Does Stress Predict the Development of Internalizing Symptoms in Middle Childhood?:

An Examination of Additive and Interactive Effects

of Early, Daily, and Physiological Stress

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Arts

Approved April 2020 by the
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May 2020
ABSTRACT

Stress in individuals presents in various forms and may accumulate across development to predict maladaptive physical and psychological outcomes, including greater risk for the onset of internalizing symptoms. Early life stress, daily life experiences, and the stress response of the hypothalamic-pituitary-adrenal (HPA) axis have all been examined as potential predictors of the development of psychopathology, but rarely have researchers attempted to understand the covariation or interaction among these stress domains using a longitudinal design when looking at the influence of stress on internalizing psychopathology. Further, most research has examined these processes in adulthood or adolescence with much less attention given to the influence of these dynamic stress pathways in childhood. Guided by the biopsychosocial model of stress, this study explored early life stress, daily life stress, diurnal cortisol (cortisol AM slope), and internalizing symptoms in a racially/ethnically and socioeconomically diverse sample of twins participating in an ongoing longitudinal study (N=970 children; Arizona Twin Project; Lemery-Chalfant et al. 2013). An additive model of stress and a stress sensitization framework model were considered as potential pathways of stress to internalizing symptoms in middle childhood. Based on a thorough review of relevant literature, it was expected that each stress indicator would individually predict internalizing symptoms. It was also predicted that early life stress would moderate the associations between diurnal cortisol and internalizing symptoms, as well as daily life stress and internalizing symptoms. Multilevel modeling analyses showed that early life stress and cortisol AM slope, but not daily life stress, predicted internalizing symptoms.
Early life stress did not moderate the associations between daily life stress and internalizing symptoms or cortisol AM slope and internalizing symptoms. Results support independent additive contributions of both physiological stress processes and early life parental stressors in the development of internalizing symptoms in middle childhood. Future investigation is needed to better understand the sensitizing effects of early parental life stress during this developmental stage.
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Introduction

Stress experienced in early childhood, ranging from adverse early life experiences to daily hassles, is associated with poor physical health outcomes and low psychological well-being in later childhood, adolescence, and adulthood (Grant et al., 2003; McMahon et al., 2003; Gershon et al., 2013). Experiences of stress in children is quite varied and is a critical component in children’s maladaptive health, specifically increased risk for internalizing symptoms (Brumariu & Kerns, 2010). Early life environment, current life stress, and diurnal cortisol are three indicators of stress experiences that need further examination given their potential individual and interactive effects on the emergence of internalizing symptoms in children. Although these pathways are often theorized, empirical research has yet to examine in one all-encompassing study how these various stress experiences may individually contribute and interact to lead to the onset of anxiety and depression symptoms in children.

Anxiety and depression are among the most common forms of psychopathology in children and adolescents (Costello et al., 2011). A recent meta-analysis of worldwide-pooled prevalence of mental disorders in children and adolescents included 41 studies and 27 countries and found the worldwide prevalence of any anxiety disorder and any depressive disorder was 6.5% and 2.6%, respectively (Polanczyk et al., 2015). Examining community samples allows for the detection of sub-threshold symptoms and the ability to detect whether these symptoms are present at an earlier age. One study of a community sample of children ages 11, 13, and 15 found 18% of youth self-reported symptoms of depression, with a higher proportion reported in females (25%) as compared to males (10%), but overall prevalence of depressive symptoms increased with age for both males
and females (Saluja et al., 2004). A more recent study of a community sample of adolescents ages 12 to 17 also found depressive symptoms to be more common in females and older age (Wartberg et al., 2018). Similarly, self-reported levels of anxiety increased with age in a community sample of children with 7.3% of the sample overall in the anxious range, including 2.3% 8-10 years old, 7.9% 11-13 years old, and 15.9% 14-16 years old reporting symptoms of anxiety (Mazzone et al., 2007). In a sample of children and adolescents ages 8 to 17, girls scored significantly higher on all anxiety disorders and symptoms were higher in adolescents than in children for all anxiety disorders with the exception of separation anxiety, which decreased with age (Orgilés et al., 2012). This overall increase in symptoms with age supports the importance of studying internalizing symptoms in children to determine what factors may be contributing to the development of these symptoms.

Studying the onset and development of stress calls for the need to include different time courses and manifestations of stress, as stress incorporates multiple factors. Lazarus (1966) proposed that stress occurs when perceived demands from the environment exceed an individual’s ability to cope with them. This definition has been amended to consider stress as a process, including a stimulus, appraisal, and response (Cohen et al., 1995; Miller et al., 2011). Aside from stress being a process, it is also understood to span across multiple realms. Prior research on stress and coping has supported the biopsychosocial model of stress (Blascovich et al., 1999; Mendes et al., 2003), which serves as a framework to explore pathways through which biological, psychological, and social factors impact health and adjustment. The three components of
stress and adaptation in this model include external or environmental stimuli, internal or physiological reactions to stress, and reciprocal relations between external and internal processes (Bernard & Krupat, 1994). It is necessary to focus not solely on external or internal reactions, but to study these together as there is a mutual course of stress processing that includes the environment and an individual’s physiology.

Within these stress processes, acute stress and chronic stress are two broad categories of stressors that have been studied across development. Both types of stress have been linked to indicators of health and adjustment ranging from flu symptoms to depression (e.g., Compas, 1987; DeLongis et al., 1988; Grant et al., 2003; Gunnar & Quevado, 2007). Developmental psychology emphasizes the enduring effects of chronic stress, such as early life adversity and recurring stressors experienced by those growing up in adverse environments (e.g., Blair & Raver, 2012; Miller et al., 2011). However, researchers have also considered the health relevance of acute stressors that change day to day and involve seemingly small, but potentially compounding disruptions to an individual’s typical functioning (e.g., DeLongis et al., 1988; Hanson & Chen, 2010). Theoretical and empirical evidence suggests that physiological stress processes and perceived stressors, ranging from chronic stressors to daily hassles, have collective and potentially interactive effects on subsequent negative health outcomes (Brown, 1993; Heim et al., 2000; Kessler, 1997). Therefore, it is important to study both acute and chronic stress to fully understand the body’s response to stress and downstream effects of these various stressors.
Early Life Stress and Internalizing Symptoms

Early life stress and adversity in childhood, such as trauma, neglect, and violence, have been associated with later physical and mental health problems (Johnson et al., 2013; Shonkoff et al., 2012) at various stages of development. The Adverse Childhood Experiences (ACES) study connected negative childhood experiences to later health conditions and research has continued to demonstrate a range of negative health consequences in adulthood, including depressive symptoms (Felitti et al., 1998; Windle et al., 2018). There is also evidence for a dose–response relationship between the number of experienced childhood adversities and the presence of a depressive episode (Chapman et al., 2004), suggesting that the combination of adversities leads to more severe consequences. ACES has also been found to have consequences that present earlier on in development, including increased alcohol use (Dube et al., 2006), smoking (Anda et al., 1999), and depressive symptoms, drug abuse, and antisocial behavior (Schilling et al., 2007) in adolescence. In children, increased ACES scores are related to increased risk of learning and behavior problems and obesity (Burke et al., 2011) and poor mental health, chronic medical conditions, and social development (Kerker et al., 2015).

The majority of early adversity literature has focused on severe adversities, such as trauma, abuse, and neglect. More subtle sources of adversity in childhood, such as early life parental stress, may also affect a child’s well-being from a young age, warranting further investigation. Parenting-related stress variables including parenting daily hassles (Kliwer & Kung, 1998; Shaw et al., 1997), parental depressive symptoms (Billings & Moos, 1983; McCarty & McMahon, 2003), lack of social support (Foster et
al., 2008; Owen et al., 2008), lack of parent-child emotional availability (Easterbrooks et al., 2012; Sturge-Apple et al., 2006), chaos in the home (Evans et al., 2005; Fiese & Winter, 2009), and use of punitive punishment (Laskey & Cartwright-Hatton, 2009; Silk et al., 2011; van der Sluis et al., 2015), have individually been found to have an effect on child internalizing symptoms. While a large amount of literature focuses on severe adversity and health outcomes, a composite of the parenting-related stress variables was used in the present study to determine how more subtle disruptions during infancy may impact a child’s development and have negative repercussions. The role of parenting within a child’s early life environment is especially critical to examine in this study, as it used a normative community sample, and, therefore, parenting stress may have a greater association with child internalizing symptoms compared to that of extreme adversity.

**Daily Experiences of Stress and Internalizing Symptoms**

Whereas major life events and early adversity are recognized as important precursors to the etiology and maintenance of internalizing disorders, stress in the form of daily hassles, plays a role at all stages of development as well. Research in adults has shown that daily hassles, such as recent stressful life events and interpersonal relationship difficulties, are positively associated with depressive symptoms (O’Sullivan, 2004). In adolescents, experience of daily stressful interpersonal events has been found to be prospectively associated with overall depressive symptoms (Hankin et al., 2007). Sex differences in stress and the prediction of internalizing symptoms has been found, with adolescent girls reporting more stressful events, higher perceived stressor intensity, and higher levels of internalizing symptoms (Jose & Ratcliffe, 2004).
Effects of daily stress have also been observed even earlier in development. Prior research has concluded that higher child-reported daily stress levels are associated with increased reports of physical health symptoms (Burkhart et al., 2017) and difficulties with emotional adjustment (Bai & Repetti, 2018), especially for girls and youth from low socioeconomic backgrounds (Escobar et al., 2008). Additionally, children’s self-reported anxiety and depressive symptoms have been found to be correlated with their daily stressors (Banez & Compas, 1990). Higher depressive symptoms have been found in children with higher negative emotion reactivity to daily school problems (Bai & Repetti, 2018) and greater exposure to daily hassles (Keles et al., 2017). It is critical to continue examining children’s daily life stress, as these experiences may have more of an impact on the onset of internalizing symptoms in children than major and traumatic life events have at these young ages (Escobar et al., 2008).

**Physiological Stress Processes and Internalizing Symptoms**

In addition to children’s experiences of stressful events and daily hassles, one must also consider the physiological stress processes that occur to fully understand the stress response and regulation system in children. The HPA axis, one of the body’s two major physiological stress systems, is a major neuroendocrine stress response system that is intended to maintain stability and health by allowing for adaptation to changes in demand (McEwen, 2004). The three components of the HPA axis, the hypothalamus, pituitary gland, and adrenal glands, connect the autonomic nervous system to the endocrine system and work together to regulate an individual’s response to stress, mood, motivation, metabolism, energy levels, and immune system.
Cortisol, the primary hormonal end product of the HPA axis, is an important marker of stress and it can be measured various ways depending on the study procedures and outcomes of interest. Cortisol is secreted by the adrenal glands to decrease stress levels and return the body to homeostasis (Kirschbaum & Hellhammer, 1989). Two widely used methods of measuring cortisol are cortisol reactivity to lab stressors and diurnal cortisol patterns. Cortisol reactivity can be examined when timing is carefully measured in a controlled lab environment following implementation of a stressor, with cortisol peaks in saliva approximately 20–25 minutes after the stressor followed by a return to baseline within the hour (Nicolson, 2008). Within the diurnal pattern, normative levels of cortisol are high upon waking in the morning, increase by 50% to 60% in the first 30 to 40 minutes after waking (known as the cortisol awakening response [CAR]), and decline throughout the day reaching the lowest point near midnight (Adam & Kumari, 2009; Pruessner et al., 1997). Diurnal cortisol can be measured by diurnal slopes, CAR, and overall cortisol output, referred to as area under the curve (AUC) (Adam & Kumari, 2009).

Researchers have argued that the individual’s perception of the stressors, as opposed to the stressors themselves, is responsible for determining the magnitude of the response and recovery of cortisol following a stressor (Guilliams & Edwards, 2010). There is a large existing literature providing cross-sectional evidence of altered HPA axis activity in youth with depression (Lopez-Duran et al., 2009), but few studies have examined whether indicators of the HPA axis prospectively predict internalizing symptoms and disorders in younger healthy populations. Prospective interpretation is
called for, as previous research has suggested that relations between HPA axis functioning and internalizing symptoms may be dynamic (Hastings et al., 2011).

Diurnal cortisol research thus far has focused mostly on specific populations of adolescents and minimally in children. Using the CAR as the diurnal indicator, studies of late adolescents at risk for the development of internalizing psychopathology have predicted onsets of MDD (Adam et al., 2010) and anxiety disorders (Adam et al., 2014), but it has yet to be determined whether the CAR predicts internalizing symptoms independently of subsequent acute stress or whether a greater CAR indicates vulnerability to later acute stress (Vrshek-Schallhorn et al., 2013). Most recently, research has suggested that greater CAR acts both independently and interactively with acute interpersonal stress to predict depressive symptoms in early adolescent girls (Stroud, Vrshek-Schallhorn, et al., 2019). Cortisol has also been found to interact with greater early life adversity and be prospectively predictive of later adjustment for certain subpopulations, including lower levels of latent trait cortisol and increases in internalizing symptoms in adolescent girls (Stroud, Vrshek-Schallhorn, et al., 2019), as well as lower diurnal cortisol and increases in adjustment problems (externalizing problems, internalizing problems, and hyperactivity) in preschool age children (Lengua et al., 2019). Few studies have examined whether diurnal cortisol may be concurrently or prospectively associated with internalizing symptoms in childhood. One study found low basal cortisol to be associated with concurrent symptom severity and high cortisol representing adolescents at risk for increasing mental health symptoms (Shirtcliff & Essex, 2008), but more research is needed to fully understand these associations.
A focus on prediction of symptom level variability, rather than diagnostic status, may be a potential direction. Increased HPA axis activity has demonstrated neurobiological alterations early in development, but has not yet concluded whether these changes are markers of the onset of mental disorders (Hatzinger et al., 2007). Variations in diurnal cortisol have been linked with internalizing problems in young children, such that a flatter diurnal rhythm is linked to higher internalizing problems (Saridjan et al., 2014). This finding holds importance and calls for further exploration as it suggests that variations in diurnal cortisol may precede internalizing symptoms early in life before onsets of disorders that typically occur during puberty and adolescence (Costello et al., 2011). Longitudinal studies are needed to explore HPA axis dysregulation as a causal or contributing factor to child psychopathology (Buitelaar, 2013). Given evidence of direct and interactive effects of cortisol on psychopathology, further research on physiological stress regulation in children is needed.

**Stress Sensitization Theoretical Framework**

The stress sensitization model suggests that, over time, an individual requires less of a stress exposure to be triggered and reach heightened levels of stress (Hammen et al., 2000). Specifically, there is evidence that early life stress sensitizes individuals to subsequent proximal stress, placing them at greater risk for the onset of psychopathology in childhood, adolescence, and adulthood (Stroud, Chen, et al., 2019). A range of major life events, including acculturation, trauma, and sexual abuse in childhood have been shown to alter physiological sensitivity to stressful events in adulthood and influence subsequent health and well-being (Kendler et al., 2004; Mangold et al., 2010). Multiple
studies looking at negative life events and internalizing symptoms in adolescents also supported the model of stress sensitization, finding that those who experienced high levels of life stress showed a stronger relation between negative life events and internalizing symptoms (Ruttle et al., 2014). Specifically, adolescents experiencing a greater number of stressful life events showed higher levels of depression (Jenness et al., 2019). These findings suggest that early life stress may create a susceptibility to internalizing symptoms with successive stress. Although the child literature is lacking in regard to how stress may increase risk for psychopathology in children, there is evidence that maternal stress during infancy may sensitize children’s HPA axis response to subsequent stress exposure, as children exposed to high levels of early maternal stress had elevated cortisol levels and exhibited greater mental health symptoms (Essex et al., 2002).

There is also some evidence that early life stress may moderate the impact of daily stress on psychological symptoms. The stress sensitization model predicts the frequency of major life events decreases, while the frequency of minor events increases, as the occurrence of minor events prevent major events from having the opportunity to effect the onset of psychological episodes due to their increasing impact and generally high base rate (Stroud, 2018). Past research has concluded that those with a history of early life stress are more sensitive to the depressogenic effects of stressful life events, and this relation is greatest for those who have experienced the most severe types of abuse (Kendler et al., 2004; Shapero et al., 2013). One study aimed to replicate and extend this work in adult samples to a sample of young children and found that the trauma-exposed
youth with current life stress had higher levels of internalizing and externalizing symptoms compared to those without trauma exposure (Grasso et al., 2012). In contrast to the adult literature, accounting for the number of traumatic events did not change the results, suggesting that any type of early trauma exposure sensitizes young children to subsequent, non-traumatic life stress and places them at increased risk for various psychological symptoms (Grasso et al., 2012). A recent study indicated that childhood adversity was associated with increased stress perception and higher morning cortisol levels in children, suggesting a stress vulnerability following childhood adversity exposure (LoPilato et al., 2019). Previous research has yet to fully understand the associations between early life stress, cortisol levels, and resulting psychopathology. The current study aimed to further explore associations between early life stress and current stress to determine whether early stress moderates associations between physiological stress and daily stress in the prediction of internalizing symptoms in children.

**Present Study and Hypotheses**

The current study extended existing literature by examining all three previously discussed indicators of stress as independent predictors, as well as early life stress as a potential moderator of daily life stress and diurnal cortisol in the prediction of internalizing symptoms in children. The study aimed to expand on the existing literature in adult and adolescent populations by looking at the relation of stress and psychopathology in children to determine whether internalizing symptoms may start to manifest at an even earlier age as a result of early adversity, daily stress, and physiology. Early parental life stress was used as the early adversity indicator to help determine
whether parental stress has longitudinal effects on the development of internalizing psychopathology in children. Analyses controlled for internalizing symptoms at the 8 year assessment and used internalizing symptoms at the 9 year assessment as the main outcome of interest. The study aims and hypotheses were as follows:

The first aim was to longitudinally assess each independent indicator of stress (early parental life stress, daily life stress, and diurnal cortisol) as predictors of internalizing symptoms at 9 years while controlling for prior symptoms (Figure 1).

**(Hypothesis 1)** I hypothesized an additive effect such that higher levels of each stress indicator (early life stress, daily life stress, diurnal cortisol) would independently predict greater internalizing symptoms at age 9 (Doane et al., 2013; Herringa et al., 2013). In terms of diurnal cortisol, I expected that flatter diurnal cortisol AM slopes and greater AUC would predict greater internalizing symptoms, as alterations in physiology have been associated with depressive symptomology in children (Ruttle et al., 2011; Shirtcliff & Essex, 2008).

The second aim was to examine a moderation model based on the stress sensitization theoretical framework (Hammen et al., 2000). The stress sensitization model examined early parental life stress as a moderator of the effects of daily life stress and diurnal cortisol on internalizing symptoms (Figure 2).

**(Hypothesis 2)** I hypothesized that there would be significant interactions between early parental life stress and both diurnal cortisol and daily life stress in the prediction of later internalizing symptoms, such that children who experience high levels of early parental life stress would experience stronger associations between a) cortisol AM slopes and
AUC (i.e. flatter slopes, greater AUC) and later internalizing symptoms and b) daily life stress and later internalizing symptoms, as compared to children who experienced lower levels of early parental life stress (Essex et al., 2002; Grasso et al., 2012).

Method

Participants

Families were recruited through birth records as part of an ongoing longitudinal study of twins (N=970 children; Arizona Twin Project; Lemery-Chalfant et al. 2013). Data for the current study were collected in 2011 for the 30 month assessment, 2017 for 8 year, and 2018 for 9 year, with 510, 698, and 714 children, respectively, participating at each of these time points. This community-based sample includes twin children and their primary caregivers (95% mothers) that are part of a racially/ethnically (>31% Hispanic/Latino) and socioeconomically diverse (45.8% growing up in households below middle class) cohort.

New participants were recruited at each of these three time points. A total of 970 children participated in at least one of the three time points, with 321 children participating in all three time points. 100 children participated in the 30 month time point only, while 410 children continued participation in the 8 year and/or the 9 year assessment in addition to the 30 month time point. There was high overlap in children participating in the 8 and 9 year time points, as 542 children participated in both the 8 year and 9 year assessment.

Attrition analyses were conducted to compare children who participated in the 30 month assessment only to children who participated at 30 month, as well as the 8 year
and/or the 9 year time points. These groups differed in family SES \((t = -2.503 [95\% CI: -0.421, -0.051]; p = .013)\), such that lower SES families were less likely to participate at the follow-up assessments. The groups were not statistically different in age \((t = 1.063 [95\% CI: -0.030, 0.100]; p = .290)\), sex \((t = 0.612 [95\% CI: -0.076, 0.145]; p = .541)\), or ethnicity \((t = -1.733 [95\% CI: -0.162, 0.011]; p = .085)\). These groups also did not differ on their level of early life stress at the 30 month assessment \((t = 1.420 [95\% CI: -0.070, 0.420]; p = .157)\).

For those children that participated in the 8 year time point, analyses were conducted to determine whether there were differences between the children who completed saliva sampling (89% of children) and those children who did not participate in the saliva sampling portion of the study. These two groups did not show any differences in age \((t = 0.620 [95\% CI: -0.120, 0.230]; p = .536)\), sex \((t = -0.528 [95\% CI: -0.126, 0.073]; p = .598)\), ethnicity \((t = 1.338 [95\% CI: -0.308, 1.614]; p = .182)\), or SES \((t = 0.207 [95\% CI: -0.199, 0.246]; p = .836)\).

**Procedure**

Institutional Review Board approval was obtained for all phases of the study. Caregivers provided written informed consent before all assessments; twins verbally assented at the 8 year and 9 year assessments. Caregivers completed questionnaires via phone when the twins were approximately 12 and 30 months of age. As the twins approached 8 years of age, families were contacted to participate in an intensive assessment of daily activities including sleep, physiological health, and the home environment. The 8 year assessment included surveys, two home visits, and a week of daily assessments, including wearing a wrist-based accelerometer (Ambulatory
Monitoring, Inc, Ardsley, NY USA) to measure objective sleep, daily diaries, and three
days of salivary cortisol collection. Two trained research assistants administered
questionnaires, cognitive and interaction tasks, and took biological assessments of the
twins and caregivers during the first home visit. Also, at this time the research assistants explained the actigraph, cortisol, and daily diary procedures to the families. Primary caregivers were instructed to complete a daily diary each night for seven consecutive
days, which included a subjective assessment of the twins’ daily stress. Parents reported on each twin separately in the diary. Additionally, an assessment table tracking bedtimes, wake times, and saliva sampling times of the twins was completed by primary caregivers to supplement times provided on the salivary cortisol vials. During the assessment week, study staff contacted the families every evening to ensure the protocols were being followed and to address any concerns. Approximately a week later, two research assistants returned to the families’ homes for the second home visit in which the actigraph watches, cortisol samples, and any paper daily diaries were retrieved.

As the twins approached 9 years of age, families were contacted to participate in an annual follow-up assessment consisting of a single home visit. Two trained research assistants administered questionnaires, cognitive and interaction tasks, and took biological assessments of the twins and caregivers during this home visit. Both the twins and their caregivers reported on psychological symptoms at this time point. Families were compensated for their time at each wave of the study.
Measures

30 Month Parental Stress and Strain Composite. Six measures (described in Miadich et al., 2019) made up the parental stress composite: Parenting Daily Hassles frequency (α = .81; Crnic & Greenberg, 1990); MOS Social Support Survey (reverse scored; α = .83; Sherbourne & Stewart, 1991); Confusion, Hubbub, and Order Scale (α = .71; Matheny et al., 1995); Center for Epidemiological Studies – Depression (α = .79; Radloff, 1977); Emotional Availability Scale (reverse scored; α = .74; Biringen et al., 2000); and Parent Responses to Child Misbehavior – Punitive Discipline (α = .59; Holden & Zambarabo, 1992). The first principal component explained 37.71% of the variance (scale loadings .46-.71) and regression values were retained as the parental stress composite (Miadich et al., 2019). Full information on the measures in the parental stress composite is included in Table 1.

Daily Diaries. On each evening of the study week at the 8 year assessment, primary caregivers completed daily diaries (55.66% online, 40.63% paper, 3.71% both) reporting on twins’ subjective sleep and bedtime behaviors, mood, activities, and food intake. As part of these daily diaries, primary caregivers reported each evening on the most stressful event that occurred for each twin during the course of that day and responded on a one to five Likert scale by answering the question “How stressful was the most stressful event your twin encountered today?” and typical responses included interpersonal stressors, such as a disagreement with a friend or co-twin (i.e. “Her brother teasing her,” “Fighting with brother over car seat belt”), losing privileges (i.e. “Not getting to get on video games,” “He wasn’t allowed to build his LEGO set), and school
expectations (i.e. “Working on a written project for school.”). The weekly average of the 7 days was used in analyses. There was excellent compliance with daily diary procedures from caregivers (85.19% of the sample completing all 7 diaries; 8.59% completed 6; 2.19% completed 5, and 4.03% completed fewer than 5).

**Salivary Cortisol.** Samples were gathered three times per day over three consecutive typical weekdays: at 30 minutes after waking, afternoon, and bedtime. Cortisol was gathered by either salivettes or passive drool, in which participants expressed their saliva through a small straw into a polypropylene tube. Caregivers of participants labeled saliva tubes and assessment table (i.e., placed in the refrigerator) with the time and date of the sample. Participants were instructed not to eat or drink or brush their teeth in the half hour before sampling. Families were asked to refrigerate cortisol samples until retrieved by study staff. Samples were collected at participants’ residences or returned by mail. The samples were refrigerated at -80 degrees Celsius until sent by Courier on dry ice over 3 days to the Biochemisches Labor at the University of Trier in Germany to be assayed for cortisol. Cortisol levels are stable at room temperature for several weeks and are unaffected by the conditions associated with a postal journey (Clements and Parker, 1998). Assays were conducted in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA) (Dressendorfer et al., 1992).

Measures of diurnal cortisol activity used in analyses include cortisol AM slope, or the slope of the diurnal cortisol rhythm from morning to afternoon (afternoon minus wake-up level divided by total time between the two samples), and average cortisol (calculated by taking the area under the curve (AUC) defined by all cortisol data points.
across the day; Pruessner et al., 2003). Each measure was averaged across the 3
collection days. Compliance with cortisol collection procedures was high with 89% of
families providing at least one day of cortisol samples at the 8 year assessment.

**Child Symptomatology.** The MacArthur Assessment Battery for Middle
Childhood was used to determine child internalizing symptoms at the 9 year assessment.
The MacArthur Health and Behavior Questionnaire (HBQ) was collected at both the 8
year and 9 year assessments and was used for parent-report of child symptoms at age 8.
The internalizing symptoms subscale (α = .85) consists of 29 items, including 7 items for
depression (α = .56), 12 overanxious items (α = .75), and 10 separation anxiety items (α =
.75) subscales. The Berkeley Puppet Interview (BPI) was used for child-report and was
collected only at the 9 year assessment. The BPI includes symptomatology, social and
academic modules, with a total of 62 items. The internalizing composite (α = .80) of the
BPI contains 11 items for depression (α = .59), 7 items for separation anxiety (α = .70),
and 8 items for over-anxiousness (α = .62). The HBQ and BPI do not yield clinical
diagnoses, but provide dimensional scales to assess children’s mental, social, and
academic well-being.

**Covariates.** Several variables (child age, sex, ethnicity, family SES, 8 year HBQ
internalizing symptoms) were explored as potential covariates based on known
associations with primary study variables (Cohen et al., 2006; DeSantis et al., 2007; Ice,
2005; Kirschbaum et al., 1999). If covariates were significantly associated with primary
study variables in preliminary analyses, they were included in the final analytic models.
Other variables (vacation, batch, time since waking) were conceptually included as covariates in all models due to their inherent relation with cortisol and/or daily life stress, regardless of their associations with primary study variables. Vacation, or whether the participant was in school or on vacation during participation, was included as a covariate due to the potential of variable sleep and stress while not in school (Kunz-Ebrecht et al., 2004; Martel et al., 1999). Saliva samples from participants were analyzed in a total of three assay batches, so batch was included as a covariate to control for any differences between batches (Franz et al., 2010). Time between waking and the morning cortisol sample was also calculated based on actigraphy. Each child wore a Motion Logger Micro Watch, a wrist-based accelerometer (Ambulatory Monitoring, Inc, Ardsley, NY USA) on their non-dominant wrist to detect waking time and primary caregivers reported exact time of each saliva sample in a provided assessment table (Kudielka & Kirschbaum, 2003). Time between waking and first sample was included as a covariate to control for any differences in sampling time which are particularly important in the first hour of waking due to the cortisol awakening response (i.e., the increase in cortisol levels that occur 30-45 minutes after waking, Adam & Kumari, 2009).

**Socioeconomic Status.** SES was assessed at the 8 year visit as a standardized composite of the family-income-to-needs ratio for 2016, primary caregiver education level, and spouse/partner education level (Doane et al., 2019). Primary caregivers reported their total household income in the past year (before taxes), and response categories included: (1) less than $30,001; (2) $30,001–$40,000; (3) $40,001–$50,000; (4) $50,001–$60,000; (5) $60,001–$70,000; (6) $70,001–$80,000; (7) $80,001–$90,000;
(8) $90,001–$100,000; (10) $100,001–$150,000; (11) More than $150,001. The income-to-needs ratio was calculated based on the number of individuals in the household and the federal poverty standards published for 2016. The mean of the family income range was divided by the federal poverty threshold for that household size (U.S. Census Bureau). Families scoring <1 were considered to be living in poverty (7.4% of sample at 8-year), near the poverty line if scored 1–2 (22.7% of sample at 8-year), lower middle class if scored 2–3 (15.9% at 8-year), and middle to upper class if scored above a 3 (54.0% at 8-year).

Primary caregivers also reported on their and their spouse/partner’s level of education using the following response categories: (1) less than high school; (2) high school or equivalent; (3) some college, not graduated; (4) college degree (e.g., BA, BS); (5) two or more years of graduate school; (6) graduate or professional degree.

**Data Analysis**

Potential covariates (e.g., child sex, age, ethnicity, and family SES), skewness, and kurtosis of variables were examined prior to analyses. Independent variables were centered prior to analyses and covariates were controlled for throughout analyses. Internalizing symptoms at age 8 measured by the HBQ was used as a baseline measure and controlled for throughout analyses with age 9 BPI internalizing symptoms as the outcome of interest. Values three standard deviations above or below the mean were considered outliers and treated as missing in all analyses. A logistic regression was conducted to determine whether variables at the 30 month assessment predicted participation in the 8 or 9 year time points. Continued participation after the 30 month
assessment was not predicted by early life stress ($OR = .875; SE = .128; p = .652$), ethnicity ($OR = 2.29; SE = .280; p = .198$), or sex ($OR = .838; SE = .259; p = .767$), but was predicted by age ($OR = .016; SE = .515; p = .001$) and SES ($OR = 2.858; SE = .172; p = .008$).

Structural equation modeling was employed to test study hypotheses using Mplus 8.0 (Muthén & Muthén, 1998-2017). Structural equation modeling is a useful approach for diary data because it makes use of all available data and allows for the inclusion of individuals with missing data in analyses, as some families were not recruited until later assessment points. New families were recruited into the study at each assessment point to ensure sufficient sample size for behavioral genetic modeling. A sandwich estimator was used to adjust the standard errors to account for the nesting of twins within families. Analyses were first conducted to determine whether there were correlations between the three stress indicators. Correlations were expected, especially between diurnal cortisol and daily life stress. If highly correlated, a latent factor composed of diurnal cortisol and daily life stress would be created to represent stress at the 8 year assessment.

Path models were used to test additive and moderating pathways and full information maximum likelihood (FIML) estimation was used to handle missing data. First, to examine independent or additive pathways, all independent variables were entered simultaneously in the model to predict internalizing symptoms. Second, to test moderating pathways, interaction terms between early parental life stress and the cortisol parameter, as well as daily life stress were computed and then entered one by one into the first model.
Results

Descriptive Statistics and Preliminary Analyses

Table 2 shows the general descriptive statistics of all variables used in the study analyses. Participants were 50.7% female and 58.8% were non-Hispanic White/Euro American. Table 3 shows the correlations between all study variables, including a negative correlation between cortisol AM slope and AUC \((r = -.58, p < .01)\). BPI internalizing symptoms at 9 years was positively correlated with early life stress \((r = .19, p < .01)\) and daily life stress \((r = .13, p < .05)\), and negatively correlated with cortisol AM slope \((r = -.12, p < .05)\). Cortisol AM slope was chosen as the cortisol measure to be used in the following models due to its stronger relation to internalizing symptoms at 9 years than AUC.

Preliminary analyses included mixed model regressions to account for the nesting of twins within families of individual potential covariates (child age, sex, ethnicity, and family SES) and BPI internalizing symptoms. None of these variables were significant predictors of BPI internalizing symptoms (all \(ps > .165\)). As such, none of these were included in final multivariate model testing with the exception of sex and SES. Sex was included due to the known sex differences between males and females in the onset of anxiety and depression (Altemus et al., 2014). SES was included in the model due to its significance in attrition analyses demonstrating that lower SES families at the 30 month assessment were less likely to participate in the subsequent assessments. Based on correlation analyses, HBQ internalizing symptoms (8 year) was included as a covariate in
all models. Vacation, batch, and time since waking were conceptually included as 
covariates in all models due to their inherent relation to cortisol and/or daily life stress.

Additive Stress Model

Prior to testing moderation, the direct association of each stress indicator with 
internalizing symptoms was examined. Both early life stress and cortisol AM slope 
significantly predicted internalizing symptoms at age 9 (Table 4). Specifically, greater 
earlier life stress at 30 months was associated with higher internalizing symptoms at age 9 
(\( \beta = .191 \) [95% CI: .053, .330]; \( SE = .071; \ p = .007 \)). Additionally, flatter (more positive 
or less difference across the day) AM slope was associated with lower internalizing 
symptoms at age 9 (\( \beta = -.128 \) [95% CI: -.227, -.030]; \( SE = .050; \ p = .011 \)). Daily life 
stress did not significantly predict later internalizing symptoms. None of the covariates 
were significant in this model with the exception of HBQ internalizing symptoms at age 8 
predicting BPI internalizing symptoms at age 9 (\( \beta = .126 \) [95% CI: .012, .241]; \( SE = 
.058; \ p = .030 \)).

Moderation of Early Life Stress

Table 5 shows the moderation models for early life stress with daily life stress 
(Model 1) and with cortisol AM slope (Model 2). In Model 1, there were significant main 
effects of early life stress (\( \beta = .192 \) [95% CI: .055, .330]; \( SE = .070; \ p = .006 \)) and AM 
slope (\( \beta = -.128 \) [95% CI: -.227, -.029]; \( SE = .050; \ p = .011 \)), but no significant 
interaction between early life stress and daily life stress. Similarly, Model 2 did not have 
significant moderation of early life stress on AM slope, but early life stress (\( \beta = .182 \)
[95% CI: .031, .333]; \( SE = .077; \ p = .018 \) and AM slope (\( \beta = -.123 \) [95% CI: -.225, -.022]; \( SE = .050; \ p = .018 \) were significant predictors of internalizing symptoms.

**Post-Hoc Sensitivity Analysis of Cortisol AM Levels**

A post-hoc sensitivity analysis was conducted using cortisol AM levels instead of AM slopes to determine whether morning cortisol level was a significant predictor of internalizing at 9 years (i.e., and likely driving associations between cortisol AM slope and internalizing). The same three models (additive model and two moderation models) were run and reports were consistent across all three models such that early parental life stress was the only significant stress indicator for the additive model (\( \beta = .194 \) [95% CI: .055, .332]; \( SE = .071; \ p = .006 \), moderation model 1 (\( \beta = .195 \) [95% CI: .057, .333]; \( SE = .070; \ p = .006 \), and moderation model 2 (\( \beta = .205 \) [95% CI: .071, .339]; \( SE = .069; \ p = .003 \), while daily life stress, cortisol AM levels, and the stress interaction variables were all non-significant. While cortisol AM slope was a significant predictor in the main analyses, AM level was not significant in post-hoc analyses. These results demonstrate that it is not specifically the morning cortisol level, but the change in cortisol levels from morning to the afternoon that are associated with the development of internalizing symptoms.

**Discussion**

The results of this study support the importance of studying the roles of various stress indicators and their relation to internalizing symptomatology in middle childhood. I found that early parental life stress and cortisol AM slopes, but not daily life stress, additively predicted internalizing symptoms. Specifically, higher early parental life stress
at 30 months and steeper cortisol AM slopes at age 8 predicted greater internalizing symptoms at age 9 while controlling for internalizing symptoms at age 8. I did not find support for either of our moderation models, such that early parental life stress did not significantly moderate the association between cortisol AM slopes and internalizing symptoms or daily life stress and internalizing symptoms. These findings underscore the importance of understanding multiple indicators of stress simultaneously from multiple time points, as longitudinal associations between stress and internalizing psychopathology can be detected as early as middle childhood.

The hypothesis of an additive effect of higher levels of each stress indicator predicting greater internalizing symptoms was partially supported. Although daily life stress was not a significant predictor, higher early parental life stress predicted higher levels of internalizing symptoms, which is in line with previous research using early life stress indicators in the prediction of later psychopathology (Herringa et al., 2013). There is a growing body of literature that has examined the effect of early life stress on psychopathology across the life course, and has found consistent support for the individuals who have experienced early life stress to be more likely to develop mood and anxiety disorders than those with no history of early life stress exposure (for review see McLaughlin, 2019). Exposure to early life stress is associated with heightened vulnerability to psychopathology in childhood and adolescence, as well as prospectively into adulthood (McLaughlin et al., 2012; McLaughlin, 2019). A previous study focused on children found that early adversity before the age of 6 was associated with internalizing symptoms between the age of 7 and 13 (Jensen et al., 2015).
Aside from the evident associations between early adversity and internalizing symptoms, some research has focused on the role of parental life stress on the development of their children’s psychopathology. However, little research has focused on *early life* parental life stress and the majority has only examined associations between parental life stress and child internalizing symptoms cross-sectionally. For example, concurrent parental distress and discord have been linked to higher internalizing symptoms in adolescent females (Crawford et al., 2001). In a community sample of mother-child dyads, maternal parenting stress was significantly associated with children’s anxious and depressive symptoms at age 10 (Rodriguez, 2011). Our results complement previous research on the importance of early parenting stress and we expand previous work by demonstrating that early parental stress predicts *increases* in psychopathology across middle childhood.

There are multiple potential explanations for the direct effect between early parental life stress and internalizing symptoms in children. One explanation is that the various forms of early life stress may accumulate and pose higher cumulative risk, resulting in higher levels of allostatic load in the repeated attempts at regulation of the stress response system (McLaughlin, 2019). When considering the underlying mechanism behind this association, threatening experiences in childhood have led to the facilitation of rapid identification of potential threats in the environment (McLaughlin & Lambert, 2017). This enhanced perceptual salience and attention toward threatening stimuli for children raised in threatening environments has been linked to disruptions in social and emotional processing, as well as the onset of internalizing and externalizing
problems (McLaughlin, 2019; McLaughlin & Lambert, 2017). Aside from major life events that might be considered threatening stimuli, parenting daily hassles have also been associated with child internalizing and externalizing behavior problems (Crnic & Low, 2002). In this way, it may be the perceived stress of the child, or the experience that demands exceed abilities, that is contributing to child emotional and behavioral problems (Lazarus & Folman, 1984). It is also possible that children may perceive parental life stress as an unsafe aspect of their environment due to decreases in access to supportive resources and in their own self-confidence and competence (Willemen et al., 2008).

While our measure of early parental life stress may not include the same level of threatening experiences compared to studies that have examined early life adversity including abuse and neglect (e.g., Johnson et al., 2013; Shonkoff et al., 2012), parental life stress may accumulate over time and affect a child’s typical physiological stress processes.

The association between flatter (more positive or less difference across the day) AM slope and lower internalizing symptoms at age 9 was the opposite of my hypothesis and adds to the mixed findings of research examining relations between diurnal cortisol rhythms and internalizing psychopathology in middle childhood and adolescence (Ruttle et al., 2011; Shirtcliff & Essex., 2008). A recent meta-analysis on diurnal cortisol slopes and mental and physical health outcomes found that flatter diurnal cortisol rhythms across the day were associated with poorer physical and mental health outcomes, including increased risk of internalizing disorders (for review and meta-analysis see Adam et al., 2017; Adam & Kumari, 2009). Importantly, the meta-analysis focused
primarily on studies of adult samples, so results of the present study may differ due to
developmental differences in physiological stress processes (Stroud et al., 2009). Further,
there are multiple methodological differences between the present study and previous
research that may explain the opposing findings. Unlike the present study, most of the
studies in the meta-analysis were cross-sectional in nature, so they do not allow for
certainty in terms of direction of causality of the cortisol rhythms and health outcomes
(Adam et al., 2017). Further, the majority of the studies used wake-to-bedtime slopes,
which showed moderate effect sizes ($r = .118 (\text{.082 - .153}), p < .001$), while estimated
slopes over a shorter portion of the day than the full wake-to-bedtime span (i.e. 2 hours
post-awakening to bedtime) were the only type of slope measure that did not reveal
significant associations with health outcomes ($r = .029 (-.168 - .224), p = .773$; Adam et
al., 2017). Given that the present study focused on times that best fit the schedule of
children in middle childhood, results may complement other existing research in
childhood and early adolescence, given the variability in number of days of data
collection and number of samples per day in studies collecting cortisol data (Adam et al.,
2017).

A recent study identified similar patterns of children who were high on irritability
in toddlerhood, such that youth who were high in irritability showed significant
associations between steeper diurnal cortisol slope at age 9 and higher levels of
internalizing symptoms at age 12 as compared to their peers (Kessel et al., 2019). This
association between steeper diurnal cortisol slopes and greater internalizing symptoms is
consistent with the cortisol findings of the present study. When comparing these results to
the cross-sectional studies in the meta-analysis, it seems that taking a developmental approach will be key to understanding prospective associations between diurnal cortisol patterns and the development of internalizing symptoms. Given the numerous developmental changes in cortisol patterns across the pubertal transition (i.e. greater cortisol reactivity to stress, higher overall cortisol output; Gunnar et al., 2009; Stroud et al., 2009), our findings demonstrate the need to examine these associations over longer developmental periods and across developmental transitions (i.e., puberty). Looking at both chronicity of physiological stress processes and the development of psychopathology may help illuminate when and under what contexts steeper, or flatter, cortisol slopes may predict more adaptive or maladaptive outcomes.

Our findings did not support the stress sensitization framework, as early life stress did not moderate associations between cortisol indicators or daily life stress and internalizing symptoms. Specifically, the results of the present study do not support the hypothesis that early life stress sensitizes children to later stress, placing them at greater risk for internalizing problems with subsequent stress exposure (Essex et al., 2002; Grasso et al., 2012). There are multiple potential explanations for the lack of moderation that should be explored in future research. The existing literature has not been able to distinguish whether early stress increases the impact of stress, the frequency of stressful episodes, or both (Stroud, 2018). Second, although there is evidence that individuals with early life stress develop psychopathology in response to lower or average levels of stress compared to those without early life stress, it is unclear whether the subsequent stress includes major or minor stressful life events (Stroud, 2018). There is support for the
concept that individuals may be selectively sensitized to recent stress, such that sensitization effects are limited to recent stressors that match the early adverse experience (Slavich et al., 2011). The measures of early parental life stress and daily life stress in the present study may not be able to capture the match or mismatch between the early and recent life stress indicators. Additionally, sensitizing effects may vary according to developmental stage and gender, as differences have been found between prepubertal and pubertal boys and girls (Rudolph & Flynn, 2007). Many studies have found support for stress sensitization, but the types and amount of stressors required to see sensitizing effects are not fully understood (La Rocque et al., 2014; Monroe & Harkness, 2005; Rudolph & Flynn, 2007). The indicators of physiological stress in the present study have not been examined as thoroughly in the literature. A small number of prospective studies have shown that alterations in the stress response system mediate the prospective association between early life stress and internalizing psychopathology (McLaughlin et al., 2010). Subsequent studies should examine whether there are mechanisms (i.e. alterations in HPA axis regulation) that mediate stress sensitization effects in the context of early life stress (Stroud, 2018).

Limitations and future directions

There are several limitations of the current research. First, the daily life stress indicator was parent-report of their child’s most stressful event during that day. Parents may not be accurate reporters, as their perceptions of their children’s stress may not be representative of each child’s actual level of stress. This study used a sample of twins that also required parents to report on each child’s daily stress independently. Parents may not
have fully distinguished one child’s stress experiences from the other, and may have reported stress levels to be more similar than they actually were. It is also possible that parents underreported their children’s stress, as they were not with their children throughout the course of the day and may have relied only on their observations or what their children reported to them. Second, internalizing symptoms at age 8 were parent-report, while internalizing symptoms at age 9 were child-reported symptoms. Different informants posed challenges to the interpretation of findings because parents and children may provide discrepant ratings of children’s internalizing symptoms. It is inherently difficult to obtain ratings of psychopathology, as parents cannot directly observe children’s thoughts emotional states, and children may lack objectivity in their rating of their own cognitive and emotional processes (Hourigan et al., 2011). Third, the cortisol sampling method requiring three samples per day (30 minutes after waking, afternoon, and bedtime) for three consecutive days was impressive for cortisol sampling in children, but did not allow for the calculation of all commonly-used cortisol indicators (i.e., cortisol awakening response). The study used only one morning sample, as opposed to waking sample and a 30-minutes after waking sample, so I was not capturing the cortisol awakening response (CAR), or the spike in cortisol that occurs upon awakening which has been one of the common cortisol indicators to prospectively predict internalizing psychopathology in adolescence (Adam et al., 2010; Vrshek-Schallhorn et al., 2013). With only one morning sample collected, differences in morning cortisol levels could be attributed to differences in sample collection time. Therefore, variation in timing of sampling was controlled for throughout analyses by controlling for the time of waking
based on actigraphy, which is considered to be a gold standard method (Stalder et al., 2016). Fourth, the longitudinal design of the study led to some attrition and subsequent recruitment of new families. The lack of overlap between time points may have made it difficult to capture the interactive effects of early parental life stress with daily life stress and cortisol AM slopes because the interaction terms required children to have participated in both the 30 month and 8 year assessments.

This study provides multiple potential directions for future research. The lack of evidence of a moderation model in support of a stress sensitization framework calls for a better fitting model incorporating early life stress and later psychopathology. Instead of a moderating pathway, it is possible that daily life stress or physiological stress may mediate associations between early life stress and internalizing symptoms, as prospective studies have shown that alterations in stress response systems mediate the prospective relation between early adversity and internalizing psychopathology (Stroud, 2018). For example, one study found that greater early adversity within the family environment and low socioeconomic status each predicted greater stress reactivity in young adulthood, which in turn predicted the onset of anxiety and depressive disorders (McLaughlin et al., 2010). Similar findings in adult samples demonstrated that continued stress exposure in childhood and adolescence, in the form of both discrete stressful life events and ongoing chronic stress, accounted for associations between early adversity and depression in late adolescence (Hazel et al., 2008). As such, future studies should explore how daily life stress and related cortisol indicators may act as mediators in the relation between early life stress and internalizing symptoms in children. Due to differences in the stress
response system at various developmental stages, it may be beneficial to examine how these same stress indicators predict internalizing psychopathology a few years later into development. Rather than looking at internalizing symptoms as the outcome, examining depression and anxiety separately may also be a valuable next step. The stress sensitization model has empirical support in the context of major depression, but less is known regarding whether early adversity sensitizes individuals to recent stress in the context of anxiety disorders (Stroud, 2018). Looking at depression and anxiety as distinct outcomes may be telling, as these disorders come along with varied symptoms and may have differential associations with early life stress.

This study extends previous research in several ways. Using the early parental life stress composite as the early life stress indicator was novel, as the majority of previous research has focused on early adversity and relied on retrospective accounts of early life stress. An additional strength was the longitudinal examination of an ethnically and socioeconomically diverse sample using continuous measurement of internalizing symptoms demonstrating significant variability and change in internalizing psychopathology in middle childhood. The results of this study build on the strengths of the biopsychosocial model and underscore the importance of understanding multiple time courses and indicators of stress across development, rather than focusing solely on psychosocial or physiological factors in isolation. The incorporation of several stress indicators and psychopathology in naturalistic settings has allowed for further understanding of how social and psychological experiences, as well as daily
physiological functioning, collectively influence the development of internalizing psychopathology over the course of child development.
Table 1. Measures included in the Parental Stress and Strain Composite (30 month assessment) replicated from Miadich et al., 2019

<table>
<thead>
<tr>
<th>Construct</th>
<th>Measure</th>
<th>Sample Items</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily hassles</td>
<td>Parenting Daily Hassles Frequency Scale (Crnic &amp; Greenberg, 1990)</td>
<td>15 items; Continually cleaning up kids’ messes; Kids don’t listen, won’t do what they are asked without being nagged</td>
<td>.81</td>
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<td></td>
<td>Center for Epidemiological Studies – Depression Scale (Radloff, 1977)</td>
<td>20 items; I felt that I could not shake off the blues even with the help of my family/friends; I felt lonely</td>
<td>.79</td>
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<td></td>
<td>MOS Social Support Survey (reverse scored; Sherbourne &amp; Stewart, 1991)</td>
<td>6 items; Someone who shows you love and affection; Someone to help you with daily chores if you were sick</td>
<td>.83</td>
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<td>Lack of social support</td>
<td>Confusion, Hubbub, and Order Scale (Matheny, Wachs, Ludwig, &amp; Phillips, 1995)</td>
<td>10 items; No matter how hard we try we always seem to be running late; It’s a real “zoo” at our home</td>
<td>.71</td>
</tr>
<tr>
<td>Chaos in the home</td>
<td>Emotional Availability Scale (reverse scored; Biriringen, Robinson, and Emde, 2000)</td>
<td>28 items; You are usually in a good mood around Twin A; Twin A looks to you and listens to you when you try to talk to him/her</td>
<td>.74</td>
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<tr>
<td>Lack of emotional availability</td>
<td>Parent Responses to Child Misbehavior – Punitive Discipline Scale (Holden &amp; Zambarabo, 1992)</td>
<td>6 items; techniques used in response to children’s misbehaviors including spanking with hand and threatening</td>
<td>.59</td>
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Table 2. Participant Demographics and Descriptive Statistics

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<th></th>
<th>n</th>
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<th>SD</th>
<th>Skew</th>
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<tr>
<td>Female</td>
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<td>Non-Hispanic White/Euro American</td>
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<td>Vacation (8 year)</td>
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<td>Summer/Vacation</td>
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<td>Not Summer/Vacation</td>
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<td>71.4</td>
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<tr>
<td>Income-to needs Ratio (8 year)</td>
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<td>Living in Poverty</td>
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<td>Near the Poverty Line</td>
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<td>Lower Middle Class</td>
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<td>Middle to Upper Class</td>
<td>305</td>
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<td>Primary Caregiver Education (8 year)</td>
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<td>High school or equivalent</td>
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<td>Some college</td>
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<td>Daily Life Stress (8 year)</td>
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<td>AUC (8 year)</td>
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Note. 30 month N = 510. 8 year N = 698. 9 year N = 714. Participation in all 3 waves N = 321. SES (socioeconomic status) = mean composite of family income-to-needs ratio, primary caregiver education, and secondary caregiver education. HBQ Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the MacArthur Health and Behavior Questionnaire. Batch = samples from each participant were analyzed in a total of three assay batches. Time Since Waking = Time (in hours) between waking and morning sample. BPI Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the Berkeley Puppet Interview. Cortisol AM Slope = slope from morning sample to afternoon sample in nmol/L with outliers (> 3 SD above mean) treated as missing. AUC = area under the curve defined by all available points in nmol/L with outliers (> 3 SD above mean) treated as missing. Cortisol AM level = morning sample in nmol/L with outliers (> 3 SD above mean) treated as missing.
Table 3. Intercorrelations Among Study Variables

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<td>4. AUC (8 year)</td>
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<td>.06</td>
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<td>-.12**</td>
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<td>.07</td>
<td>-.09*</td>
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<td>14. BPI Internalizing (9 year)</td>
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<td>-.12*</td>
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<td>.06</td>
<td>-.08</td>
<td>.19**</td>
<td>.03</td>
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Note. * p < .05. ** p < .01. SES (socioeconomic status) = mean composite of family income-to-needs ratio, primary caregiver education, and secondary caregiver education. HBQ Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the MacArthur Health and Behavior Questionnaire. Batch = samples from each participant were analyzed in a total of three assay batches. Time Since Waking = Time (in hours) between waking and morning sample. BPI Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the Berkeley Puppet Interview. Cortisol AM Slope = slope from morning sample to afternoon sample in nmol/L with outliers (> 3 SD above mean) treated as missing. AUC = area under the curve defined by all available points in nmol/L with outliers (> 3 SD above mean) treated as missing. Sex (0 = male, 1 = female). Ethnicity (0 = Non-white, 1 = White). Vacation = participation during summer or holiday break (0 = not on vacation, 1 = on vacation). Cortisol AM level = morning sample in nmol/L with outliers (> 3 SD above mean) treated as missing.
Table 4. Additive Effects of Early Life Stress (at 30 months), Daily Life Stress (age 8), and AM Slope (age 8) on Internalizing Symptoms (age 9)

<table>
<thead>
<tr>
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<th>β</th>
<th>SE</th>
<th>p</th>
<th>95% CI</th>
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<tr>
<td>Early Life Stress (30 month)</td>
<td>0.191</td>
<td>0.071</td>
<td>0.007</td>
<td>0.053, 0.330</td>
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<tr>
<td>Daily Life Stress (8 year)</td>
<td>0.087</td>
<td>0.048</td>
<td>0.071</td>
<td>-0.007, 0.182</td>
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<tr>
<td>Cortisol AM Slope (8 year)</td>
<td>-0.128</td>
<td>0.050</td>
<td>0.011</td>
<td>-0.227, -0.030</td>
</tr>
<tr>
<td>Vacation</td>
<td>0.064</td>
<td>0.051</td>
<td>0.209</td>
<td>-0.036, 0.164</td>
</tr>
<tr>
<td>Batch</td>
<td>-0.038</td>
<td>0.050</td>
<td>0.442</td>
<td>-0.136, 0.060</td>
</tr>
<tr>
<td>Time Since Waking</td>
<td>0.013</td>
<td>0.057</td>
<td>0.822</td>
<td>-0.099, 0.125</td>
</tr>
<tr>
<td>Sex</td>
<td>0.047</td>
<td>0.045</td>
<td>0.305</td>
<td>-0.042, 0.135</td>
</tr>
<tr>
<td>HBQ Internalizing Symptoms (8 year)</td>
<td>0.126</td>
<td>0.058</td>
<td>0.030</td>
<td>0.012, 0.241</td>
</tr>
<tr>
<td>SES (8 year)</td>
<td>-0.019</td>
<td>0.049</td>
<td>0.705</td>
<td>-0.116, 0.078</td>
</tr>
</tbody>
</table>

Note. Results are from standardized model and significant effects are bolded. HBQ Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the MacArthur Health and Behavior Questionnaire. Batch = samples from each participant were analyzed in a total of three assay batches. Time Since Waking = Time (in hours) between waking and morning sample. Cortisol AM Slope = slope from morning sample to afternoon sample in nmol/L with outliers (> 3 SD above mean) treated as missing. Sex (0 = male, 1 = female). Vacation = participation during summer or holiday break (0 = not on vacation, 1 = on vacation). SES (socioeconomic status) = mean composite of family income-to-needs ratio, primary caregiver education, and secondary caregiver education.
Table 5. Early Life Stress as a Moderator of Associations between Daily Life Stress (Model 1) and Cortisol AM Slopes (Model 2) and Subsequent Internalizing Symptoms

<table>
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<tr>
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<tr>
<td></td>
<td>(\beta)</td>
<td>SE</td>
<td>(p)</td>
<td>95% CI</td>
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<tr>
<td>Early Life Stress (30 month)</td>
<td>0.192</td>
<td>0.070</td>
<td>0.006</td>
<td>0.055, 0.330</td>
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<tr>
<td>Daily Life Stress (8 year)</td>
<td>0.093</td>
<td>0.048</td>
<td>0.053</td>
<td>-0.001, 0.187</td>
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<tr>
<td>Cortisol AM Slope (8 year)</td>
<td>-0.128</td>
<td>0.050</td>
<td>0.011</td>
<td>-0.227, -0.029</td>
</tr>
<tr>
<td>Early Life Stress X Daily Life Stress</td>
<td>0.020</td>
<td>0.070</td>
<td>0.778</td>
<td>-0.118, 0.158</td>
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<tr>
<td>Sex</td>
<td>0.045</td>
<td>0.046</td>
<td>0.326</td>
<td>-0.045, 0.135</td>
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<tr>
<td>HBQ Internalizing (8 year)</td>
<td>0.129</td>
<td>0.059</td>
<td>0.030</td>
<td>0.013, 0.245</td>
</tr>
<tr>
<td>Vacation</td>
<td>0.064</td>
<td>0.051</td>
<td>0.209</td>
<td>-0.036, 0.163</td>
</tr>
<tr>
<td>Batch</td>
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<td>0.050</td>
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<td>-0.136, 0.060</td>
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<tr>
<td>Time Since Waking</td>
<td>0.010</td>
<td>0.057</td>
<td>0.855</td>
<td>-0.102, 0.123</td>
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<tr>
<td>SES (8 year)</td>
<td>-0.018</td>
<td>0.049</td>
<td>0.712</td>
<td>-0.114, 0.078</td>
</tr>
</tbody>
</table>

Note. Results are from standardized model and significant effects are bolded. HBQ Internalizing = internalizing composite (depression, separation anxiety, over-anxiousness) of the MacArthur Health and Behavior Questionnaire. Batch = samples from each participant were analyzed in a total of three assay batches. Time Since Waking = Time (in hours) between waking and morning sample. Cortisol AM Slope = slope from morning sample to afternoon sample in nmol/L with outliers (> 3 SD above mean) treated as missing. Sex (0 = male, 1 = female). Vacation = participation during summer or holiday break (0 = not on vacation, 1 = on vacation). SES (socioeconomic status) = mean composite of family income-to-needs ratio, primary caregiver education, and secondary caregiver education.
Figure 1. Study Aim 1: Additive Model

H₁: Higher levels of each stress indicator will independently predict greater internalizing symptoms.
H₂: Early parental life stress will significantly moderate both diurnal cortisol (cortisol AM slope) and daily life stress in the prediction of later internalizing symptoms.
References


APPENDIX A

IRB AND FUNDING ACKNOWLEDGEMENTS
Appendix A: IRB and Funding Acknowledgements

This work was supported by the US Eunice Kennedy Shriver National Institute of Child Health and Human Development: R01 HD079520 and R01 HD086085 to Kathryn Lemery-Chalfant and Leah Doane, and Mary Davis and Kathryn Lemery-Chalfant. Approval was obtained by the Institutional Review Boards at Arizona State University (IRB# STUDY00000637 and STUDY00004309).