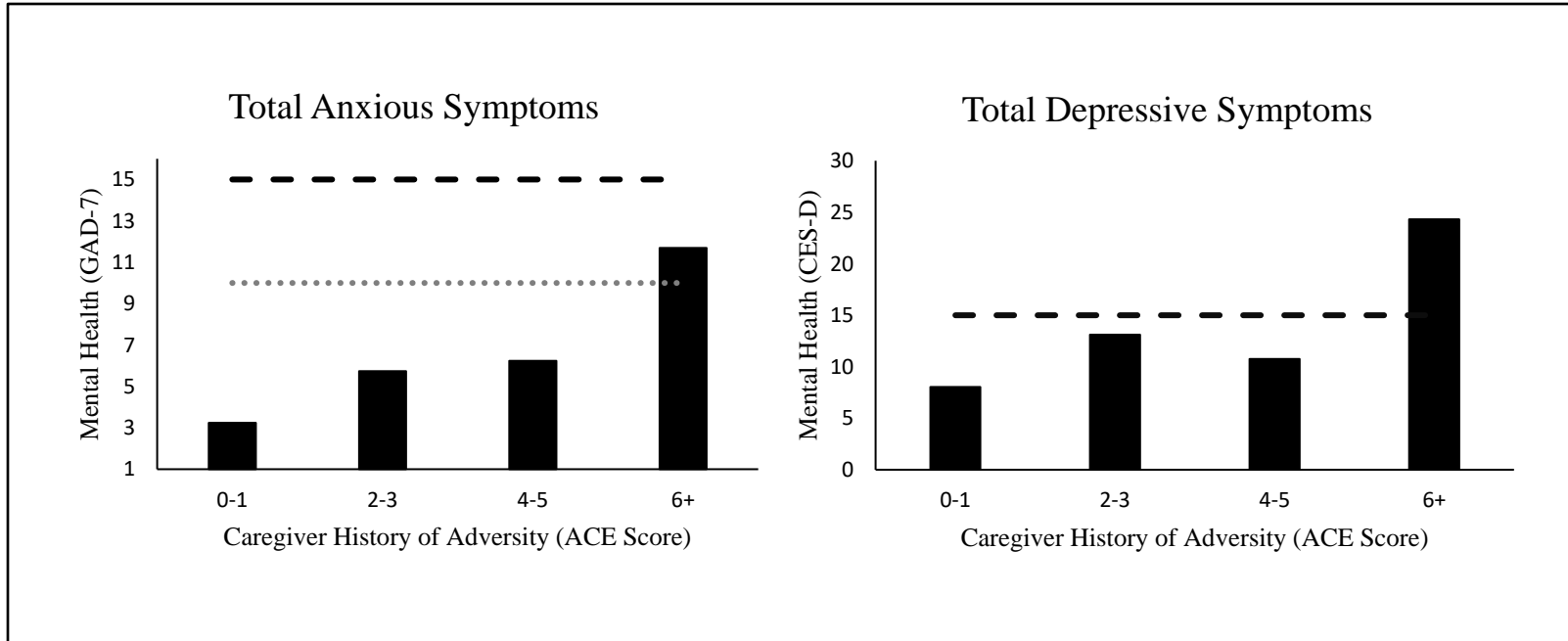
Figure 3. Caregiver individual mental health symptoms for anxiety and depression are displayed by risk group. The reference lines illustrate clinical cutoffs on the standardized mental health measures. For GAD-7, the grey dotted line represents moderate anxiety (score > 10) and the black dashed line represents severe anxiety (score > 15). For CES-D, the black dashed line represents the clinical cutoff for depression (score > 15). GAD-7: Generalized Anxiety Disorder Scale, CES-D: Center for Epidemiologic Studies-Depression Scale.

Figure 4. Environmental Instability by Caregiver Adverse Childhood Experiences Score
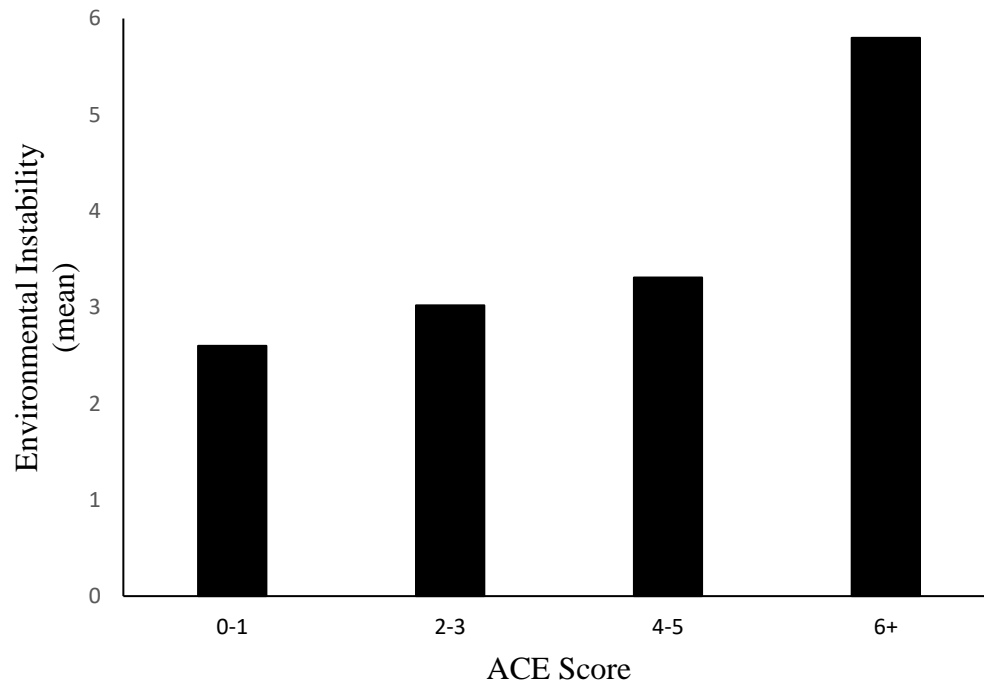
Figure 5. Child Diurnal Cortisol Profile
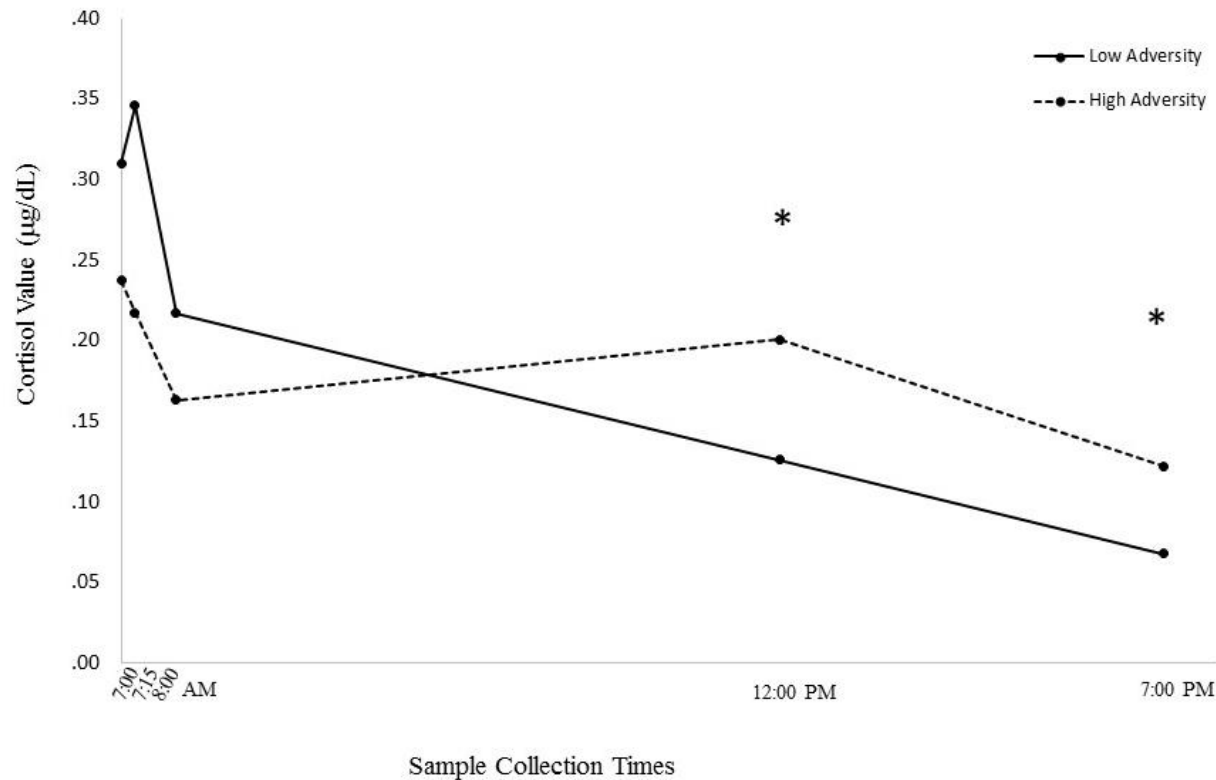
*Figure 5.*Child diurnal cortisol values averaged across two collection days controlling for income-to-needs, caregiver race, and child early life stress. Caregivers who endorsed six or more adverse childhood experiences are characterized high adversity (n=9). The Noon sample was higher for children of caregiver's with high adversity (*M* =.24, *SD* =.21) as compared to children of caregiver's with lower adversity (*M* =.12, *SD* =.08) $F(1, 65)=22.94$, $p< .001$. The Bedtime sample was higher for children of caregiver's with high adversity (*M* =.14, *SD* =.15) as compared to children of caregiver's with lower adversity (*M* =.06, *SD* =.06), $F(1, 65)=14.78$, $p< .00$.

Figure 6. Child Stress Reactive Cortisol Profile
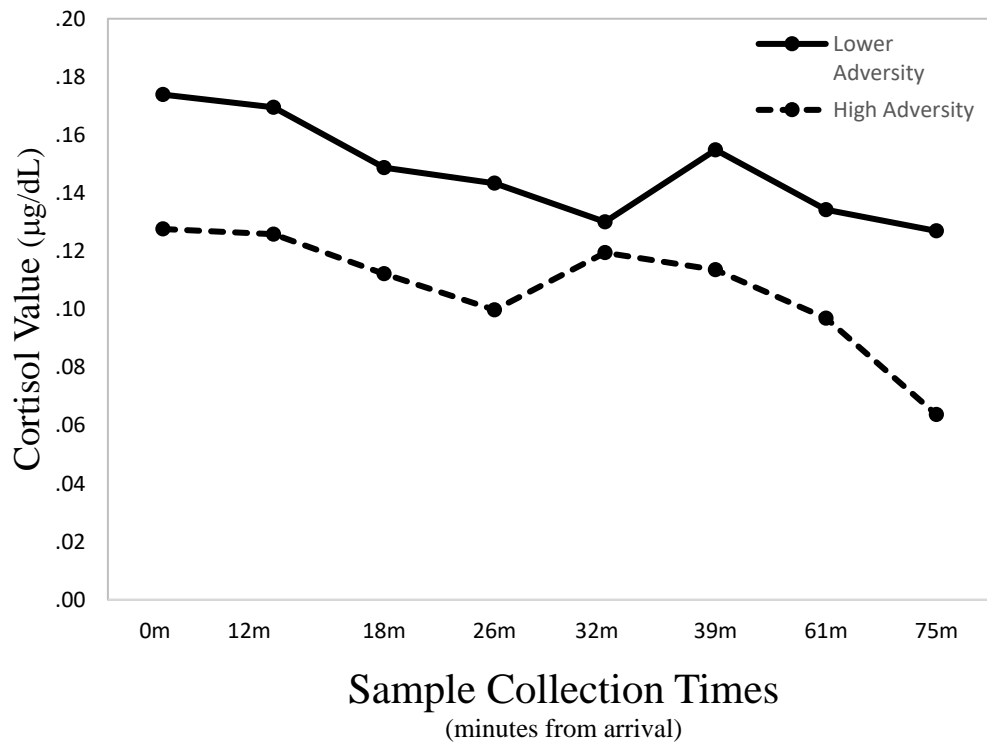
Sample Collection Times
(minutes from arrival)

Figure 6. Child stress reactive values during the second home visit which included a 7- minute structured stress paradigm. The caregiver-infant dyad participated in the 3-bag task (0-11m), stress paradigm (12m - 18m), free play and interview (26m – 75m). Peak reactivity values are expected between 32m and 39m as cortisol spikes are detectable 15-20m after an experienced stressor (18m). Cortisol values for children of lower and high adversity caregivers were not significantly different.
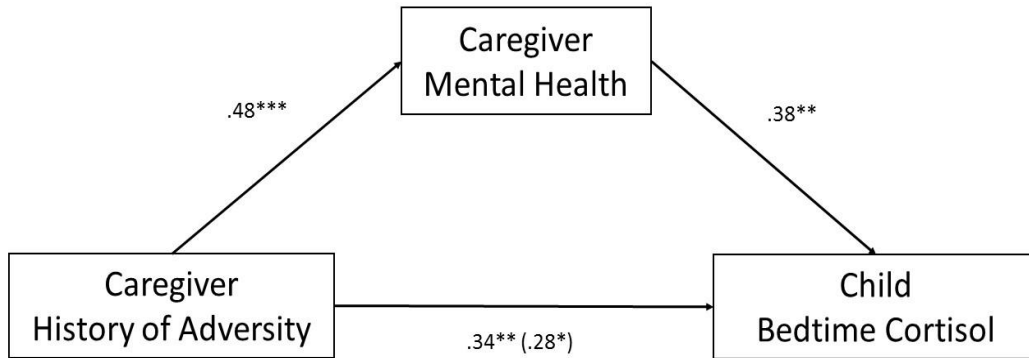
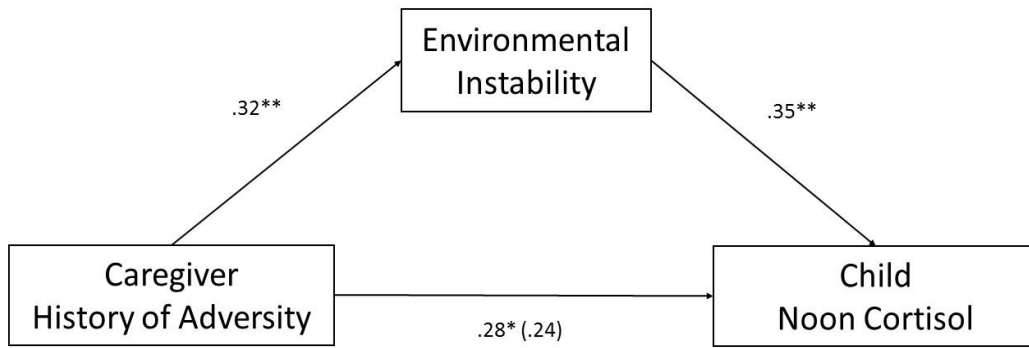Figure 7. Mediation Model Caregiver Mental Health
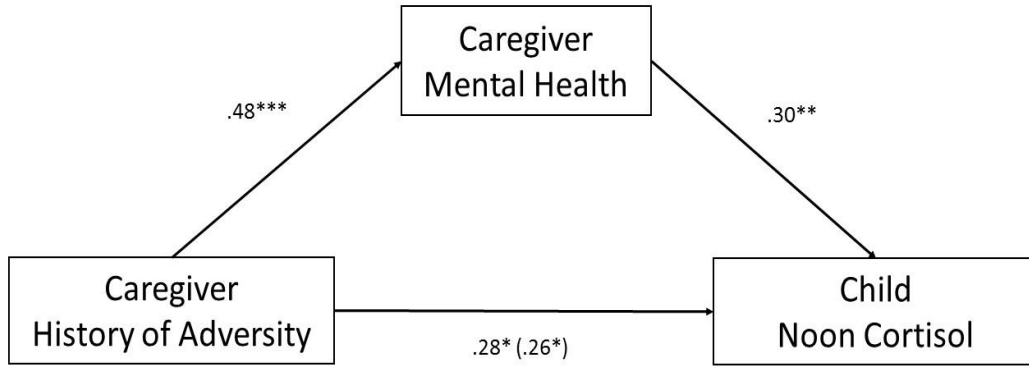
Figure 7. Standardized regression coefficients for the relationship between caregiver adverse experiences and child noon cortisol value as mediated by caregiver mental health. The standardized regression coefficient between caregiver adverse experiences and child noon cortisol value, controlling for caregiver mental health, is in parentheses.
*p<.05, **p<.01, ***p<.001

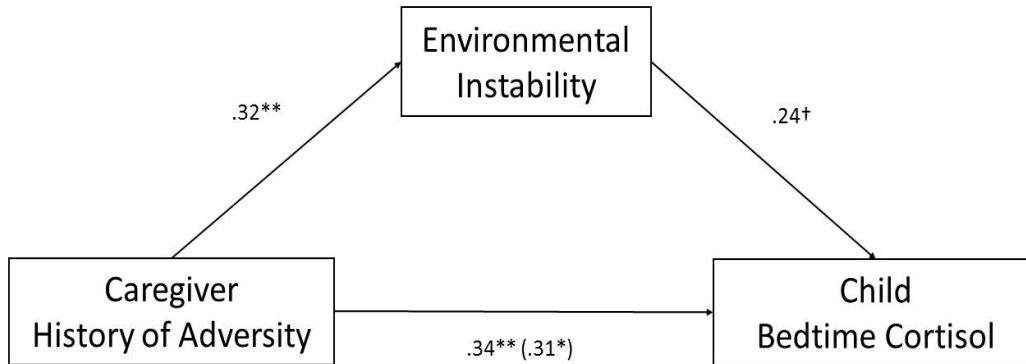Figure 8. Mediation Model Caregiver Mental Health

Figure 8. Standardized regression coefficients for the relationship between caregiver adverse experiences and child bedtime cortisol value as mediated by caregiver mental health. The standardized regression coefficient between caregiver adverse experiences and child bedtime cortisol value, controlling for caregiver mental health, is in parentheses.
*p<.05, **p<.01, ***p<.001*

Figure 9. Mediation Model Environmental Instability


```
                        ┌─────────────────┐
                        │  Environmental  │
                        │   Instability   │
                        └─────────────────┘
            .32**                              .35**

┌──────────────────────┐                    ┌──────────────────┐
│     Caregiver        │                    │      Child        │
│ History of Adversity │ ─────────────────► │  Noon Cortisol    │
└──────────────────────┘    .28* (.24)      └──────────────────┘
```

Figure 9. Standardized regression coefficients for the relationship between caregiver adverse experiences and child noon cortisol value as mediated by environmental instability. The standardized regression coefficient between caregiver adverse experiences and child noon cortisol value, controlling for environmental instability, is in parentheses.
*$p<.05$, **$p<.01$

Figure 10. Mediation Model Environmental Instability


```
                    ┌──────────────────┐
                    │  Environmental   │
                    │   Instability    │
                    └──────────────────┘
           .32**                          .24†
  ┌──────────────────┐              ┌──────────────────┐
  │    Caregiver     │              │      Child        │
  │History of Adversity│──────────→ │ Bedtime Cortisol  │
  └──────────────────┘   .34** (.31*)└──────────────────┘
```

Figure 10. Standardized regression coefficients for the relationship between caregiver adverse experiences and child bedtime cortisol value as mediated by environmental instability. The standardized regression coefficient between caregiver adverse experiences and child bedtime cortisol value, controlling for environmental instability, is in parentheses.
$^{†}p < .10, *p<.05, **p<.01$

Figure 11. Methylation by CpG Site for Lower and High Adversity
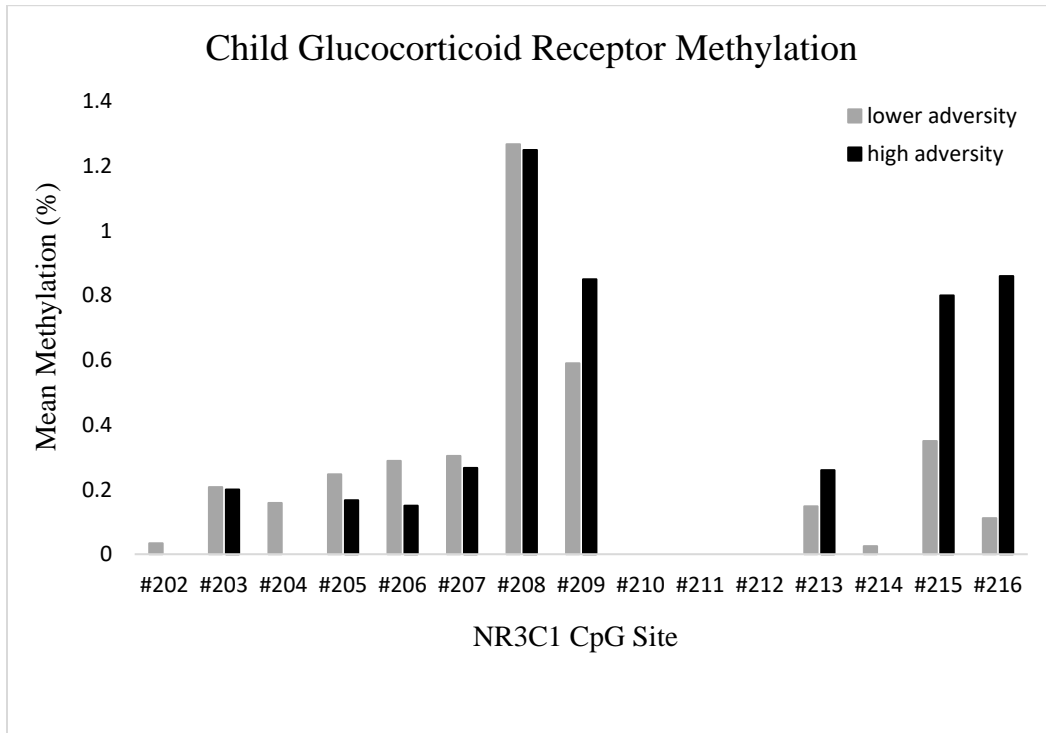
Figure 12. Two-Way Interaction: Caregiver History of Adversity X Chronic Warmth and Responsivity
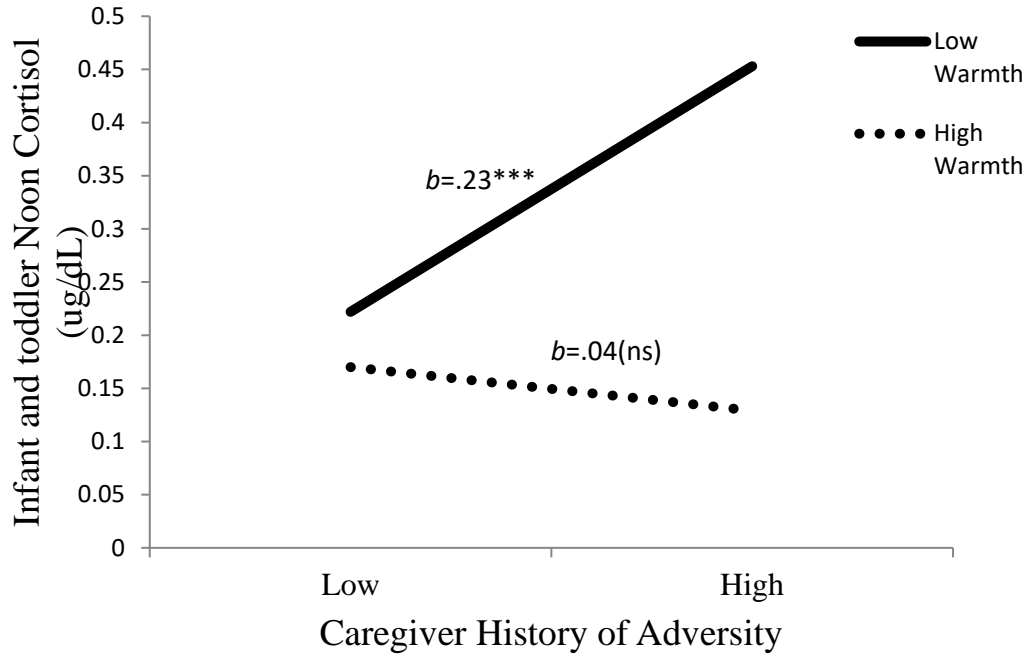
Figure 12. Moderating effect of chronic warm and responsive caregiving on the association between caregiver history of adversity and child diurnal noon cortisol values.

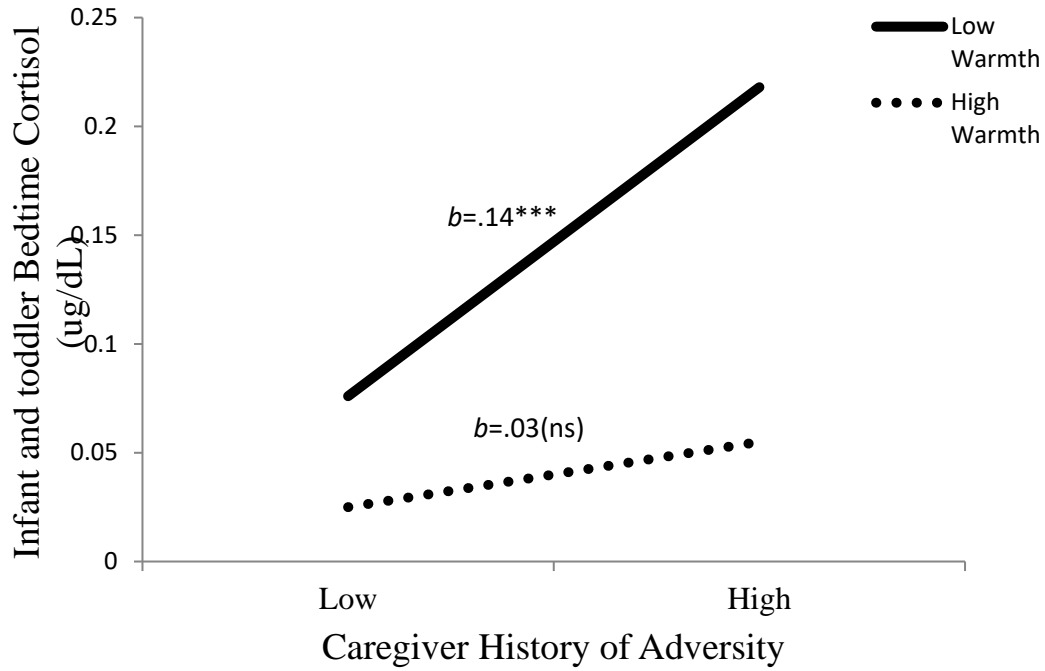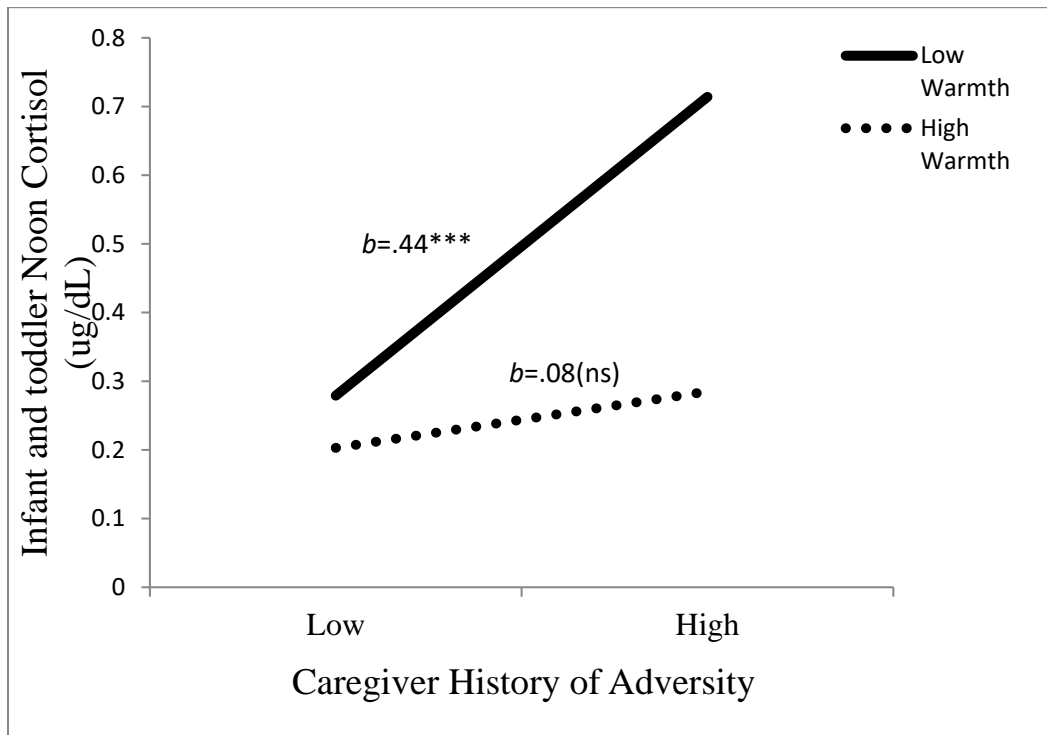Figure 13. Two-Way Interaction: Caregiver History of Adversity X Chronic Warmth and Responsivity
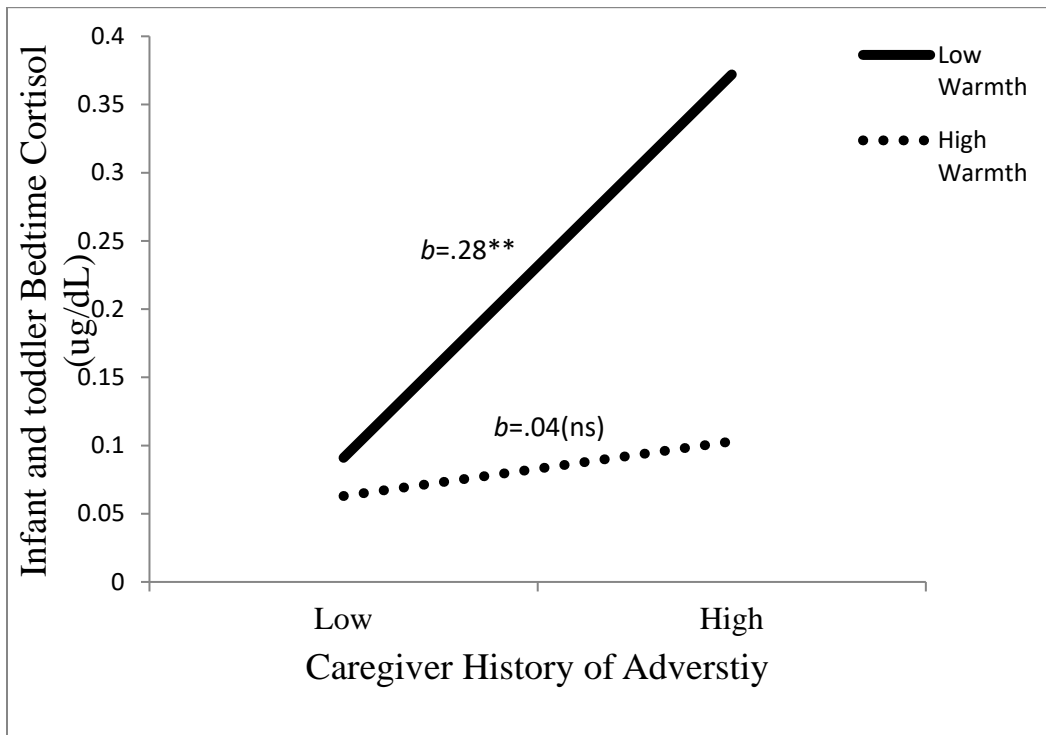

Figure 13. Moderating effect of chronic warm and responsive caregiving on the association between caregiver history of adversity and child diurnal bedtime cortisol values.

Figure 14. Two-Way Interaction: Caregiver History of Adversity X Acute Warmth and Responsivity

Figure 14. Moderating effect of acute warm and responsive caregiving on the association between caregiver history of adversity and child diurnal noon cortisol values.

Figure 15. Two-Way Interaction: Caregiver History of Adversity X Acute Warmth and Responsivity

Figure 15. Moderating effect of acute warm and responsive caregiving on the association between caregiver history of adversity and child diurnal bedtime cortisol values.

Figure 16. Child Diurnal Cortisol Profile Plotted as Four Groups of Differing Risk
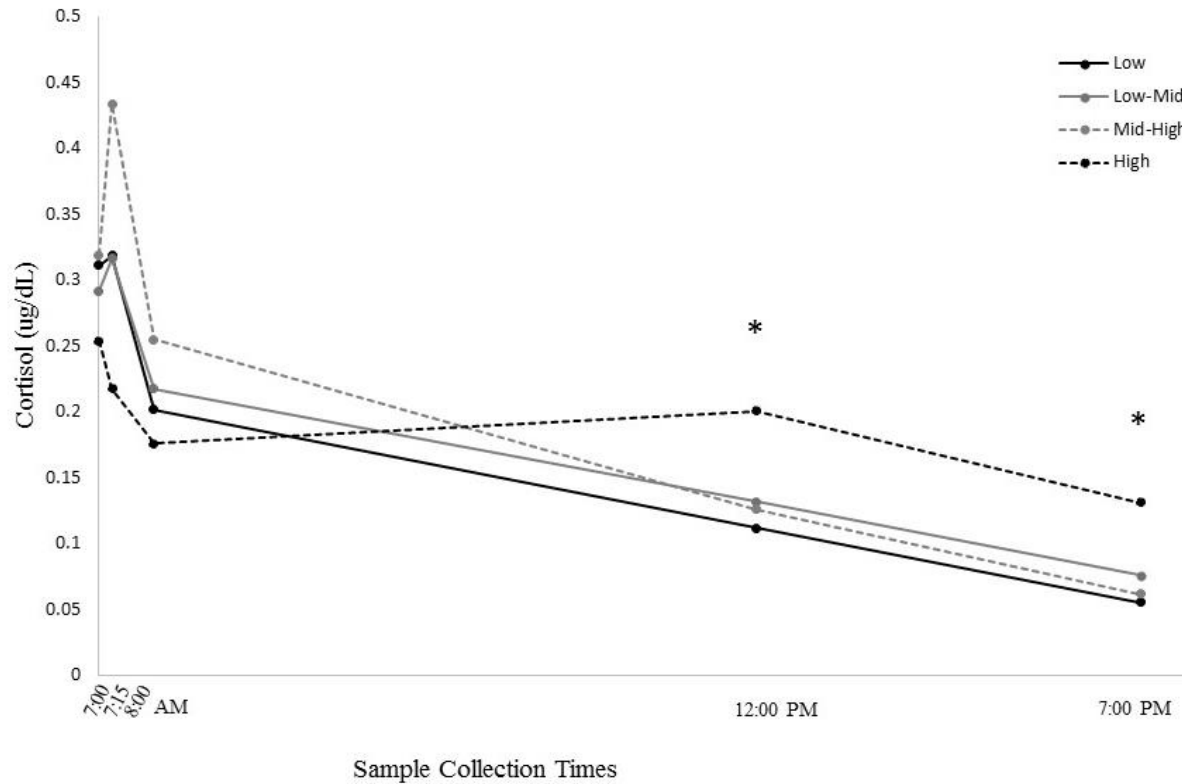

Figure 16. Child diurnal cortisol values averaged across two collection days controlling for income-to-needs, caregiver race, and child early life stress. History of adversity is capture in four groups of increasing risk; low adversity (n=30), low-to-mid (n=23), mid-high (n=11), and high (n=6). The Noon sample was higher for children of caregiver's with high adversity (M =.31, SD =.22) as compared to children of caregiver's with low (M =.10, SD =.08), low-mid (M =.11, SD =.08), mid-high (M =.13, SD =.07), $F(3,63)$= 7.55, $p<$ .001. The Bedtime sample was higher for children of caregiver's with high adversity (M =.19, SD =.17) as compared to children of caregiver's with low (M =.06, SD =.04), low-mid (M =.07, SD =.08), mid-high (M =.06, SD =.02), $F(3,63)$=4.81, $p<$ .01

Figure 17. Child Stress Reactive Cortisol Profile Plotted as Four Groups of Differing Risk

Sample Collection Times
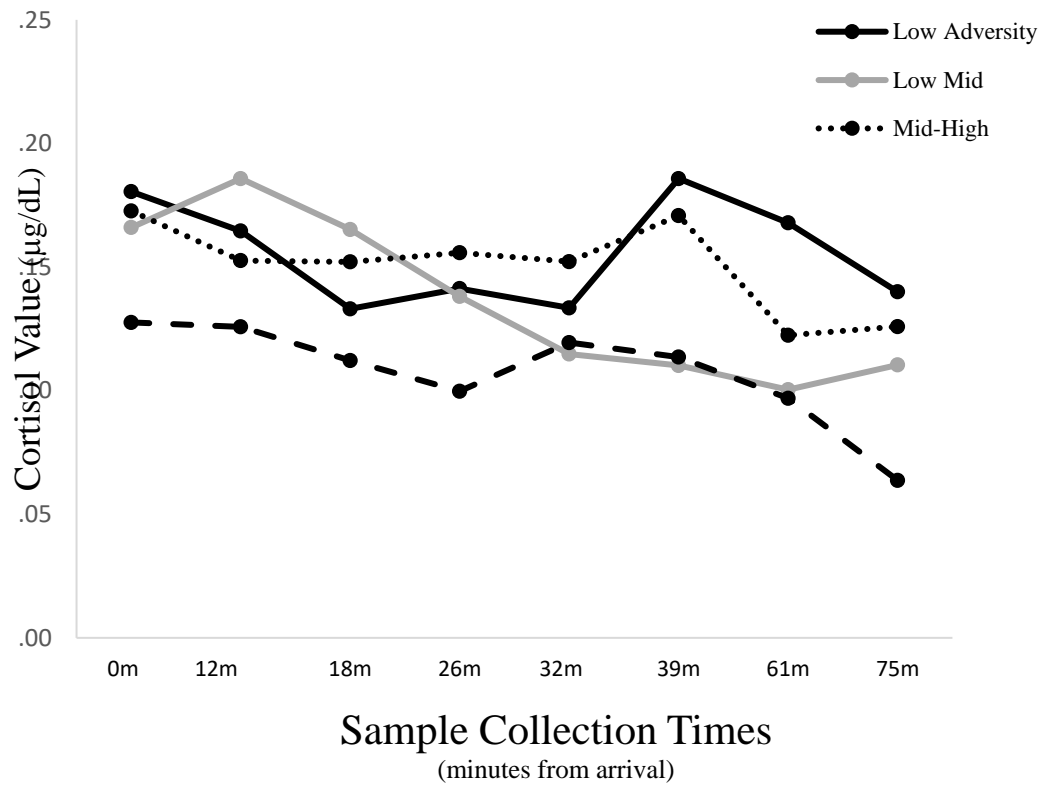(minutes from arrival)

Figure 17. Child stress reactive values during the second home visit which included a 7- minute structured stress paradigm. The caregiver-infant dyad participated in the 3-bag task (0-11m), stress paradigm (12m - 18m), free play and interview (26m – 75m). Peak reactivity values are expected between 32m and 39m as cortisol spikes are detectable 15-20m after an experienced stressor (18m). No group differences were observed for child stress reactive cortisol values.
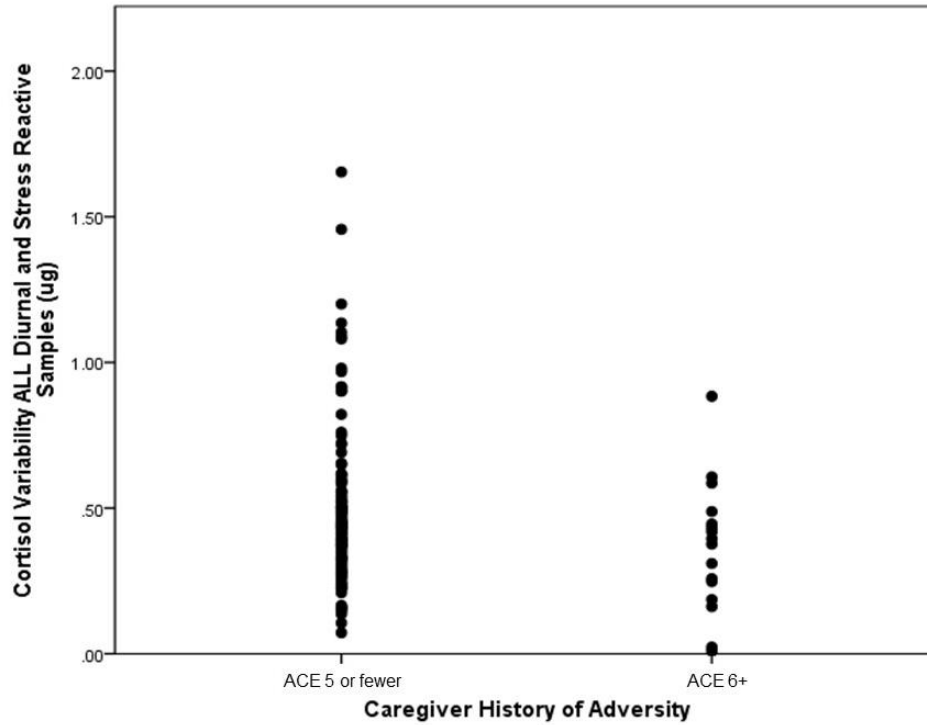
.Figure 18. Child Physiologic Range

Figure 18. The difference between the highest and lowest cortisol value for each child (diurnal and stress reactivity) is plotted above. Caregivers who endorsed six or more adverse childhood experiences are characterized as high adversity (n=15). The physiologic range was marginally smaller for children of caregiver's with high adversity (*M* =.36, *SD* =.22) as compared to children of caregiver's with lower adversity (*M* =.49, *SD* =.27) *t*(131)=1.82, *p*=.07.

Appendix C: Measures

Measure 1. Frequency of Risk Items in Environmental Instability Composite Score

Environmental Instability
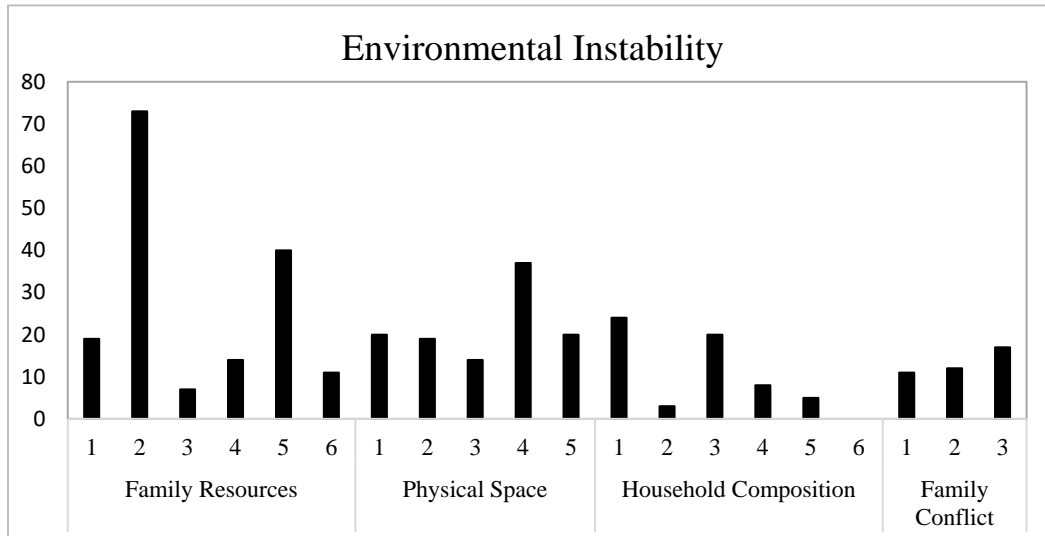
Family Resources
1. Are you now having serious or disturbing problems with your financial matters?
2. Was this a typical monthly income for you? (reverse coded; income stability)
3. (Employment) How stable is it? (reverse coded; income stability: varies=1)
4. Does your family have a car? (reverse coded)
5. In the last 12 months, we relied on government based breakfast/lunch food programs to feed our children. (dichotomized; 0-no; 1- sometimes or usually)
6. In the last 12 months, were you ever hungry but didn't eat because you couldn't afford enough food?

Physical Space
1. Has your family moved two or more times in the last year?
2. Does your home need emergency repairs?
3. Does your home have any problems with heating or plumbing?
4. Does your family have enough space? (reverse coded; perceived crowding)
5. Total people/Total bedrooms (dichotomized; physical crowding: 3 or more to a bedroom)

Household Composition
1. Have other people moved in with you in the last year?
2. Your child got a new guardian or step-parent.
3. Your child's parents separated or divorced.
4. Many times there was no one to take care of your child.
5. Your child had to live with a relative or friend for a while.
6. Your child was in a foster home (*excluded from composite variable due to no endorsements*)

Family Conflict
1. Are you now having serious or disturbing problems with your marriage?
2. Are you now having serious or disturbing problems with your family?
3. Your child was upset by family arguments.