Influence of Life Events on the Stress Response in Healthy Children and Adolescents

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Abstract

A life event is as an occurrence that involves a subsequent change in the life pattern of an individual (Holmes & Rahe, 1967). The current study investigated whether exposure to life events over the past year influenced hypothalamic-pituitary-adrenocortical (HPA) axis function in healthy children and adolescents, and explored whether sex, age, behavioural inhibition, trait anxiety, anxiety sensitivity, perceived parental bonding, and parental history of anxiety moderated this relationship. The sample included 147 healthy children and adolescents. Participants were administered Coddington's Life Events Scale (CLES) and salivary cortisol was collected for the determination of the cortisol awakening response (CAR), diurnal cortisol, and cortisol reactivity to a laboratory stressor. Separate linear regression models were conducted for each cortisol profile. Results revealed that life events significantly predicted total CAR output, diurnal cortisol response, and cortisol reactivity to a laboratory stressor. Further, behavioural inhibition, trait anxiety, not having a parental history of anxiety, and paternal caring positively moderated some of the relationships between life events and cortisol profiles. Considering the physiological and psychological effects of early exposure to stress, this study is significant in understanding the impact of life events to improve the health of children and adolescents.

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Exposure to stressful life events in childhood or adolescence requires a process of readjustment or adaptation (Coddington, 1972a), with life events defined as an occurrence that involves a subsequent change in the life pattern of an individual (Holmes & Rahe, 1967). Life events is an umbrella term that encompasses a wide variety of different events, ranging from mild to severe forms of stressors. According to Cohen et al. (2006), approximately 70% of individuals are exposed to adverse childhood experiences, with nearly 33% reporting more than two stressful life events. Early adversity has been found to have substantial long-term physiological and psychological consequences (Essex et al., 2011), with studies indicating that stressful life events in childhood and adolescence is associated with depressive symptoms (Stikkelbroek et al., 2016), suicidal ideation (Cohen-Sandler et al., 1982), and somatic complaints (Robinson et al., 1988). Exposure to stressful events in childhood has also been reported to increase risk for developing depression and anxiety later in life (McLaughlin & Hatzenbuehler, 2009; Michl et al., 2013), with neglect, family conflict, violence, and emotional abuse in childhood associated with depressive symptoms in adulthood (Cohen et al., 2006). In addition, exposure to stressful life events may be associated with cognitive deficits in adulthood, possibly due to the long-term impact of stress on the brain (Lupien et al., 2009). Exposure to adverse experiences can also result in long-lasting or permanent changes in the stress response system (Hunter et al., 2011). Considering the high prevalence of early exposure to stress and its possible detrimental health consequences, examining the factors that influence the stress response is an important area of research. Thus, the current study explored the relationship between life events and HPA axis function biomarkers in healthy children and adolescents.

Stress and the HPA Axis

Stress is a dynamic state in which the body engages in efforts to adapt and maintain equilibrium in stressful situations (Lazarus & Folkman, 1984), with stress as a state of disequilibrium that challenges homeostasis of the organism (Chrousos & Gold, 1992). Compas (1987) distinguishes between acute stressors, which involve changes in pre-existing conditions, and chronic stressors, which are recurring life events that pose a continuous challenge to the individual. In addition, stress has been defined as a relationship between the person and their cognitive appraisal of the stressful environment, in which a stress response or physiological

reaction is initiated when an individual perceives the environment as demanding, capable of endangering their well-being, or exceeding their resources (Lazarus & Folkman, 1984). Thus, the stress response is influenced by individual characteristics, as well as the nature of the stressful event (Lazarus & Folkman, 1984).

When exposed to a stressor, individuals experience physiological and behavioural changes that prepare them for the "fight or flight response" (Cannon, 1932). This response is attributable to changes in the central and peripheral nervous system, and promotes adaptive functions and inhibits non-adaptive ones, as a means to protect the individual from real or potential threats (Chrousos, 2009). A key brain structure that orchestrates the body's response to a stressor is the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis consists of the hypothalamus, the pituitary glands, and the adrenal cortex. When exposed to a real or perceived threat, the hypothalamus secretes a corticotropin-releasing hormone (CRH), signaling the anterior pituitary gland to secrete adrenocorticotropin hormone (ATCH), which is followed by the release of cortisol from the adrenal glands (McEwen, 1998; Miller et al., 2007). Cortisol is the primary hormone responsible for the stress response; it heightens attentiveness, alters memory and learning, and terminates the stress response through a negative feedback loop, which is activated once the threat has passed or through habituating or adjusting to the stressor based on the individual's perception of the event as non-threatening (Hunter et al., 2011; McEwen, 1998). Thus, the increase in cortisol secretion following a stressor is deemed a necessary physiological reaction (Jessop & Turner-Cobb, 2008).

During a normal state with non-stressful conditions, cortisol levels follow a circadian rhythm with higher levels in the morning, known as the cortisol awakening response (CAR), and lower levels in the evening, indicating a diurnal slope (Glaser, 2000; King et al., 2017). The CAR has been shown to be distinct from diurnal cortisol secretion (Clow et al., 2010) and involves a rapid increase of cortisol into the blood stream, reaching a maximum peak at approximately 30 minutes post awakening (Pruessner et al., 1997). Peaks in early morning cortisol levels assist in waking the body and increasing one's appetite (Klimes-Dougan et al., 2001), with the CAR hypothesized to be associated with the days demands and orientating oneself in time and space (Fries et al., 2009). Cortisol is able to cross the blood-brain barrier and therefore has a direct effect on the brain (Essex et al., 2011). This is supported by research that

has found that prolonged elevated cortisol levels reduce the volume and function of the hippocampus, resulting in deficits in memory and learning (Lupien et al., 1998).

Research has shown that genetic and environmental factors are both implicated in the regulation of the HPA axis (Linkowski et al., 1993). During infancy and childhood, the HPA axis is still developing and may be susceptible to dysregulation (Tarullo & Gunnar, 2006), especially as a result of early adversity and maltreatment (De Bellis & Zisk, 2014; Kuhlman, Vargas, et al., 2015). Increased levels of diurnal cortisol secretion (i.e., hypercortisolism) have been reported in children reared in orphanages (Gunnar, Morison, et al., 2001), and physically and sexually abused children (Cicchetti & Rogosch, 2001). With increased cortisol reactivity observed in bullied children in response to a laboratory stressor (Chen et al., 2017). Similarly, Pesonen and Räikkönen (2012) found increased cortisol reactivity to a social stress test in adults who were separated as toddlers from their family during WWII, with the highest cortisol concentrations found in adults who were separated at ages 4-7. Although it is difficult to make a causal link, Pesonen and Räikkönen (2012) suggested that separation for this age group may have been perceived as difficult to cope with, due to underdeveloped self-regulation mechanisms, subsequently impacting uncontrollability and contributing to heighted HPA axis reactivity. Indeed, stressors that are perceived as uncontrollable, involve trauma, and threaten physical well-being are related to elevated cortisol levels (Miller et al., 2007). Hypercortisolism has been associated with reduction in hippocampus functioning (Lupien et al., 1998) and risk for developing depression in children and adolescents (Kaufman et al., 1997).

Other research has demonstrated that exposure to prolonged stress may be associated with blunted cortisol levels (i.e., hypocortisolism), which results, in part, from the suppression of the HPA axis due to prolonged exposure to stress (Gunnar & Vazquez, 2001). For example, blunted basal cortisol levels have been observed in maltreated children (Hart et al., 1995) and sexually abused girls (King et al., 2001), and a blunted CAR has been reported in parentally bereaved children (Kaplow et al., 2013). Blunted cortisol levels in response to a laboratory stressor have also been found in maltreated children (MacMillan et al., 2009; Trickett et al., 2014). Thus, chronic stress may reduce the body's neuroendocrine response to future stressors, possibly as a form of adaptation. It has been suggested that under activation of the HPA axis may heighten one's vulnerability to the negative consequences of future stress (Danielson et al., 2015). This is supported by research that has shown that early life stress results in the

sensitization of the central nervous system, resulting in vulnerability to later stress and development of psychopathology (Heim & Nemeroff, 2001). Indeed, a review of the literature revealed that a blunted response to acute stress is associated with eating disorders, substance use, depression, obesity, poor cognitive function, and self-reported poor health (Phillips et al., 2013). While research indicates that both blunted and heightened cortisol levels are associated with negative physiological and psychological consequences, the relationship between life events and the stress response remains unclear, with some studies reporting hypercortisolism in response to life events while others reporting hypocortisolism.

Daily and Common Stressors

In contrast to the aforementioned research on the effects of trauma and maltreatment on the HPA axis in children, few studies have examined the impact of daily or common stressors on the stress response system. Existing research suggests that major life events and daily hassles both contribute to a process of adjustment in adolescence (Rowlison & Felner, 1988). For example, in a study on daily negative events in children and adolescents, peer problems on average were associated with flatter diurnal cortisol slopes, with children who reported more peer or academic problems than usual exhibiting an elevated CAR (Bai et al., 2017). In a meta-analysis on psychosocial factors impacting the CAR, higher levels of cortisol increase following awakening was associated with general life stress and job stress (Chida & Steptoe, 2009). Indeed, cortisol levels have been shown to increase during stressful days, with a steeper diurnal slope found in individuals who experienced frequent daily stressors than those who did not (between-group estimated diurnal linear slope= -.31; Stawski et al., 2013). Jacobs and colleagues (2007) found that minor stressors increased diurnal cortisol levels and negative affect, such as anxious and depressed mood, and decreased positive affect. Bruce et al. (2002), studied first graders at the beginning of a new school year and found that a steeper diurnal cortisol profile was detected on the first day of school in comparison to the weekend, as evidenced by higher morning and lower afternoon and evening cortisol levels. On the fifth day of school cortisol levels were relative to weekend days, however, among children with high activity levels and impulsivity, cortisol profiles observed on the first day of school were maintained at day five (Bruce et al., 2002). Thus, common stressors, such as the first day of school, may elicit increased diurnal cortisol in children during the onset of the event, with levels returning to baseline

following adaptation. However, temperamental factors appear to be important moderators of adaptation to the stress response in children and adolescents.

Frequency of Life Events

The existing literature on the relationship between frequency of life events and the stress response in children and adolescents is also limited. Armbruster et al. (2011) found that an increased number of stressful life events was related to smaller increases in cortisol levels following a laboratory stress test, suggesting a possible resiliency or adaptation to stressors. Similarly, Elzinga et al. (2008) observed blunted cortisol levels in response to a laboratory stressor in individuals who reported a high number of adverse experiences, in comparison to individuals who were exposed to none or one adverse experience. In a study on cumulative adversity and diurnal cortisol, children exposed to moderate cumulative adversity (i.e., one to two adverse experiences) exhibited an increased CAR and diurnal cortisol profile, whereas children with high exposure to adverse experiences (i.e., three or more adversities) exhibited a lower CAR and diurnal cortisol profile, which was similar to children that were not exposed (Gustafsson et al., 2010).

Research on the implications of distal and recent life events on the stress response is sparse. Bevans et al. (2008) found that life stress within the last 12 months was associated with high afternoon cortisol levels in children, whereas exposure to early adversity and recent life stress was associated with high afternoon cortisol levels and decreased morning cortisol levels. Hair cortisone concentrations in female children were found to be significantly related to life events occurring in the past 0 – 6 months, whereas the relationship between cortisone and life events in the past 0 – 3 months and 4 – 6 months were insignificant (Vanaelst et al., 2013). In contrast, Cullen et al. (2014) found that the total number of life events within the past 6 months was not associated with diurnal cortisol area under the curve with respect to ground (AUC_G) or CAR area under the curve with respect to increase (AUC_I) in children (Cullen et al., 2014). A meta-analysis conducted by Fogelman and Canli (2018) found no significant relationship between early life stress and current cortisol levels in studies consisting of adult samples.

Perception of Stress

Studies on children's perception of stress have shown that the appraisal of stress is more strongly associated with the cortisol response than the actual stress experience (Allwood et al., 2017). This is supported by research which found that when daily events were appraised as more

stressful, morning CAR levels were decreased (Gartland et al., 2014). Similarly, Duan et al. (2013) observed lower cortisol levels 30 minutes post awakening and a lower AUC_I in males preparing for school examinations, with high perceived stress and anxiety associated with a lower CAR. In comparison, research has found that adolescent reported stress during a school test was associated with an increase in cortisol levels during the test (Lindahl et al., 2005).

Moderators of the Stress Response

Although a multitude of factors may moderate the relationship between life events and HPA axis function, the present study focused on puberty, age, sex, temperament, parental bonding, and presence of parental psychopathology.

Puberty

One possible mechanism by which early exposure to stressful life events alters the HPA axis is puberty. Research has shown that age and pubertal maturation is associated with an increase in basal cortisol levels and heightened cortisol reactivity to laboratory stressors (Gunnar, Wewerka, et al., 2009; Kiess et al., 1995). Hankin et al. (2010) found that prepubertal children at risk for depression exhibited a hyporeactive cortisol response to a laboratory stressor, whereas post-pubertal at-risk adolescents displayed a hyperactive cortisol response. Later stages of puberty have also been associated with higher daytime cortisol levels, reduced CAR, and a steeper diurnal cortisol curve (Adam, 2006). In contrast, King et al. (2017) observed that elevated levels of early exposure to stressors was associated with a blunted CAR in early pubertal stages, whereas in later stages of puberty, elevated levels of early exposure to stressors was associated with an increase in the CAR. Hence, puberty may be a factor in the relationship between stress reactivity and life events, although findings are mixed.

Age

Another factor that may impact the relationship between life events and the HPA axis is the age in which the individual was exposed to the adverse experience. Kuhlman, Vargas, et al. (2015) found that exposure to trauma during infancy was associated with delayed cortisol recovery in relation to subsequent stress. Research has also found that prenatal and postnatal adversity is related to higher cortisol levels in adolescents following a laboratory stressor (Bosch et al., 2012). Von Klitzing et al. (2012) observed that experiencing peer victimization at age five was a predictor of increased shyness, depression, and anxiety symptoms at age six for children who displayed decreased cortisol reactivity to a stress test one year prior. However, Bosch et al.

(2012) found no relationship between cortisol secretion and adverse experiences occurring between ages 0-5, but observed higher basal cortisol levels in children exposed to adverse experiences during ages 6-11 and lower basal cortisol levels in children exposed at ages 12-15.

Sex

Research has also proposed a relationship between sex and the HPA axis, with some findings indicating that females secrete higher levels of cortisol at baseline (Bloch et al., 2007), in the morning (Reynolds et al., 2013), midday and late afternoon (Klimes-Dougan et al., 2001) than males. In comparison, Michels et al. (2012) found higher morning cortisol levels in boys with emotional problems and lower overall cortisol levels in girls with peer problems. In a study on early life trauma and stress reactivity, females were shown to release more CRH following a stress challenge (DeSantis et al., 2011). In contrast, Kirschbaum et al. (1999) observed increases in ATCH levels and salivary cortisol in males following a laboratory stressor, in comparison to females in the follicular phase of their menstrual cycle. This is similar to research that found lower cortisol levels in female children compared to male children following a laboratory stressor (Trickett et al., 2014). In a meta-analysis on sex differences and the stress response in children and adolescents, males ages seven and younger displayed higher basal cortisol levels compared to females, whereas males ages 8-18 exhibited lower basal cortisol levels than females (Van der Voorn et al., 2017), suggesting a possible interaction between age and sex on the stress response.

Temperament and Personality

Individual differences in temperament, personality traits, and externalizing and internalizing behaviours may also moderate the relationship between life events and the stress response in children and adolescents. Behavioural inhibition is an early appearing temperamental predisposition that is characterized as being fearful, avoidant, and reactive to unfamiliar situations (Kagan & Snidman, 1999). Behaviourally inhibited children have been found to exhibit increased baseline cortisol levels and increased CAR (Kagan et al., 1987). Similarly, Gunnar et al. (2011) found that behavioural inhibition moderated the relationship between elevated diurnal cortisol levels and increased internalizing symptoms in preschoolers. Kuhlman, Geiss et al. (2018) reported that among children with increased internalizing and externalizing behaviours, physical abuse was associated with a blunted cortisol response to a laboratory stressor, whereas emotional abuse was associated with an elevated cortisol response.

Internalizing symptoms have also been shown to be associated with immediate and delayed cortisol reactivity to a psychosocial stressor (Klimes-Dougan et al., 2001). Additionally, research has found that an increase in cortisol in adolescents in response to a laboratory stressor was associated with more stressful family life events and internalizing and externalizing behaviours (Steeger et al., 2016).

In a study that measured children's cortisol levels in response to the beginning of a school year, Bruce et al. (2002) found a correlation between shyness and elevated basal cortisol levels on the fifth day of school in comparison to weekdays. Dougherty and colleagues (2012) observed that negative child affect measured at age three was a predictor of elevated evening cortisol levels at age six. Similarly, a study of preschoolers also noted that lower self-control and emotional negativity were associated with elevated increases in cortisol levels from morning to afternoon (Dettling et al., 2000). Mixed results have been observed in studies on neuroticism and cortisol profiles, with high levels of neuroticism associated with a greater CAR AUC_G (Portella et al., 2005) and elevated diurnal cortisol output (Garcia-Banda et al., 2014), whereas, no association between neuroticism and diurnal cortisol profiles have been found (Hauner et al., 2008).

Trait anxiety, which is characterized as a stable tendency to experience anxiety (Speilberger, 1973), has been shown to be associated with the stress response, with decreases in the CAR AUC_G (Walker et al., 2011) and lower salivary cortisol levels (Jezova et al., 2004) observed in response to a laboratory stressor. Indeed, Therrien et al. (2008) noted lower CAR in females with high trait anxiety, whereas, Kapsdorfer et al. (2017) found that children with high trait anxiety exhibited elevated morning cortisol levels. In addition, high trait anxiety in adolescents has been associated with increases in evening cortisol levels and a flat diurnal cortisol profile (Van den Bergh et al., 2008). In contrast, Adam (2006) found that trait anxiety was not associated with diurnal cortisol in adolescents. Anxiety sensitivity, a trait associated with beliefs regarding the negative consequences of anxiety (Silverman et al., 1991), has been found to mediate the relationship between trait anxiety and the stress response, with anticipatory anxiety predicting high trait anxiety and a low CAR (Walker et al., 2011). However, Van Santen et al. (2011) found no relationship between anxiety sensitivity and the CAR.

Parenting Styles and Attachment

Another possible moderator of the relationship between life events and HPA axis reactivity is parenting and attachment style. Parenting and attachment styles have been found to predict cortisol levels in offspring, with children with a secure parental attachment demonstrating increased levels of cortisol in response to a fearful situation and decreased levels in response to a positive event (Roque et al., 2011). In a study on attachment as a moderator of the relationship between stress and the HPA axis, children with high exposure to life events within the past year and who reported high attachment security exhibited less cortisol reactivity in response to a psychosocial stressor, whereas children with low attachment security exhibited greater cortisol reactivity (Bendezú et al., 2019). In contrast, nonresponsive parenting has been associated with low levels of cortisol reactivity to a laboratory stressor in children exposed to traumatic experiences (Jaffee et al., 2014). Roque et al. (2011) observed that cortisol reactivity levels in insecure children did not change in response to positive or negative events in a laboratory emotion regulation paradigm, indicating that an insecure attachment style may be associated with the suppression of the HPA axis. However, Smyth and colleagues (2015) found that an insecure anxious attachment style was related to an increase in cortisol secretion following a laboratory stressor. Other research has found that lower parental warmth in childhood is associated with elevated levels of diurnal cortisol following a stressful event in adulthood (Hanson & Chen, 2010), whereas higher maternal warmth is associated with lower morning cortisol levels and lower diurnal cortisol AUC_G in response to daily hassles (Lucas-Thompson, 2013).

In a study on the relationship between cortisol levels in children and family functioning, Pendry and Adam (2007) found that marital functioning and higher quality of maternal parenting was associated with steeper diurnal cortisol in children. In comparison, poor parental monitoring has been shown to predict a flatter diurnal cortisol slope and an increase in externalizing behaviours (Martin et al., 2014). According to Kopala-Sibley et al. (2017), HPA axis activity may moderate the association between the parent-child relationship and changes in the child's affect. Research has shown that a positive parent-child relationship predicts an increase in positive affect for children with low levels of cortisol reactivity to a laboratory stressor and a reduction in negative affect for children with high levels of cortisol reactivity to the stressor (Kopala-Sibley et al., 2017). This is further supported by a study which found that HPA axis reactivity moderated the relationship between family environment and emotional symptoms in

children, with an unfavourable environment and high cortisol reactivity to a stress task predicting increased levels of emotional symptoms (Von Klitzing et al., 2012).

Although an extensive literature exists on parental attachment, the majority of the research on parental factors and the dysregulation of children's HPA axis focuses on maternal influences. However, Pereira et al. (2013) found that both maternal anxiety and paternal overprotection contributed to offspring's anxiety. Similarly, maternal and parental overprotection have been shown to be negatively associated with cortisol in response to a DEX/CRH test (Narita et al., 2012). In a study on HPA axis function and interparental conflict, children whose fathers reported increases in interparental conflict exhibited a faster cortisol secretion and slower stress recovery in response to a laboratory stressor, while no relationship existed between mother-reported conflict and the child's stress response (Kuhlman, Repetti, et al., 2018). Further, higher reported relationship quality with fathers has been related to less emotional reactivity to daily stressors in adult males (Mallers et al., 2010).

Parental Psychopathology

A number of studies have examined the potential influence of various parental psychopathology on HPA axis functioning in offspring. For example, in healthy female adolescents of mothers with post-traumatic stress disorder (PTSD), higher CAR and greater daily total cortisol secretion have been reported (Liu et al., 2016). Other studies have found that offspring of mothers with PTSD exhibit blunted basal cortisol levels (Yehuda & Bierer, 2007) and blunted cortisol reactivity to a laboratory stressor (Danielson et al., 2015). Among offspring of parents with anxiety disorders, elevated morning cortisol (Dougherty et al., 2012), CAR (Vreeburg et al., 2010), and basal cortisol levels (Warren et al., 2003) have been detected. As well, increased (Poole et al., 2017) and blunted (Koszycki et al., 2019) cortisol response to a laboratory stressor have been reported in offspring of parents with anxiety disorders.

Research on offspring of parents with mood disorders also report altered HPA axis functioning. For example, offspring of parents with bipolar disorder have been found to exhibit higher afternoon levels of cortisol (Ellenbogen et al., 2010) and an increase in the CAR associated with experiencing chronic stress (Ostiguy et al., 2011). In comparison, Schreuder and colleagues (2016) found no difference in the CAR and diurnal cortisol levels in offspring with or without a parent with bipolar disorder, although they did note that offspring with parental bipolar disorder reported higher levels of adverse experiences and these experiences were associated

with lower daytime cortisol levels. Offspring of parents with depression have been found to exhibit a higher CAR (Mannie et al. , 2007; Vreeburg et al., 2010), elevated basal cortisol levels (Brennan et al., 2008; Halligan et al., 2004; Lupien et al., 2000), and a blunted cortisol response to a laboratory stressor (Bouma et al., 2011). Interestingly, elevated morning cortisol levels at age 13 were found to predict depressive symptoms at age 16 in offspring of mothers with postnatal depression (Halligan et al., 2007), suggesting that altered HPA axis function may confer risk for depression in offspring at familial risk for depression. There is also suggestive evidence of a relationship between child temperament, parental depression, and child HPA axis functioning. In this regard, Dougherty and colleagues (2012) found that maternal depression and positive affect in offspring at age three predicted decreased morning cortisol levels at age six. In comparison, Ashman et al. (2002) found that children who possessed high levels of internalizing symptoms and whose mothers had depression, exhibited heightened basal cortisol levels. Further, Mackrell et al. (2014) observed that paternal depression and child behavioural inhibition predicted higher basal cortisol levels and cortisol reactivity to a laboratory stressor.

Limitations in the Literature

In summary, while the literature on the stress response system in children and adolescents is limited, research suggests that exposure to early adversity has a negative impact on the HPA axis in children (De Bellis & Zisk, 2014). However, the nature of the stress response in children and adolescents is inconsistent, with some research reporting hypercortisolism (e.g., Gunnar, Morison, et al., 2001) and others reporting hypocortisolim (e.g., Kaplow et al., 2013). There are also mixed findings regarding early adversity and alterations in the CAR, diurnal cortisol response, and cortisol reactivity to a laboratory stressor. Further, while most studies have focused on the impact of childhood maltreatment on stress reactivity, there is a paucity of research on the relationship between a range of life events and HPA axis function in children and adolescents. In addition, there is limited literature studying life events over the duration of a year, with most studies focusing on early maltreatment or one specific event in the child's life, such as parental death (Kaplow et al., 2013). There is also a scarcity of research exploring distal and recent life events on HPA axis function, as well as the relationship between the number of life events a child experiences and the implications on the stress response system.

This thesis aims to address the aforementioned gaps in the literature. Specifically, I examined the influence of distal and recent life events on different cortisol profiles (CAR,

diurnal cortisol response, and cortisol reactivity to a laboratory stressor) in healthy children and adolescents. Second, I explored the impact of life events combined with potential moderating factors on different cortisol profiles. There is a paucity of research on moderators of the relationship between stress exposure and HPA axis function in children and adolescents, and available studies have yielded inconsistent findings (e.g. Hankin et al., 2010; Hudson et al., 2011; Kuhlman, Geiss, et al., 2018; Reynolds et al., 2013; Roque et al., 2011). Identifying reliable moderator variables is an important research goal as this may help identify factors that contribute to adaptive regulation of the HPA axis function in response to life stressors in children and adolescents.

Research Objectives

The primary aim of the current study was to investigate the relationship between exposure to life events over the past year and cortisol profiles in healthy children and adolescents. It was hypothesized that life events would predict variation in the CAR, diurnal cortisol secretion, and cortisol reactivity to a laboratory stressor. Further, it was hypothesized that cortisol profiles would vary based on exposure to distal and recent life events. Given the preliminary nature of the current study, the direction of the stress response (hypercortisolism or hypocortisolism) was not hypothesized. A secondary aim of the study was to identify moderators of the relationship between life events and HPA axis function. The secondary hypothesis was that demographic (sex and age), psychological (behavioural inhibition, trait anxiety, and anxiety sensitivity), genetic (having a parent with or without an anxiety disorder), and environmental (perceived parental bonding) factors would moderate the relationship between life events and cortisol profiles. Due to the preliminary nature of this research, the direction of the moderator's possible influence on the primary outcomes was not hypothesized.

Methodology

Participants

The sample included 150 healthy children and adolescents between the ages of 7-18 years who participated in a larger study on biological, psychological, and environmental risk factors for anxiety. Participants were excluded if they had current or past history of any psychiatric disorder, unstable and/or clinically significant medical conditions, participated in a study that involved medication within the last three months, or currently use medication that may affect central or peripheral nervous system function. Families were recruited via advertisements placed

in local newspapers, the Internet, and flyers posted on university and community bulletin boards. The study was approved by the University of Ottawa's Office of Research Ethics and Integrity. Written informed consent was obtained from the child's legal guardian and the child's assent was obtained. Participants ages 16 years and older provided their own consent. Financial compensation was provided to the families who participated in the study.

Assessment

An initial telephone pre-screen with the parent who contacted the research unit was conducted by a research assistant to explain the purpose of the study and obtain information on the psychiatric status of both biological parents and history of psychiatric symptoms of the child participating in the study. Information was also collected on medication use and medical illness of the offspring. If the families were potentially eligible, a second telephone screen was scheduled to formally evaluate the diagnostic status of the child's parents. Interviews of parents with anxiety disorders were conducted by a licensed psychologist and current or lifetime diagnosis of psychiatric disorders was confirmed with a DSM-IV based structured clinical interview (Diagnostic Statistical Manual of Mental Disorders (SCID); First et al., 1995). Trained research assistants under the supervision of a psychologist conducted the interviews with parents who did not have a history of anxiety disorders. After parental diagnostic status was confirmed, their offspring were invited to the University of Ottawa for a face-to-face interview to confirm the absence of psychopathology. This was assessed using the Anxiety Disorders and Interview Schedule for Children (ADIS-C), a well-established diagnostic instrument (Silverman & Albano, 1996). Eligible children completed self-report questionnaires and their body mass index (BMI) was calculated based on their weight and height measured during the assessment. Pubertal status was also determined by using Tanner's Scale (Tanner, 1962). Younger participants were supported by the research assistants in completing the self-report questionnaires.

Collection of Cortisol for Determination of CAR and Diurnal Variation

Participants collected salivary cortisol at home with a collection kit (Sarstedt, Inc., Newton, NC), consisting of a cotton swab fitted into pre-labelled plastic holders resting inside a centrifuge tube. Sampling was conducted upon waking up, + 30, and +60 minutes after awakening, and at 4 pm and 8 pm on two consecutive days. Samples collected at wake-up and +30 and +60 minutes post wake-up were used for determination of the CAR. Samples collected in the morning (average of samples collected at wake-up, wake-up + 30 minutes, wake-up + 60

minutes), 4 pm, and 8 pm were used to calculate diurnal cortisol secretion. Participants were given instructions on food and medication to avoid during the days of sampling. Additionally, participants were instructed to not collect samples on days they were ill and for females when they were menstruating. The participants were also required to not drink, eat, smoke, or brush their teeth less than 60 minutes prior to sampling. Participants were provided with a diary to record the time they took the samples. Parents were asked to refrigerate the samples until sampling was completed and bring the tubes to the laboratory at the child's next scheduled visit.

Cortisol Reactivity to a Laboratory Stressor

Children completed a stress test within the first two weeks following the assessment interview. Participants were asked to not take any medication or food that may influence psychological measures 24 hours prior to the stress test. The stress test was completed between 2-3 pm and the child's parents were not present during the test. Post-pubertal girls completed the test during the follicular phase of their menstrual cycle. The stressor was as an impromptu speech task modified from the Trier Social Stress Test (Kirschbaum et al., 1993), a well-established protocol to measure stress reactivity. When the child arrived at the laboratory, they were seated in a comfortable chair and baseline measures of salivary cortisol were collected (T1). Following the baseline condition, children were informed that they would be required to give a 10-minute speech while standing on three of four topics, which included their favourite books or movies, school, friends, and hobbies or sports they enjoy. They were also told that they had two minutes to prepare the speech. During the speech task, two research assistants made observations and took notes. Salivary cortisol samples were collected at 15 (T2), 30 (T3), 45 (T4), and 60 (T5) minutes post-speech.

Measures

Baseline Screening Questionnaire

A baseline screening questionnaire was administered to the parents to obtain demographic information, including maternal pre-natal and post-natal development, parental stressors, parental drug use, and child medical and development history.

Structured Clinical Interview for Axis I Disorders (SCID-I)

The SCID-I is considered a gold standard semi-structured interview to facilitate DSM-IV Axis I disorders (First et al., 1995) in adults ages 18 and older. Inter-rater agreement has been found to be fair to excellent with values between 0.61 – 0.83 (Lobbestael et al., 2011).

Anxiety Disorders and Interview Schedule for Children (ADIS-C)

The ADIS-C is a semi-structured interview used to diagnose major childhood psychiatric disorders based on DSM-IV criteria in individuals 6 to 18 years of age (Silverman & Nelles, 1988). Responses for the ADIS-C include whether the symptom is present, absent, or other (Silverman et al., 2001). A total symptom scale score based on the number of "present" responses is used to determine if the child meets DSM criteria. The ADIS-C is widely used in clinical studies (Silverman et al., 2001) and has demonstrated high test-retest reliability and excellent inter-rater reliability (Silverman et al., 2001).

Coddington's Life Events Scales (CLES)

The CLES is a well validated and reliable self-report questionnaire that measures a variety of life events within the past year (Coddington, 1972a). The CLES is based on the Social Readjustment Scale, constructed by Holmes and Rahe, that assesses positive and negative life events in adults that requires readjustment (Coddington, 1972b). The CLES consists of two separate questionnaires applicable to children (CLES-C) and adolescents (CLES-A), which were administered to the corresponding age group at the screen visit. The CLES-C has 35 items and the CLES-A has 50 items. The CLES measures how often (0, 1, 2+ times) the life event occurred during the past year, as well as the time period in which the life event occurred (0 - 3 months, 0 - 6 months, 0 - 9 months, and 0 - 12 months). The units used in the scale are Life Change Units (LCU), with more stressful, repetitive, and recent events corresponding to a higher LCU. The CLES includes items such as death of a parent, being hospitalized for illness or injury, birth of a brother or sister, and moving to a new school district. The CLES also determines children who are at risk for physical and emotional problems based on greater exposure to life events. Children are reported to be at risk if they are in the 75^{th} percentile based on an age-specific cut-off point corresponding to the life event time points (0 – 3 months, 0 – 6 months, 0 – 9 months, and 0 – 12 months). For clarity, children and adolescents who met the cut-off point for being at risk will henceforth be referred to as the "high stress exposed group". Concurrent validity measuring parent and child reporting of life events, was found to have a total score correlation of 0.27 (Coddington, 1999). The inter-rater reliability has been found to be 0.45 (Coddington, 1999).

Childhood Self-Report of Inhibition (CSRI)

The CSRI Version 2 (Reznick, n.d) is a 30-item scale that measures behavioural inhibition in children and is based on the Retrospective Self-Report of Inhibition, which

retrospectively measures childhood behavioural inhibition in an adult sample (Reznick et al., 1992). The CSRI assess the behaviours of children, such as social withdrawal, separation anxiety, and fears. The CSRI parallels items of the RSRI, which consists of two factors measuring illness and fears (12 items), and social and school-related experiences (12 items) (Reznick et al., 1992). Items are rated using a 5-point Likert scale. A higher total score indicates higher levels of behavioural inhibition. While no published psychometric data is available for the CSRI, the RSRI has been shown to demonstrate a high internal consistency of $\alpha = 0.83$ in undergraduate students (Caulfield et al., 2013).

State-Trait Anxiety Inventory for Children – Trait Form (STAIC-T)

The STAIC-T (Speilberger, 1973) is a well-established instrument that measures trait anxiety, a stable tendency to experience anxiety, in children and adolescents from ages 6 to 18 years. The STAIC-T is comprised of a 20-item scale and consists of questions measuring how children feel generally. Items are rated using a three-point Likert scale, with higher scores indicating higher levels of trait anxiety. Re-test reliability for the trait form has been found to be between .65 and .71 (Southam-Gerow et al., 2003). STAIC-T has also been found to have a concurrent validity of .75 with the Children's Manifest Anxiety Scale (Southam-Gerow et al., 2003).

Parental Bonding Instrument (PBI)

The PBI (Parker et al., 1979) is one of the most widely established self-report questionnaires that measures parental bonding. The PBI measures two dimensions of parenting styles, which includes perceived caring (positive parenting) and overprotection (negative parenting). The measure consists of 25 items, 13 items for care and 12 items for overprotection. Items are rated using a 4-point Likert scale. The caring dimension focuses on affection and warmth, whereas the overprotection dimension measures constraint and control (Xu et al., 2018). The PBI is completed by participants for fathers and mothers separately and assesses perceived parenting over the first 16 years of life. In a non-clinical sample, test-retest reliability was found to be 0.76 for the care scale and 0.63 for the protection scale (Parker et al., 1979). The PBI has been found to demonstrate stability over a 20-year time period (Wilhelm et al., 2005).

Childhood Anxiety Sensitivity Index (CASI)

The CASI (Silverman et al., 1991) consists of 18-items that measures children's beliefs regarding the negative consequences of anxiety. The CASI is a modified version of the Anxiety

Sensitivity Index (Reiss et al., 1986), which is the most extensively used measure for anxiety sensitivity in adult samples (Muris, 2002). Items on the CASI are rated using a 3-point Likert scale, with higher scores indicating higher levels of anxiety sensitivity. The instrument includes questions such as, “It scares me when my heart beats fast”. The CASI has been found to have internal consistency estimates of $\alpha = 0.87$ for clinical and nonclinical samples, and test-retest reliability estimates of 0.76 for nonclinical samples and 0.79 for clinical samples (Silverman et al., 1999). The instrument has been shown to correlate with measures of anxiety and fear (Silverman et al., 1999).

Salivary Cortisol

The salivary swabs were centrifuged to remove particulate matter, and the supernatant liquid stored in Eppendorf tubes at -80^{o}C until analyses. A technician performed the analyses in duplicate using the protocol and enzyme-linked immunosorbent assay cortisol kits from Salimetrics Inc.

Statistical Analyses

Prior to analyzing the data, all variables were examined for outliers or missing data. Analyses showed 30 cortisol values were outliers (3 standard deviations from the mean) and were winsorized to 3 standard deviations above the mean (Bendezú et al., 2019; Hostinar et al., 2014). Four LCU values were determined to be outliers and were also winsorized to 3 standard deviations above the mean. Participants were excluded from the analysis if they were missing four (out of ten) cortisol values for the diurnal cortisol, three (out of six) cortisol values for the CAR or missing two (out of five) cortisol values for the reactivity to a laboratory stressor. Missing data for the CAR, diurnal cortisol, and cortisol reactivity was determined to be missing completely at random (MCAR) using Little’s (MCAR) test, $p = .149$ (combined CAR and diurnal values) and $p = .989$ (cortisol reactivity). Missing values were imputed using the expectation-maximization method. Cortisol values for the CAR, diurnal, and stress task were log transformed due to skewness and to meet the normally distributed assumption for multiple regression analyses. Area under the curve (AUC) was calculated for the three cortisol measures (CAR, diurnal cortisol, cortisol reactivity). AUC with respect to increase (AUC_I), representing the change of cortisol over the specific time points (Clow et al., 2010), and area under the curve with respect to ground (AUC_G), representing the total cortisol output, were calculated using the trapezoid formula described by Pruessner, Kirschbaum, et al., (2003) (see Appendix A).

Pearson's correlations were conducted to examine the relationship between the study variables. Separate multiple linear regression analyses were conducted to determine if life events and their interaction with the moderator variables predicted each of the cortisol profiles (AUC_I and AUC_G for CAR, diurnal cortisol, and cortisol reactivity). Life events and moderator variables were mean centered and the interaction terms with life events and moderator variables were computed. In Model 1, the Life Change Units (LCU) were entered as a predictor variable, and in Model 2, the centered moderator variables and two-way interactions between the LCU and each moderator variable (sex, age, trait anxiety, anxiety sensitivity, behavioural inhibition, parental anxiety, and parental bonding) were entered. The primary interest in these analyses was whether life events predicted cortisol profiles and whether the effect of life events on cortisol profiles was influenced by age, sex, parental history of anxiety, and personality traits. When the interaction between LCU scores and the moderator variable was significant, *post hoc* analyses were conducted based on the method recommended by Aiken and West (1991). Continuous moderator variables were divided into tertiles based on ascending order of the data, and defined as low, moderate, and high groups. Follow-up simple regressions were conducted for each group to determine the strength of the relationship between life events and cortisol profiles. Analysis of the CAR and diurnal cortisol included data from 141 children. Analysis of cortisol reactivity to the laboratory stressor included 138 children. A secondary analysis was conducted using the same procedure outlined above with high stress exposure based on life events as the predictor variable.

For all analyses, SPSS version 26 was used (IBM Corp., 2019). All statistical tests were considered significant at a two-tailed level of $\alpha = 0.05$.

Results

Participant Characteristics

There were 172 participants in the study, of these individuals 12 were excluded as they did not meet the study criteria. Three participants withdrew from the study prior to completing the baseline screening questionnaire, and two participants withdrew from the study prior to the collection of cortisol data and were excluded from the analysis. Eight participants were also excluded from the analysis, as they were missing the aforementioned values required for the cortisol profiles. Demographic characteristics of the sample are described in Table 1.

Table 1

Demographic Characteristics of Participants

Characteristics	
Age (mean ± SD, years)	12.31 ± 3.21
Sex (% Female)	71%
Ethnicity (% white)	64.6%

Note. N = 147

Correlations Between Life Events and Predictor Variables

Pearson correlations between life events and the predictor variables are shown in Table 2. With one exception, correlations between life events and cortisol reactivity to the stress task were not significant. Several significant correlations emerged between life events and the CAR and diurnal cortisol secretion, although the magnitude of the correlations was small.

Table 2

Correlations for Life Events and Cortisol Measures

	Speech AUC_G	Speech AUC_I	CAR AUC_G	CAR AUC_I	Diurnal AUC_G	Diurnal AUC_I
LCU A	.13	.08	.27**	.07	.29**	-.20*
LCU B	.12	.09	.27**	.08	.31**	-.18*
LCU C	.14	.12	.27**	.10	.31**	-.19*
LCU D	.14	.12	.27**	.11	.31**	-.19*
HSE A	.09	.25*	.25*	.07	.26**	-.20*
HSE B	.10	.15	.25*	.02	.27**	-.19*
HSE C	.09	.17	.26*	.05	.25**	-.22*
HSE D	.06	.15	.25*	.05	.26**	-.21*

Note. N= 141 (CAR and Diurnal); N=138 (Stress Reactivity); AUC_G = Area Under the Curve with Respect to Ground; AUC_I = Area Under the Curve with Respect to Increase; CAR = Cortisol Awakening Response; HSE = High Stress Exposure, LCU A = Life events 0–3 months; LCU B = Life events 0-6 months; LCU C = Life events 0-9 months; LCU D = Life events 0-12 months.

* $p < .05$. ** $p < .01$.

Cortisol Awakening Response

Life Events at 0-3 Months (LCU-A)

Multiple regression analysis for life events at 0 – 3 months predicting CAR AUC_G revealed that Model 1 was statistically significant, ($F(1, 139) = 10.54$, $p = .001$, $R^2 = .07$, $R^2_{\text{Adjusted}} = .06$). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 19% of variance in CAR AUC_G ($F_{Change}(12, 127) = 2.77$, $p = .002$). Examination of the individual standardized beta coefficients showed that the interaction between life events and trait anxiety and behavioral inhibition contributed significantly to the regression model (Table 3). Follow-up regressions revealed that the association between life events and AUC_G was stronger for children with low levels of behavioural inhibition ($R^2 = .12$, $\beta = .34$, $t(45) = 2.44$, $p = .02$) than those with medium ($R^2 = .08$, $\beta = .28$, $t(45) = 1.97$, $p = .06$) and high ($R^2 = .06$, $\beta = .25$, $t(45) = 1.75$, $p = .09$) levels of behavioural inhibition. Graphical representation of the interaction is shown in Figure 1a. The associations between life events and CAR AUC_G within each level of trait anxiety were not statistically significant.

Multiple regression analysis for life events at 0 – 3 months predicting CAR AUC_I revealed that Model 1 was not statistically significant, ($F(1, 139) = .76$, $p = .38$, $R^2 = .005$, $R^2_{\text{Adjusted}} = -.002$). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 17% of variance in CAR AUC_I ($F_{Change}(12, 127) = 2.13$, $p = .02$). Examination of the individual standardized beta coefficients showed that the interaction between life events and sex and behavioral inhibition contributed significantly to the regression model (Table 4). Follow-up regressions revealed that the associations between life events and CAR AUC_I within each level of behavioral inhibition and with sex were not statistically significant.

Model 1 predicting CAR AUC_G for children in the HSE group at 0 - 3 months was statistically significant, ($F(1, 139) = 9.26$, $p = .003$, $R^2 = .06$, $R^2_{Adjusted} = .06$). Adding the other predictor variables and their interactions with high stress exposure (HSE) significantly improved the model, explaining an additional 20% of variance in CAR AUC_G ($F_{Change}(12, 127) = 2.78$, $p = .002$) (see Table B1 in Appendix B). Examination of the individual standardized beta coefficients showed that the interaction between HSE and trait anxiety contributed significantly to the regression model. A follow-up regression revealed that the association between HSE and CAR

AUC_G was stronger for children with high levels of trait anxiety (R^2 = .09, β = .30 , t(45) = 2.12, p = .04) than children with medium (R^2 = .06, β = .24, t(45) = 1.69, p = .10) and low (R^2 = .04, β = .21 , t(45) = 1.40, p = .17) levels of trait anxiety (see Figures B1 and B2 in Appendix B).

Model 1 predicting CAR AUC_I for children in the HSE group at 0 - 3 months was not statistically significant, (F(1, 139) = .71, p = .40, R^2 = .01, $R^2_{Adjusted}$ = -.002). Adding the other predictor variables and their interaction with HSE significantly improved the model, explaining an additional 16% of variance in AUC_G (F_{Change} (12, 127) = 1.99, p = .03). However, examination of the individual beta coefficients showed that neither HSE nor its interaction with the other predictor variables contributed in a meaningful way to the regression model.

Life Events at 0-6 Months (LCU-B)

Multiple regression analysis for life events at 0 – 6 months predicting CAR AUC_G revealed that Model 1 was significant, (F(1, 139) =11.06, p = .001, R^2 = .07, $R^2_{Adjusted}$ = .07). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 17% of variance in CAR AUC_G (F_{Change} (12, 127) = 2.42, p = .01). Examination of the individual standardized beta coefficients showed that the interaction between life events and behavioural inhibition contributed significantly to the regression model (Table 3). Graphical representation of the significant interaction is displayed in Figure 1b. A follow-up regression revealed that the association between life events and CAR AUC_G was stronger for children with low levels of behavioural inhibition (R^2 = .17, β = .41 , t(45) = 3.01, p = .004) than those with medium (R^2 = .07, β = .26, t(45) = 1.81, p = .08) and high (R^2 = .06, β = .25 , t(45) = 1.71, p = .09) levels of behavioural inhibition.

Multiple regression analysis for life events at 0 – 6 months predicting CAR AUC_I revealed that Model 1 was not statistically significant, (F(1, 139) = .99, p = .32, R^2 = .01, R^2_{Adjusted} = .00). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 16% of variance in CAR AUC_I (F_{Change} (12, 127) = 1.97, p = .03). Examination of the individual standardized beta coefficients showed that the interaction between life events and sex and behavioral inhibition contributed significantly to the regression model (Table 4). Follow-up regressions revealed that the associations between life events and CAR AUC_I with sex and within each level of behavioral inhibition were not statistically significant.

Model 1 predicting CAR AUC_G for children in the HSE group at 0 - 6 months was statistically significant, ($F(1, 139) = 8.96$, $p = .003$, $R^2 = .06$, $R^2_{Adjusted} = .05$. Adding the other predictor variables and their interaction with life events significantly improved the model, ($F_{Change}(12, 127) = 2.25$, $p = .01$). However, examination of the individual beta coefficients showed that neither HSE nor its interaction with other predictor variables contributed in a meaningful way to the regression model. Model 1 and Model 2 predicting CAR AUC_I based on HSE at 0 – 6 months were not statistically significant.

Life Events at 0-9 Months (LCU-C)

Multiple regression analysis for life events at 0 – 9 months predicting CAR AUC_G revealed that Model 1 was significant, ($F(1, 139) = 11.12$, $p = .001$, $R^2 = .07$, $R^2_{Adjusted} = .07$. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 17% of variance in CAR AUC_G, ($F_{Change}(12, 127) = 2.30$, $p = .01$). Examination of the individual beta coefficients showed that the interaction involving life events and behavioral inhibition contributed significantly to the regression model (Table 3). Graphical representation of the significant interaction is shown in Figure 1c. A follow-up regression revealed that the association between life events and CAR AUC_G was stronger for children with lower levels of behavioural inhibition ($R^2 = .18$, $\beta = .43$, $t(45) = 3.15$, $p = .003$) than those with medium ($R^2 = .06$, $\beta = .25$, $t(45) = 1.73$, $p = .09$) and higher ($R^2 = .06$, $\beta = .24$, $t(45) = 1.67$, $p = .10$) levels of inhibition.

Multiple regression analysis for life events at 0 – 9 months predicting CAR AUC_I revealed that Model 1 was not statistically significant, ($F(1, 139) = 1.34$, $p = .25$, $R^2 = .01$, $R^2_{\text{Adjusted}} = .002$). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 16% of variance in CAR AUC_I ($F_{Change}(12, 127) = 1.97$, $p = .03$). Examination of the individual standardized beta coefficients showed that the interaction between life events and behavioral inhibition contributed significantly to the regression model (Table 4). Follow-up regressions revealed that the associations between life events and CAR AUC_I within each level of behavioral inhibition were not statistically significant.

Model 1 predicting CAR AUC_G for children in the HSE group at 0 - 9 months was statistically significant, $F(1, 139) = 9.72$, $p = .002$, $R^2 = .07$, $R^2_{Adjusted} = .06$). Adding the other predictor variables and their interaction with life events significantly improved the model,

explaining an additional 17% of variance in CAR AUC_G (F_{Change} (12, 127) = 2.32, p = .01) (see Table B1 in Appendix B). Examination of the individual standardized beta coefficients showed that the interaction between life events and behavioral inhibition contributed significantly to the regression model. A follow-up regression revealed that the association between HSE and CAR AUC_G was stronger for children with lower (R^2 = .10, β = .32 , t(45) = 2.27, p = .03) and higher (R^2 = .09, β = .29 , t(45) = 2.06, p = .05) levels of behavioural inhibition than children with medium (R^2 = .04, β = .19 , t(45) = 1.31, p = .20) levels of inhibition (see Figure B2 in Appendix B). Model 1 and Model 2 predicting CAR AUC_I based on HSE at 0 – 9 months were not statistically significant.

Life Events at 0-12 Months (LCU-D)

Multiple regression analysis for life events at 0 – 12 months predicting CAR AUC_G revealed that Model 1 was significant, F(1, 137) = 10.95, p = .001, R^2 = .07, $R^2_{Adjusted}$ = .07. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 17% of variance in CAR AUC_G (F_{Change} (12, 127) = 2.33, p =.01). Examination of the individual beta coefficients showed that the interaction term involving life events and behavioral inhibition contributed significantly to the regression model (Table 3). Graphical representation of this interaction is shown in Figure 1d. A follow-up regression revealed that the association between life events and CAR AUC_G was stronger for children with lower levels of behavioural inhibition (R^2 = .18, β = .42 , t(45) = 3.13, p = .003) than those with medium (R^2 = .07, β = .26 , t(45) = 1.81, p = .08) and higher (R^2 = .05, β = .23 , t(45) = 1.58, p = .12) levels of inhibition.

Multiple regression analysis for life events at 0 – 12 months predicting CAR AUC_I revealed that Model 1 was not statistically significant, (F(1, 139) = 1.64, p = .20, R^2 = .01, R^2_{Adjusted} = .005). However, adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 15% of variance in CAR AUC_I (F_{Change} (12, 127) = 1.91, p = .04). Examination of the individual standardized beta coefficients showed that the interaction between life events and behavioral inhibition contributed significantly to the regression model (Table 4). A follow-up regression revealed that the associations between life events and CAR AUC_I within each level of behavioral inhibition was not statistically significant.

Model 1 predicting CAR AUC_G for children in the HSE group at 0 - 12 months was statistically significant, $F(1, 139) = 9.29$, $p = .003$, $R^2 = .06$, $R^2_{Adjusted} = .06$). Adding the other predictor variables and their interactions with life events significantly improved the model, explaining an additional 18% of variance in CAR AUC_G ($F_{Change}(12, 127) = 2.47$, $p = .01$) (see Table B1 in Appendix B). Examination of the individual standardized beta coefficients showed that the interaction between HSE and behavioral inhibition contributed significantly to the regression model. A follow-up regression revealed that the association between HSE and CAR AUC_G was stronger for children with lower levels of behavioural inhibition ($R^2 = .10$, $\beta = .32$, $t(45) = 2.23$, $p = .03$) than those with medium ($R^2 = .05$, $\beta = .22$, $t(45) = 1.53$, $p = .13$) and higher ($R^2 = .06$, $\beta = .25$, $t(45) = 1.70$, $p = .10$) levels of inhibition (see Figure B2 in Appendix B).

Model 1 predicting CAR AUC_I for children in the HSE group at 0 - 12 months was not statistically significant, $F(1, 139) = .36$, $p = .55$, $R^2 = .003$, $R^2_{Adjusted} = -.005$). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 15% of variance in AUC_G $F_{Change}(12, 127) = 1.85$, $p = .05$). However, examination of the individual beta coefficients showed that neither HSE nor its interaction with other variables in the model contributed to the regression model in a meaningful way.

Summary of Significant Findings for the CAR

A positive association between life events and CAR AUC_G was observed for all the life event time points. Further, a positive relationship between HSE and CAR AUC_G was found. The relationship between life events and CAR AUC_G was significantly moderated by low levels of behavioural inhibition. This was observed for all the life event time points. Low and high levels of behavioural inhibition moderated the relationship between HSE at 0 – 9 months and CAR AUC_G. Low levels of behavioural inhibition also moderated the association between HSE at 0 – 12 months and CAR AUC_G. Lastly, high levels of trait anxiety moderated the relationship between HSE at 0 – 3 months and CAR AUC_G.

Table 3

Multiple Linear Regression for Life Events Predicting AUC_G for Cortisol Awakening Response

		Model 1				Model 2			
Time Point	Predictor	*B*	*β*	*t*	*p*	*B*	*β*	*t*	*p*
0 – 3 Months	LCU A	.02	.27	3.25	.00	.04	.54	1.82	.07
	LCU A x STAIC					.00	.29	2.43	.02
	LCU A x CSRI					-.05	-.30	-2.64	.01
	LCU A x CASI					-.001	-.07	-.54	.59
	LCU A x PA					-.02	-.37	-1.41	.16
	LCU A x Age					-.00	-.04	-.50	.62
	LCU A x Sex					.00	.01	.05	.96
0 – 6 Months	LCU B	.02	.27	3.33	.00	.04	.60	1.90	.06
	LCU B x STAIC					.00	.24	1.91	.06
	LCU B x CSRI					-.05	-.32	-2.73	.01
	LCU B x CASI					-.00	-.09	-.73	.47
	LCU B x PA					-.02	-.37	-1.34	.18
	LCU B x Age					-.00	-.06	-.69	.49
	LCU B x Sex					-.01	-.06	-.52	.61
0 – 9 Months	LCU C	.02	.27	3.33	.00	.03	.58	1.83	.07
	LCU C x STAIC					.00	.17	1.36	.18
	LCU C x CSRI					-.04	-.31	-2.64	.01
	LCU C x CASI					-.00	-.06	-.45	.65
	LCU C x PA					-.02	-.33	-1.22	.22
	LCU C x Age					-.00	-.07	-.81	.42
	LCU C x Sex					-.00	-.05	-.42	.68
0 – 12 Months	LCU D	.01	.27	3.31	.00	.03	.58	1.82	.07
	LCU D x STAIC					.00	.17	1.36	.18
	LCU D x CSRI					-.04	-.32	-2.65	.01
	LCU D x CASI					-.00	-.06	-.50	.62
	LCU D x PA					-.01	-.34	-1.23	.22
	LCU D x Age					-.00	-.08	-.81	.42
	LCU D x Sex					-.00	-.06	-.44	.66

Note. N = 141. Only interactions with predictor variables shown. LCU = Life Change Units; STAIC = State-Trait Anxiety Inventory for Children Trait Form; CSRI = Childhood Self-Report of Inhibition; CASI = Childhood Anxiety Sensitivity Index; PA = Parental history of anxiety.

Table 4

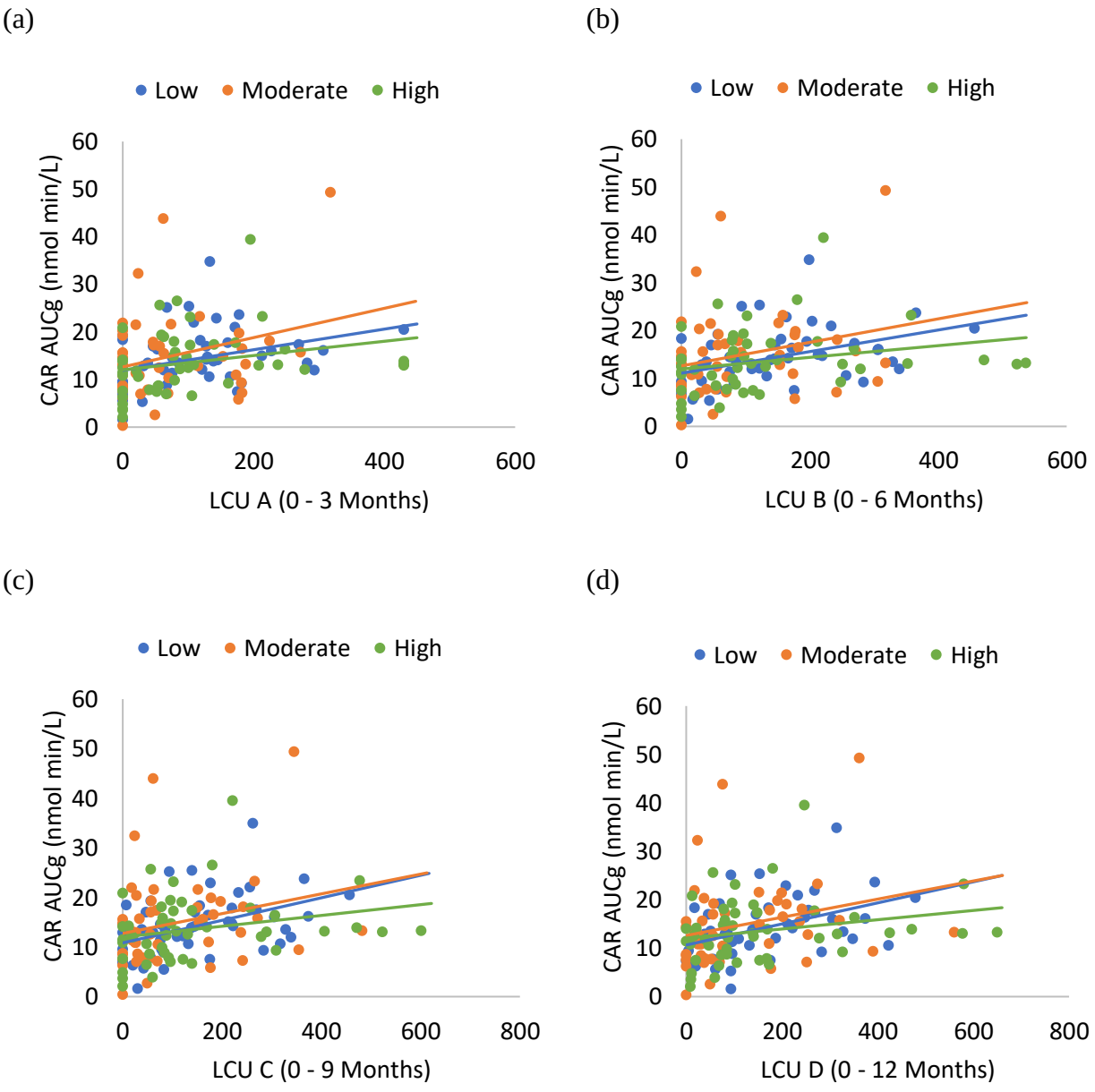
Multiple Linear Regression for Life Events Predicting AUC_I for Cortisol Awakening Response

		Model 1				Model 2			
Time Point	Predictor	*B*	*β*	*t*	*p*	*B*	*β*	*t*	*p*
0 – 3 Months	LCU A	.01	.07	.87	.38	.04	.64	2.07	.04
	LCU A x STAIC					.00	.14	1.09	.28
	LCU A x CSRI					-.03	-.25	-2.11	.04
	LCU A x CASI					-.00	-.06	-.45	.66
	LCU A x PA					-.02	-.51	-1.82	.07
	LCU A x Age					.00	-.02	-.22	.82
	LCU A x Sex					-.02	-.23	-2.01	.05
0 – 6 Months	LCU B	.00	.08	.99	.32	.03	.58	1.73	.09
	LCU B x STAIC					.00	.10	.76	.43
	LCU B x CSRI					-.03	-.36	-2.07	.04
	LCU B x CASI					.00	-.04	-.31	.76
	LCU B x PA					-.02	-.42	-1.44	.15
	LCU B x Age					.00	-.02	-.22	.83
	LCU B x Sex					-.02	-.25	-2.06	.04
0 – 9 Months	LCU C	.01	.10	1.16	.25	.03	.63	1.92	.06
	LCU C x STAIC					.00	.07	.55	.58
	LCU C x CSRI					-.03	-.26	-2.12	.04
	LCU C x CASI					.00	-.04	-.28	.78
	LCU C x PA					-.02	-.44	-1.54	.13
	LCU C x Age					.00	-.02	-.17	.86
	LCU C x Sex					-.02	-.25	-1.94	.06
0 – 12 Months	LCU D	.01	.11	1.28	.20	.03	.65	1.92	.06
	LCU D x STAIC					.00	.06	.42	.68
	LCU D x CSRI					-.03	-.27	-2.13	.04
	LCU D x CASI					.00	-.02	-.13	.89
	LCU D x PA					-.02	-.45	-1.55	.12
	LCU D x Age					.00	-.02	-.24	.81
	LCU D x Sex					-.02	-.24	-1.78	.08

Note. N = 141. Only interactions with predictor variables shown. LCU = Life Change Units; STAIC = State-Trait Anxiety Inventory for Children Trait Form; CSRI = Childhood Self-Report of Inhibition; CASI = Childhood Anxiety Sensitivity Index; PA = Parental history of anxiety

Figure 1

Interaction Between Life Events and Levels of Behavioural Inhibition Predicting AUC_G for Cortisol Awakening Response



Note. N = 141. (a) Life Change Units 0 – 3 months; (b) Life Change Units 0 – 6 months; (c) Life Change Units 0 – 9 months; (d) Life Change Units 0 – 12 months.

Diurnal Cortisol Response

Life Events at 0-3 Months (LCU-A)

Multiple regression analyses for life events at 0 – 3 months predicting diurnal AUC_G revealed that Model 1 was statistically significant, ($F(1, 139) = 12.37$, $p = .001$, $R^2 = .08$, $R^2_{\text{Adjusted}} = .08$). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 25% of variance in diurnal AUC_G ($F_{Change}(12, 127) = 3.89$, $p < .001$). Examination of the individual standardized beta coefficients showed that the interaction terms involving life events and parental history of anxiety, behavioral inhibition, and trait anxiety contributed significantly to the regression model (Table 5). Graphical representations of these interactions are shown in Figures 2 - 4. Follow-up regressions revealed that the association between life events and diurnal AUC_G was stronger for children without a parental history of anxiety ($R^2 = .15$, $\beta = .39$, $t(91) = 19.81$, $p < .001$) and negligible for children with parental history of anxiety ($\beta = -.36$, $t(46) = -.25$, $p = .81$); stronger for children with high levels of behavioural inhibition ($R^2 = .11$, $\beta = .34$, $t(45) = 2.41$, $p = .02$) versus those with low ($R^2 = .06$, $\beta = .25$, $t(45) = 1.75$, $p = .09$) and medium ($R^2 = .07$, $\beta = .27$, $t(45) = 1.86$, $p = .07$) levels of inhibition; and stronger for children with high ($R^2 = .11$, $\beta = .28$, $t(45) = 2.34$, $p = .02$) levels of trait anxiety than children with medium ($R^2 = .08$, $\beta = .28$, $t(45) = 1.93$, $p = .06$) and low levels of trait anxiety ($R^2 = .05$, $\beta = .22$, $t(45) = 1.51$, $p = .14$).

Analysis for life events at 0 – 3 months predicting diurnal AUC_I revealed that Model 1 was statistically significant, $F(1, 139) = 5.67$, $p = .02$, $R^2 = .04$, $R^2_{Adjusted} = .03$). Adding the other predictor variables and their interaction with life events did not significantly improve the model ($F_{Change}(12, 127) = 1.54$, p = .12). Thus Model 1 was retained. The standardized beta coefficient for LCU-A in Model 1 was -.20 (t = -2.38, p = .02), indicating that high exposure to life events was associated with lower AUC_I.

Multiple regression analysis for children in the HSE group at 0 - 3 months predicting diurnal AUC_G revealed that Model 1 was statistically significant, $F(1, 139) = 10.26$, $p = .002$, $R^2 = .07$, $R^2_{Adjusted} = .06$. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 25% of variance in diurnal AUC_G ($F_{Change}(12, 127) = 3.85$, $p < .001$) (see Table B2 in Appendix B). Examination of the individual standardized beta coefficients showed that the interaction between HSE at 0 - 3 months and parental history of anxiety and trait anxiety contributed significantly to the regression model.

Follow-up regressions showed that the association between HSE and diurnal AUC_G was stronger for children with no parental history of anxiety (R^2 = .17, β = .41 , t(91) = 4.28, p = < .001) and negligible for children with a parental history of anxiety (R^2 =.01, β = -.11 , t(45) = -.75, p = .45); and stronger for children with high trait anxiety (R^2 =.09, β = .29 , t(45) = 2.05, p = .05) compared to those with medium (R^2 = .07, β = .26 , t(45) = 1.84, p = .07) and low (R^2 = .05, β = .21 , t(45) = 1.47, p = .15) trait anxiety (see Figures B3 and B4 in Appendix B).

For diurnal cortisol AUC_I, the regression model revealed that Model 1 was statistically significant, F(1, 139) = 5.73, p = .018, R^2= .04, $R^2_{Adjusted}$ = .033. However, adding the other predictor variables and their interactions with HSE at 0 - 3 months did not improve the model (F_{Change} (12, 127) = 1.43, p=.16). Thus, Model 1 was retained. The standardized beta coefficient for HSE and AUC_I in Model 1 was -.199 (t = -2.40, p = .02), indicating that high exposure to life events was associated with lower AUC_I.

Life Events at 0-6 Months (LCU-B)

Multiple regression analysis for life events at 0 – 6 months predicting diurnal AUC_G showed that Model 1 was statistically significant, F(7, 139) = 15.20, p < .001, R^2 = .10, $R^2_{Adjusted}$ = .09. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 22% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.37, p < .001). Examination of the individual beta coefficients showed that the interaction between life events at 0 - 6 months and parental anxiety, trait anxiety, and behavioral inhibition contributed significantly to the regression model (Table 5). Graphical representations of these interactions are shown in Figures 2 - 4. Follow-up regressions revealed that the association between life events and diurnal AUC_G was stronger for children with no parental history of anxiety (R^2 = .16, β = .41 , t(91) = 4.22, p < .001) and negligible for those with a parental history of anxiety (R^2 = .00, β = .03 , t(46) = .19, p = .85); stronger for children with higher (R^2 = .13, β = .36 , t(45) = 2.58, p = .01) and lower (R^2 = .11, β = .32 , t(45) = 2.29, p = .03) levels of behavioural inhibition than those with medium levels of inhibition (R^2 = .07, β = .27 , t(45) = 1.85, p = .07); and stronger for children with medium (R^2 = .15, β = .39 , t(45) = 2.83, p = .01) levels of trait anxiety than those with low (R^2= .08, β = .28 , t(45) = 1.98, p = .054) and high levels of trait anxiety (R^2= .08, β = .28 , t(45) = 1.92, p = .06).

Analysis for life events at 0 – 6 months predicting diurnal AUC_I revealed that Model 1 was statistically significant, $F(1, 139) = 4.72$, $p = .03$, $R^2 = .03$, $R^2_{Adjusted} = .03$). Adding the other predictor variables and their interaction with life events did not significantly improve the model (F_{Change} (12, 127) = 1.41, p = .17). Thus Model 1 was retained. The standardized beta coefficient for LCU-B in Model 1 was -.001 (t = -2.17, p = .03), indicating that the lower the LCU-B score the higher the diurnal cortisol AUC_I value.

Model 1 of the multiple regression analyzing children in the high stress exposed group at 0 - 6 months to predict diurnal AUC_G was significant, $F(1, 139) = 10.47$, $p = .002$, $R^2 = .070$, $R^2_{Adjusted} = .063$. Adding the other predictor variables and their interaction with HSE improved the regression model, explaining an additional 22% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.32, $p < .001$) (see Table B2 in Appendix B). Inspection of the individual standardized beta weights indicated that the interaction between HSE at 0 - 6 months and trait anxiety contributed significantly to the regression model. Follow-up regression revealed that the association between HSE and AUC_G was stronger for children with medium levels of trait anxiety ($R^2 = .12$, $\beta = .35$, $t(45) = 2.49$, $p = .02$) than those with high ($R^2 = .074$, $\beta = .27$, $t(45) = 1.90$, $p = .07$) and low ($R^2 = .03$, $\beta = .17$, $t(45) = 1.12$, $p = .27$) levels of trait anxiety (see Figure B4 in Appendix B).

For diurnal cortisol AUC_I, the regression model revealed that Model 1 was statistically significant, $F(1, 139) = 5.43$, $p = .02$, $R^2 = .04$, $R^2_{Adjusted} = .03$. However, adding the other predictor variables and their interaction with HSE at 0 – 6 months did not improve the model (F_{Change} (12, 127) = 1.33, $p = .21$). Thus Model 1 was retained. The standardized beta coefficient for HSE and diurnal AUC_I, in Model 1 was -.19 (t = -2.33, $p = .02$), indicating that HSE was associated with lower diurnal AUC_I.

Life Events at 0-9 Months (LCU-C)

Model 1 for life events predicting diurnal AUC_G was also significant, $F(1, 139) = 14.61.$, $p < .001$, $R^2 = .10$, $R^2_{Adjusted} = .09$. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 21% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.15, $p = .001$). Further analysis of standardized beta coefficients showed that interactions between life events and family history of anxiety and behavioural inhibition contributed significantly to the regression model (Table 5). Graphical representations of the interactions appear in Figures 2 and 3. Follow-up regressions revealed that the association

between life event at 0 - 9 months and diurnal AUC_G was stronger for children with no parental history of anxiety (R^2 = .15, β = .39, t(91) = 4.05, p < .001) and negligible for those with a family history of anxiety (β = .03 , t(46) = .21, p = .84); and stronger for children with high (R^2 = .12, β = .35 , t(45) = 2.52, p = .02) and low levels of behavioural inhibition (R^2 = .11, β = .33 , t(45) = 2.32, p = .03) than those with medium (R^2 = .06, β = .25 , t(45) = 1.73, p = .09) levels of inhibition.

Analysis for life events at 0 – 9 months predicting diurnal AUC_I revealed that Model 1 was statistically significant, F(1, 139) = 5.10, p = .03, R^2 = .04, $R^2_{Adjusted}$ = .03). Adding the other predictor variables and their interaction with life events did not significantly improve the model (F_{Change} (12, 127) = 1.42, p = .17). Thus Model 1 was retained. The standardized beta coefficient for LCU-C in Model 1 was -.001 (t = -2.26, p = .03), indicating that the lower the LCU-C score the higher the diurnal cortisol AUC_I value.

Model 1 of the multiple regression analyzing children in the HSE group at 0 - 9 months to predict diurnal AUC_G was significant, F(1, 139) = 9.54, p = .002, R^2 = .06, $R^2_{Adjusted}$ = .06. Adding the other predictor variables and their interaction with HSE improved the regression model, explaining an additional 22% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.31, p < .001). However, examination of the individual standardized beta coefficients showed that neither HSE nor its interaction with other predictor variables contributed in a meaningful way to the regression model. For diurnal cortisol AUC_I, the regression model revealed that Model 1 was statistically significant, F(1, 139) = 7.14, p = .008, R^2 = .05, $R^2_{Adjusted}$ = .04. However, adding the other predictor variables and their interaction with HSE at 0 – 9 months did not improve the model (F_{Change} (12, 127) = 1.45, p = .15). Thus, Model 1 was retained. The standardized beta coefficient for HSE and AUC_I, in Model 1 was -.221 (t = -2.67, p =.008), indicating that HSE was associated with lower AUC_I levels.

Life Events at 0-12 Months (LCU-D)

Multiple regression analysis also revealed that Model 1 for life events at 0 – 12 months predicting diurnal AUC_G was significant, F(1, 139) = 14.67, p < .001, R^2 = .10, $R^2_{Adjusted}$ = .09. Adding the other predictor variables and their interaction with life events improved the model, explaining an additional 21% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.12, p = .001). Inspection of the standardized beta weights showed that the interaction between life events and parental history of anxiety and behavioral inhibition contributed significantly to the regression

model (Table 5). Graphical representations of these interactions appear in Figures 2 and 3. Follow-up regressions showed that the association between life events at 0 - 12 months and diurnal AUC_G was stronger for children with no parental history of anxiety (R^2= .15, β = .39 , $t(91) = 4.06$, $p < .001$) and negligible for those with a parental history of anxiety (β = .01 , $t(46)$ = .09, p = .93), and stronger for children with high (R^2 = .12, β = .35 , $t(45) = 2.47$, p = .02) and low levels of behavioral inhibition (R^2= .12, β = .34 , $t(45) = 2.42$, p = .02) than those with medium (R^2 = .06, β = .25 , $t(45) = 1.73$, p = .09) levels of inhibition.

Analysis for life events at 0 – 12 months predicting diurnal AUC_I revealed that Model 1 was statistically significant, $F(1, 139) = 5.03$, p = .03, R^2 = .04, $R^2_{Adjusted}$ = .03. Adding the other predictor variables and their interaction with life events did not significantly improve the model (F_{Change} (12, 127) = 1.48, p = .14). Thus Model 1 was retained. The standardized beta coefficient for LCU-D in Model 1 was -.001 (t = -2.46, p = .03), indicating that the lower the LCU-D score the higher the diurnal cortisol AUC_I value.

Model 1 for children in the HSE group at 0 - 12 months was also significant, $F(1, 139)$ = 10.28, p = .002, R^2 = .07, $R^2_{Adjusted}$ = .06. Adding the other predictor variables and their interaction with HSE improved the model, explaining an additional 23% of variance in diurnal AUC_G (F_{Change} (12, 127) = 3.38, $p < .001$) (see Table B2 in Appendix B). Investigation of individual beta coefficients demonstrated that the interaction between HSE and trait anxiety predicted diurnal cortisol AUC_G. A follow-up regression showed that the association between HSE and diurnal AUC_G was stronger for the group of children with medium levels of trait anxiety (R^2 =.12, β = .35 , $t(45) = 2.50$, p = .02) versus those with high (R^2 =.07, β = .27 , $t(45)$ = 1.84, p = .07) or low (R^2 = .03, β = .17 , $t(45) = 1.12$, p = .27) levels of trait anxiety (see Figure B4 in Appendix B). For diurnal cortisol AUC_I, the regression model revealed that Model 1 was statistically significant, F (1, 139) = 6.07, p = .02, R^2 = .04, $R^2_{Adjusted}$ = .04. However, adding the other predictor variables and their interaction with HSE at 0 – 12 months did not improve the model (F_{Change} (12, 127) = 1.48, p = .14). Thus Model 1 was retained. The standardized beta coefficient for HSE and AUC_I, in Model 1 was -.21 (t = -2.46, p = .02), indicating that HSE was associated with lower AUC_I levels.

Summary of Significant Findings for Diurnal Cortisol

Life events significantly predicted diurnal AUC_G and AUC_I. A significant relationship between HSE and diurnal AUC_G and AUC_I also emerged. High levels of trait anxiety moderated

the relationship between life events at 0 – 3 months and diurnal AUC_G, and HSE at 0 – 3 months and diurnal AUC_G. Medium levels of trait anxiety moderated the relationship between life events (0 – 6 and 0 – 12 months) and diurnal AUC_G, and HSE at 0 – 6 months and diurnal AUC_G. Not having a parental history of anxiety moderated the relationship between all the life event time points and diurnal AUC_G, as well as the association between HSE at 0 – 3 months and diurnal AUC_G. High levels of behavioural inhibition moderated the relationship between all the life event time points and diurnal AUC_G. Lastly, low levels of behavioural inhibition moderated the relationship between life events (0 – 6, 0 – 9, and 0 – 12 months) and diurnal AUC_G.

Table 5

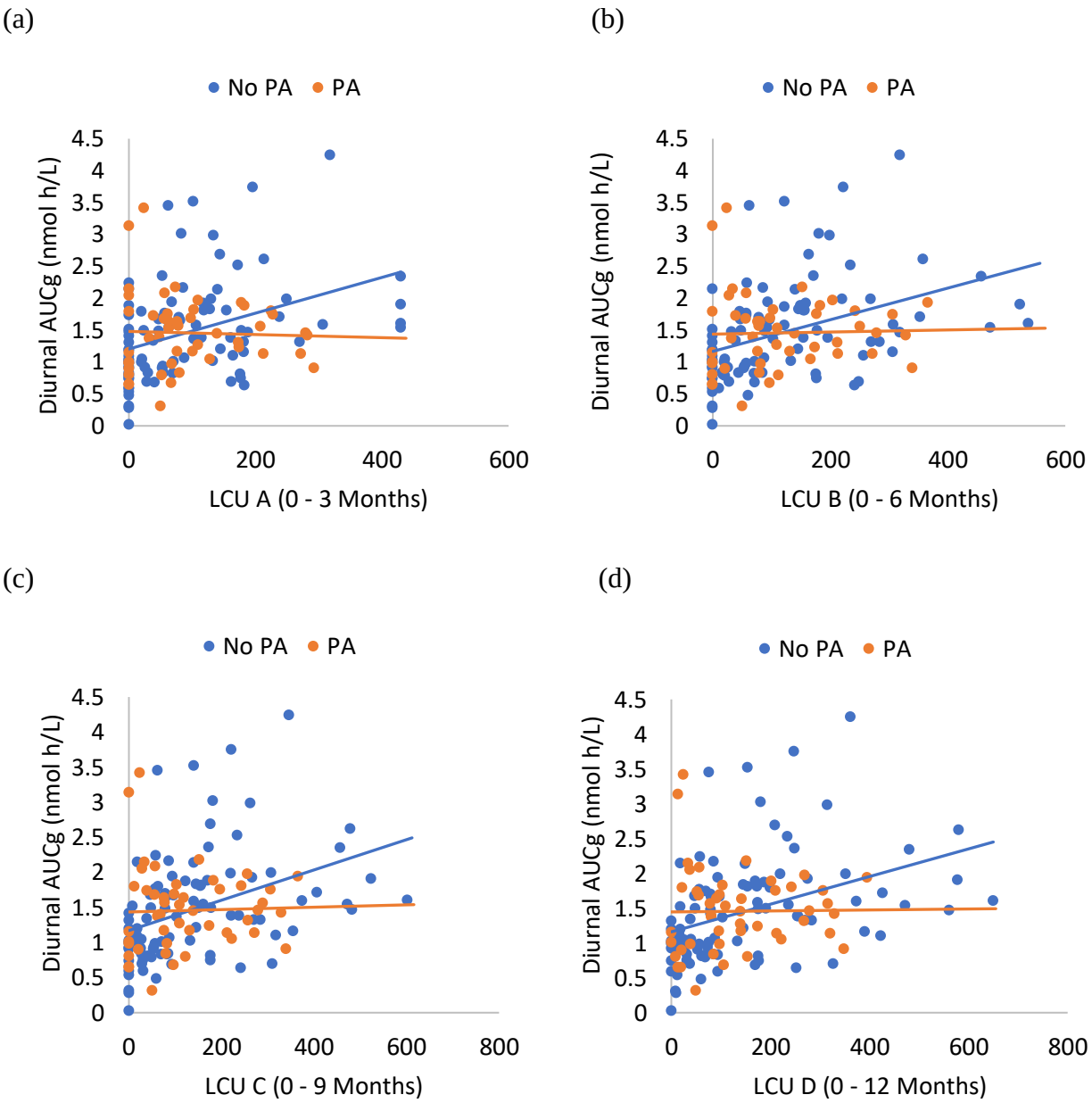
Multiple Linear Regression for Life Events Predicting AUC_G for Diurnal Cortisol Response

		Model 1				Model 2			
Time Point	Predictor	*B*	*β*	*t*	*p*	*B*	*β*	*t*	*p*
0 – 3 Months	LCU A	.00	.29	3.52	.001	.01	.69	2.44	.02
	LCU A x STAIC					.00	.30	2.62	.01
	LCU A x CSRI					-.00	-.23	-2.12	.04
	LCU A x CASI					.00	-.09	-.76	.45
	LCU A x PA					-.00	-.55	-2.16	.03
	LCU A x Age					.00	-.06	-.70	.48
	LCU A x Sex					.00	.01	.07	.94
0 – 6 Months	LCU B	.00	.31	3.90	.00	.00	.82	2.70	.01
	LCU B x STAIC					.00	.25	2.12	.04
	LCU B x CSRI					-.00	-.27	-2.43	.02
	LCU B x CASI					.00	-.11	-.86	.39
	LCU B x PA					-.00	-.59	-2.23	.03
	LCU B x Age					.00	-.06	-.71	.48
	LCU B x Sex					-.00	-.07	-.62	.54
0 – 9 Months	LCU C	.00	.31	3.82	.00	.00	.77	2.57	.01
	LCU C x STAIC					.00	.19	1.53	.13
	LCU C x CSRI					-.00	-.27	-2.34	.02
	LCU C x CASI					-.00	-.06	-.50	.62
	LCU C x PA					-.00	-.55	-2.09	.04
	LCU C x Age					.00	-.08	-.94	.35
	LCU C x Sex					-.00	-.07	-.57	.57
0 – 12 Months	LCU D	.00	.31	3.83	.00	.00	.81	2.63	.01
	LCU D x STAIC					.00	.18	1.48	.14
	LCU D x CSRI					-.00	-.26	-2.26	.03
	LCU D x CASI					-.00	-.26	-.57	.56
	LCU D x PA					-.00	-.58	-2.20	.03
	LCU D x Age					-.00	-.07	-.81	.42
	LCU D x Sex					-.00	-.08	-.6	.52

Note. N = 141. Only interactions with predictor variables shown. LCU = Life Change Units; STAIC = State-Trait Anxiety Inventory for Children Trait Form; CSRI = Childhood Self-Report of Inhibition; CASI = Childhood Anxiety Sensitivity Index; PA = Parental history of anxiety.

Figure 2

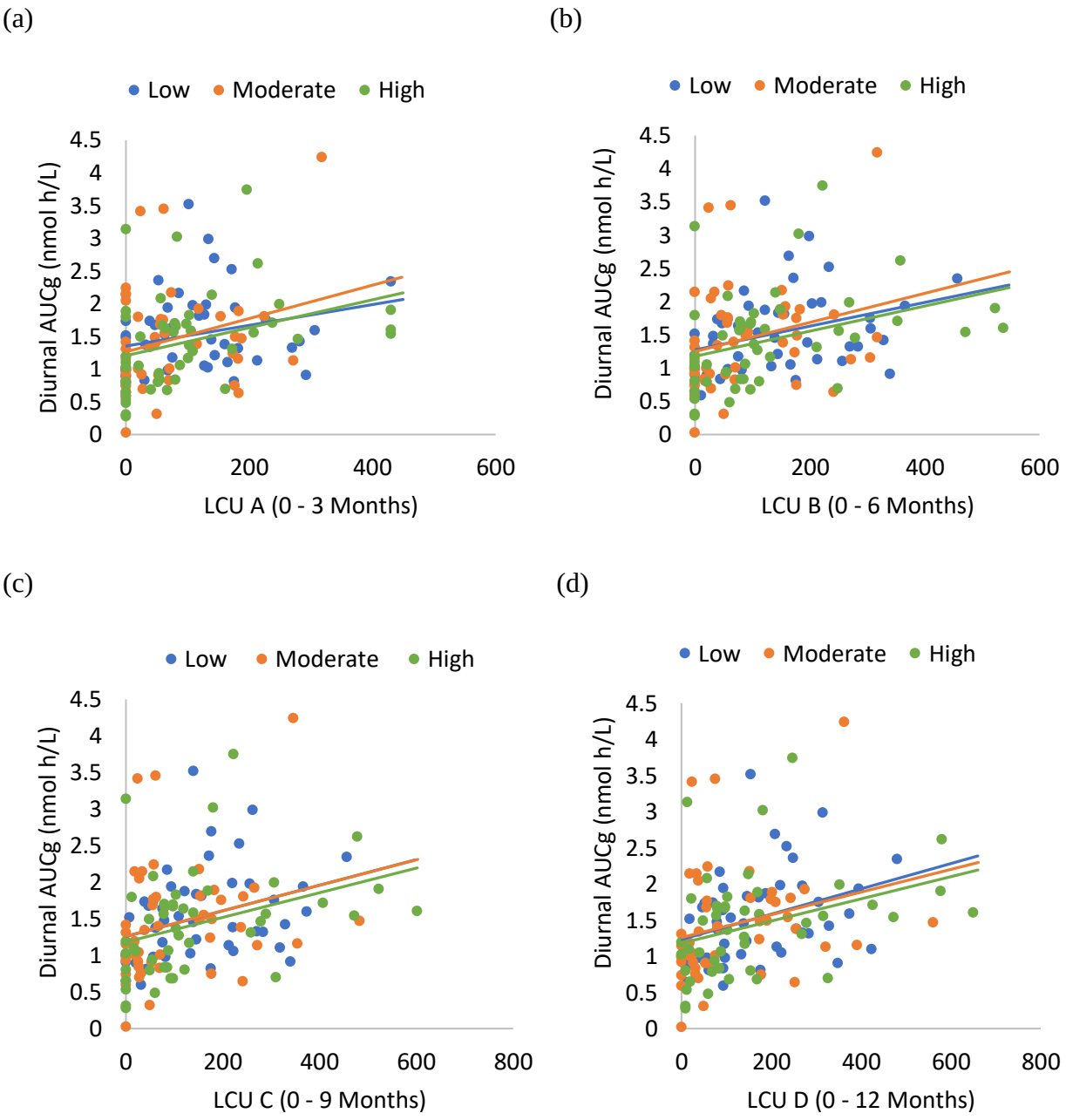
Interaction Between Life Events and Parental Anxiety Predicting AUC_G for Diurnal Cortisol Response



Note. N = 141. (a) Life Change Units 0 – 3 months; (b) Life Change Units 0 - 6 months; (c) Life Change Units 0 – 9 months); (d) Life Change Units 0 – 12 months.

Figure 3

Interaction Between Life Events and Levels of Behavioural Inhibition Predicting AUC_G for Diurnal Cortisol Response

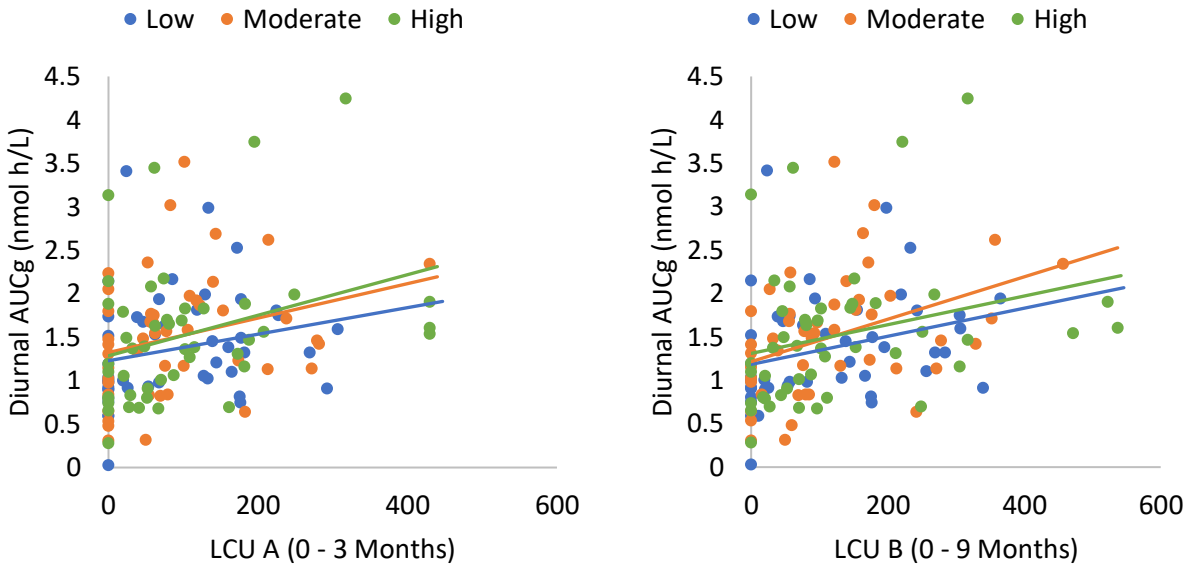


Note. N = 141. (a) Life Change Units 0 – 3 months; (b) Life Change Units 0 - 6 months; (c) Life Change Units 0 – 9 months); (d) Life Change Units 0 – 12 months.

Figure 4

Interaction Between Life Events and Levels of Trait Anxiety Predicting AUC_G for Diurnal Cortisol Response

(a) (b)



Note. N = 141. (a) Life Change Units 0 – 3 months; (b) Life Change Units 0 - 6 months.

Cortisol Reactivity to a Laboratory Stressor

Life Events at 0 - 3 Months (LCU-A)

None of the models involving cortisol reactivity AUC_G were statistically significant. Multiple regression analysis for life events at 0 – 3 months predicting AUC_I revealed that Model 1 was not statistically significant, $F(1, 136) = .91$, $p = .34$, $R^2 = .01$, $R^2_{Adjusted} = -.001$. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 18% of variance in cortisol reactivity AUC_I ($F_{Change}(12, 124) = 2.18$, $p = .01$). However, examination of the individual beta coefficients showed that neither life events nor its interaction with other variables contributed in a meaningful way to the regression model.

Model 1 predicting cortisol reactivity AUC_I for children in the HSE group at 0 - 3 months was statistically significant, $F(1, 136) = 8.89$, $p = .003$, $R^2 = .06$, $R^2_{Adjusted} = .05$). Adding the other predictor variables and their interactions with HSE significantly improved the model, explaining an additional 18% of variance in cortisol reactivity AUC_I ($F_{Change}(12, 124) = 2.49$, $p = 001$). However, examination of the individual standardized beta coefficients showed that

neither HSE nor the interaction with other predictor variables contributed in a meaningful way to the regression model. Thus Model 1 was retained. The standardized beta coefficient for HSE and AUC_I in Model 1 was .51 (t = 2.98, p = .003), indicating that HSE at 0 – 3 months was associated with increased AUC_I levels.

Life Events at 0-6 Months (LCU-B)

None of the models involving cortisol reactivity AUC_G were statistically significant. Multiple regression analysis for life events at 0 – 6 months predicting cortisol reactivity AUC_I revealed that Model 1 was not statistically significant (F(1, 136) = 1.21, p = .27, R^2 = .01, $R^2_{Adjusted}$ = .002). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 18% of variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.26, p = .01). However, examination of the individual beta coefficients showed that neither life events nor its interaction with other variables in the model contributed in a meaningful way.

Model 1 predicting cortisol reactivity AUC_I for children in the HSE group at 0 - 6 months was not statistically significant, F(1, 136) = 3.12, p = .08, R^2 = .02, $R^2_{Adjusted}$ = .02. Adding the other predictor variables and their interactions with HSE significantly improved the model, explaining an additional 18% of variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.38, p = 01). However, examination of the individual standardized beta coefficients showed that neither HSE nor the interaction with other predictor variables contributed in a meaningful way to the regression model.

Life Events at 0-9 Months (LCU-C)

None of the models involving cortisol reactivity AUC_G were statistically significant. Multiple regression analysis for life events at 0 – 9 months predicting AUC_I revealed that Model 1 was not statistically significant, F(1, 136) = 1.93, p = .17, R^2 = .01, $R^2_{Adjusted}$ = .01). Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 19% variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.48, p = .005). Examination of the individual standardized beta coefficients showed that neither life events nor its interaction with other variables in the model contributed in a meaningful way to the regression model.

Model 1 predicting cortisol reactivity AUC_I for children in the HSE group at 0 - 9 months was not statistically significant, F(1, 136) = 3.85, p = .052, R^2 = .03, $R^2_{Adjusted}$ = .02). Adding the

other predictor variables and their interactions with HSE significantly improved the model, explaining an additional 16% of variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.18, p = .01). However, examination of the individual standardized beta coefficients showed that neither HSE nor the interaction with other predictor variables contributed in a meaningful way to the regression model.

Life Events at 0-12 Months (LCU-D)

None of the models involving cortisol reactivity AUC_G were statistically significant. Multiple regression analysis for life events at 0 – 12 months predicting cortisol reactivity AUC_I revealed that Model 1 was not statistically significant, F(1, 136) = 2.06, p = .15, R^2 = .02, $R^2_{Adjusted}$ = .01. Adding the other predictor variables and their interaction with life events significantly improved the model, explaining an additional 21% variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.65, p = .003). Examination of the individual beta coefficients showed that neither life events nor its interaction with other variables in the model contributed in a meaningful way to the regression model.

Model 1 predicting cortisol reactivity AUC_I for children in the HSE group at 0 - 12 months was not statistically significant, F(1, 136) = 2.97, p = .09, R^2 = .02, $R^2_{Adjusted}$ = .01). Adding the other predictor variables and their interactions with HSE significantly improved the model, explaining an additional 17% of variance in cortisol reactivity AUC_I (F_{Change} (12, 124) = 2.19, p = .02). However, examination of the individual standardized beta coefficients showed that neither HSE nor the interaction with other predictor variables contributed in a meaningful way to the regression model.

Summary of Significant Findings for Cortisol Reactivity

HSE at 0 - 3 months was found to significantly predict cortisol reactivity AUC_I. No moderators predicted the relationship between life events and cortisol reactivity.

Interaction Between Parental Bonding and Life Events and Cortisol Response

The Parental Bonding Instrument (PBI) was not administered to all of the participants in the study, thus a separate analysis was conducted with the subset of participants who had available data for both the mother and father version of the PBI. These analyses included one hundred and four participants. Analyses were conducted for both the AUC_G and AUC_I. The model included the life events scale, the parental bonding predictor variables (maternal care, paternal care, maternal protection, paternal protection), and their interaction with life events. As

the primary interest in these analyses was whether the interactions in the model were statistically significant, only significant interaction results are reported.

Parental Bonding and Cortisol Awakening Response

None of the regression models involving the interaction between life events at 0 – 6 (LCU-B), 0 – 9 (LCU-A), and 0 – 12 (LCU-D) months and maternal and paternal bonding were statistically significant for AUC_I and AUC_G. For life events at 0 – 3 months (LCU-A) the model involving the interaction between life events and maternal and paternal bonding was not statistically significant for CAR AUC_I. However, the model was statistically significant for life events at 0 – 3 months predicting CAR AUC_G ($F(9, 94) = 1.99$, $p = .05$, $R^2 = .16$, $R^2_{Adjusted} = .08$). Examination of the individual beta coefficients showed that the interactions between life events at 0 – 3 months and paternal care significantly contributed to the prediction of CAR AUC_G (Table 6). Graphical representation of this interaction appears in Figure 5. A follow-up regression showed that the association between life events and CAR AUC_G was stronger for the group of children with higher ($R^2 = .25$, $\beta = .50$, $t(33) = 3.28$, $p = .002$) and lower ($R^2 = .15$, $\beta = .39$, $t(32) = 2.38$, $p = .02$) levels of paternal care than those with moderate ($R^2 = .00$, $\beta = -.02$, $t(33) = -.09$, $p = .93$) levels of paternal care.

Table 6

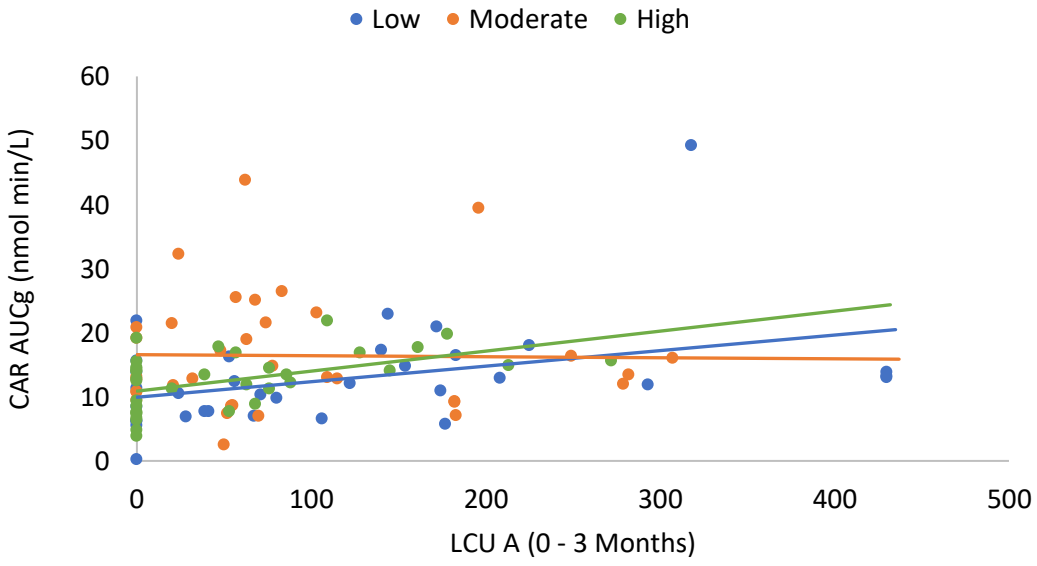
Multiple Linear regression of Life Events at 0 - 3 Months and the Interaction with Parental Bonding Predicting AUC_G for Cortisol Awakening Response

	Model 1			
Predictor	*B*	*β*	*t*	*p*
LCU A	.00	.31	3.83	.00
LCU A x Care M	-.00	-.12	-1.04	.30
LCU A x Care F	.00	.33	2.09	.04
LCU A x Protect M	.00	.11	.87	.39
LCU A x Protect F	.00	.18	1.05	.30

Note. N = 101. Only interactions with predictor variables shown. LCU A = Life Change Units 0-3 months; Care M = Maternal care; Care F = Paternal Care; Protect M = Maternal Protection; Protect F = Paternal Protection.

Figure 5

Interaction Between Life Events at 0 – 3 Months and Paternal Care Predicting AUC_G for Cortisol Awakening Response



Note. N = 101.

Parental Bonding and the Diurnal Cortisol Response

None of the regression models involving the interaction between life events and maternal and paternal bonding were statistically significant for diurnal AUC_I. For AUC_G, multiple regression analysis was significant for life events at 0 – 3 months ($F(9, 94) = 2.06$, $p = .04$, $R^2 = .17$, $R^2_{Adjusted} = .09$), 0 – 6 months ($F(9, 94) = 2.22$, $p = .03$, $R^2 = .18$, $R^2_{Adjusted} = .10$), and 0 – 9 ($F(9, 94) = 2.13$, $p = .03$, $R^2 = .17$, $R^2_{Adjusted} = .09$) months. However, for each time point, examination of the individual beta coefficients showed that the interactions between life events and parental bonding did not contribute to the regression model in a meaningful way. The model involving the interaction between life events at 0 – 12 months and maternal and paternal bonding was not statistically significant for diurnal AUC_G.

Parental Bonding and Cortisol Reactivity to a Laboratory Stressor

Results indicated that none of the regression models involving the interaction between life events and maternal and paternal bonding were statistically significant for cortisol reactivity AUC_I and AUC_G.

Discussion

The present study examined the influence of life events on HPA axis function biomarkers in healthy children and adolescents and identified moderators of this relationship. Results showed that life events were a significant predictor of different indicators of HPA axis function biomarkers in this sample. Further, behavioural inhibition, not having a parental history of anxiety, trait anxiety, and paternal care were found to positively moderate some of these associations.

Life Events and the Cortisol Measures

The CAR is the rapid increase in cortisol initiated by awakening (Pruessner et al., 1997). The hypothesis that life events would predict the CAR was partially supported. Specifically, increased life events were associated with a greater CAR AUC_G. Further, a significant association was observed between the high stress exposed group and increased CAR AUC_G. The current findings are consistent with prior research that found a relationship between life stress over the past year and increased CAR in adolescents (Miller, 2014). Similarly, associations between elevated morning cortisol secretion and higher social stress and worries over the past year in adults have been reported (Wüst et al., 2000). Although the exact function of the CAR remains unclear, the rise in cortisol upon awakening has been related to anticipation of the day's

demands (Fries et al., 2009), with increased CAR reflecting the responsiveness of the HPA axis (Chen et al., 2017). Indeed, CAR AUC_G has been shown to be positively related to life stress, with elevated CAR hypothesized to be a neuroendocrine response to prepare the individual for daily challenges (see meta-analysis conducted by Chida & Steptoe, 2009). Taken together, the elevated CAR AUC_G observed in the current study may represent normal adaptation to increased life events in healthy children and adolescents.

Contrary to expectation, no significant associations emerged between life events and the CAR AUC_I. This finding is consistent with previous research that found no relationship between CAR AUC_I and life events over the past year in adolescents (Starr et al., 2017) or total number of negative life events in children (Cullen et al., 2014). However, a recent review on guidelines for assessing the CAR suggests that AUC_I is a more accurate measure of the CAR than AUC_G, which should only be assessed as providing supplemental information on the "post-awakening cortisol concentrations" (Stalder et al., 2016). Indeed, it has been shown that if the initial cortisol wakening value is large, CAR AUC_G will be greater, even if the increase in cortisol following awakening is minimal (Chida & Steptoe, 2009). Therefore, Stalder et al. (2016) proposed that measurement of the CAR should be restricted to cortisol increase post-awakening. In light of this recommendation, the relationship between life events and the CAR AUC_G should be viewed with caution, especially as no significant association between life events and the CAR AUC_I was found. Further, findings related to potential moderators of the relationship between life events and CAR AUC_G need to be interpreted with caution based on the aforementioned recommendations.

The diurnal cortisol response consists of a peak cortisol level following awakening and a decrease in cortisol secretion throughout the day (Stone et al., 2001). Similar to the CAR, the hypothesis that life events would predict diurnal cortisol secretion was supported in this study. Specifically, increases in life events was associated with a greater total diurnal cortisol secretion (AUC_G). Participants in the high stress exposed group also demonstrated a significantly greater daily cortisol output. In line with these findings, previous work found that exposure to frequent stress is associated with elevated secretion of stress hormones (McEwen, 1998), with chronic stress related to higher daily cortisol secretion (Miller et al., 2007). In contrast, other research found no relationship between diurnal cortisol AUC_G and frequency of daily hassles within the past 6 months or total negative life events (Cullen et al., 2014). Methodological differences

likely explain these discrepant findings, with the Cullen et al.'s (2014) study focusing on school-related hassles and life events over a child's lifetime and the current study exploring life events over the past year.

The present study also found a significant negative relationship between life events and diurnal cortisol AUC_I. Further, a significant association between the high stress exposed group and diurnal AUC_I was observed. Specifically, the high stress exposed group exhibited a steeper decrease in cortisol during the day. In comparison to results of the current study, Michels et al. (2012) observed no relationship between total life events in the past year, as measured by the CLES, and diurnal cortisol in children. However, in line with the present study's findings, these researchers did find that greater negative life events in the past three months significantly predicted a steeper diurnal slope. As the current study only measured the total number of life events children experienced, it is plausible that they experienced a greater number of negative life events, which may explain the significant relationship between diurnal cortisol and all the life event time points. It is interesting to note that compared to the study of Michels et al. (2012), participants in the current study reported a greater total LCU score at each time point during the year. Thus, differences in sample characteristics as well as the measurement of diurnal slope may account for inconsistencies between the studies. As AUC_I is a measure of stress reactivity (Pruessner et al., 2003) and accounts for intensity and sensitivity (Fekedulegn et al., 2007), it could be suggested that high exposure to life events enhances the responsiveness of the stress system as a means to adapt to the stress load. However, additional research on the association between diurnal AUC_I and life events in children and adolescents is needed to confirm the study's results.

With respect to cortisol reactivity to the laboratory stressor**,** a significant relationship between life events at 0 – 3 months and cortisol reactivity emerged. Specifically, participants in the high stress exposed group at 0 – 3 months exhibited a greater AUC_I compared to those in the low stress exposed group. Similar findings have been reported in research with adults (Elzinga et al., 2008). However, Roos et al. (2018) found no relationship between the number of life events experienced over the past year and cortisol reactivity AUC_I to a laboratory stressor in their sample of adults. A plausible explanation for this discrepancy is that the study by Roos et al. (2018) did not measure life events occurring within the past three months. In a study on life events and cortisol reactivity in adolescents, Chiang et al. (2019) found that participants who

experienced a greater number of major life events in the past year exhibited lower cortisol reactivity to the TSST than those who experienced less life events. Methodological differences in the calculation of cortisol reactivity in the aforementioned study was observed and thus the findings may not be generalizable to AUC_I measures in the current study.

As only high exposure to recent life events predicted cortisol reactivity, it is plausible that the cortisol response to a laboratory stressor is more strongly influenced by proximal rather than distal life events. Indeed, a temporal pattern of stress has been observed in previous research, with the effect size of cortisol measures decreasing in relation to increases in the time of stressor onset (Miller et al., 2007). Although the meta-analysis on chronic stress and the HPA axis by Miller et al. (2007) did not include measures of cortisol reactivity AUC_I, it could be suggested that participants with high exposure to stress in the past three months were still adapting to the life events when the laboratory stress test occurred. However, further research is needed on the relationship between exposure to recent stress and cortisol reactivity in children and adolescents to confirm these findings.

In contrast to the observed relationship between HSE at 0 - 3 months and cortisol reactivity AUC_I, life events over the past year did not predict total cortisol reactivity output in children and adolescents. Similar findings have been observed in research by Bendezú and Wadsworth (2017), who found no significant association between stressful life events in the preceding year and cortisol reactivity to the TSST in children. However, findings are mixed and other studies have noted an association between cortisol reactivity to the TSST and stressful life events. For example, in a study of adolescents, chronic stress within the past six months significantly predicted cortisol reactivity AUC_G to the TSST (Rao et al., 2008). However, this study is limited by the small sample size and that the analysis combined depressed and healthy children, which may have obscured findings. In another study of healthy children, a significant relationship between stressful life events experienced over a child's lifetime and cortisol reactivity following a laboratory stressor was found (Armbruster et al., 2011). As the current study evaluated life events within the past 12 months, it is possible that different results would have emerged if lifetime exposure to stressful life events was also assessed.

Moderator Variables on Life Events and the Cortisol Response

The current study identified several moderators of the relationship between life events and the cortisol profiles, notably levels of behavioural inhibition, trait anxiety, parental anxiety,

and parental bonding. Taking these moderators into account, a more complex picture of the relationship between life events and HPA axis function biomarkers emerged. The CAR and diurnal cortisol profiles appeared to be sensitive to different moderating variables, highlighting the differences in these stress response measures. Indeed, research has demonstrated that the CAR is distinct from diurnal cortisol (Clow et al., 2010), with both measures related to psychosocial variables in differing ways (Adam, 2012). Further, both the CAR and diurnal cortisol response have been shown to be influenced by numerous factors (e.g. Adam, 2012; Fries et al., 2009), supporting their sensitivity to moderating variables. Conversely, none of the moderators in the study were found to influence the relationship between high exposure to life events and cortisol reactivity. Thus, the effect of high recent stress exposure on acute stress response appears to be more direct and less influenced by variables that moderated the other indices of HPA axis function.

Behavioural Inhibition

The present study found that the association between life events and the CAR AUC_G was significantly stronger for participants with low levels of behavioural inhibition than for those with high or moderate levels of inhibition. These results were consistent across all the life event time points. To my knowledge, this is the first study that has examined behavioural inhibition as a moderator of the relationship between life events and the CAR in healthy children and adolescents. Previous studies have examined the relationship between behavioural inhibition and the stress response system. For example, children who were fearful of novel stimuli at age two demonstrated an increased morning cortisol at age 4 (Schmidt et al., 1997). Likewise, behaviourally inhibited toddlers have been shown to exhibit higher morning cortisol, with their stress response hypothesized to have a lower threshold than non-inhibited children in response to challenges (Kagan et al., 1987). Both high and low basal cortisol levels have been observed in 10-year old shy children (Schmidt et al., 2007), with a significant relationship between shyness and lower morning cortisol levels observed for shy adults in comparison to non-shy adults (Beaton, Schmidt, Schulkin, and Hall, 2013). Based on these findings, one might have expected a stronger association between life events and the CAR in participants with high levels of behavioural inhibition in comparison to those with low or moderate levels of inhibition. Nevertheless these findings are somewhat consistent with those of Bruce et al. (2002), who found that among children starting a new school year, those with high levels of surgency

(defined as a lack of shyness) had higher morning cortisol, detected by one morning cortisol sample, on the fifth day of school relative to the weekend, whereas no relationship was found with high levels of shyness and morning cortisol. Bruce et al. (2002) propose that elevated morning cortisol secretion in non-shy children may assist in the adaptation to a new school year. This lends support to the current study's findings and suggests that for participants with low levels of inhibition, the association between life events and morning cortisol output represents an adaptive response. However, based on the inconsistent findings in the literature, further research is needed to explore behavioural inhibition as a moderator of the relationship between life events and total morning cortisol secretion.

The present study also found that low and high levels of behavioural inhibition significantly moderated the relationship between HSE and CAR AUC_G. However, findings were inconsistent and dependent on when the HSE occurred. One plausible explanation for a stronger association between high exposure to life events and CAR in participants with low levels of inhibition, is that the body secretes cortisol to cope with stressors and a greater CAR represents a normal stress response to increased exposure to life events. As stated previously, the CAR AUC_G has been shown to be related to life stress, with elevated CAR hypothesized to assist in preparing for daily challenges (Chida & Steptoe, 2009). In regard to high levels of behavioural inhibition, Schmidt et al. (1997) observed elevated morning cortisol levels in behaviourally inhibited children and proposed that heightened cortisol in inhibited children may contribute to a predisposition to expect fear. Thus, it could be suggested that behaviourally inhibited children experience heightened fear in response to increased life events, which subsequently enhances the CAR to assist the body in coping. However, this fails to clarify why the relationship between high exposure to life events at 0 – 12 months and the CAR AUC_G was stronger for participants with low levels of behavioural inhibition and insignificant for those with high levels of inhibition. Further research is warranted to confirm the results of the current study.

In addition to the CAR, behavioural inhibition also moderated the relationship between life events and diurnal cortisol secretion. Specifically, both low and high levels of behavioural inhibition moderated the association between life events and diurnal AUC_G. However, these findings appeared to vary based on the different life event time points. To my knowledge, there are no published studies on the moderating role of behavioural inhibition on the relationship between life events and diurnal cortisol response in healthy children and adolescents. However,

research on daycare as a challenging early experience, has found that social fear in children predicted changes in cortisol throughout the day (Watamura et al., 2013), suggesting partial support for high levels of behavioural inhibition. Literature on shyness and diurnal cortisol has found that shy adults exhibited lower daily cortisol than non-shy adults while a greater change in cortisol from morning to afternoon was observed in non-shy adults in comparison to their shy counterparts (Beaton et al., 2013). This lends support to the current findings, in that both high and low levels of inhibition appear to influence the diurnal cortisol response. As discussed earlier, it has been hypothesized the behaviourally inhibited children have a lower stress response threshold than non-inhibited children (Kagan et al., 1987). Indeed, in the current sample, the relationship between life events and diurnal cortisol output was strongest for participants with high levels of behavioural inhibition than those with moderate or low levels of inhibition. It is plausible that participants with low levels of behavioural inhibition exhibited increased cortisol secretion in response to cumulative exposure to life events. This may explain why high but not low levels of behavioural inhibition strongly moderated the relationship between recent life events (0 – 3 months) and diurnal AUC_G, and why the association between life events within the past year and diurnal cortisol output was significantly stronger for participants with both high and low levels of inhibition. The current study demonstrates the complexity of timing and cumulative exposure of life events on daily cortisol output based on the influence of behavioural inhibition. Thus, while the relationship between life events and the diurnal cortisol response appears to be moderated by levels of behavioural inhibition, additional research is needed to confirm these findings.

Trait Anxiety

The present study found that the association between the high stress exposed group at 0 – 3 months and total CAR output was stronger for participants with high levels of trait anxiety than those with moderate or low levels of trait anxiety. While no studies have specifically examined trait anxiety as a moderator of the relationship between life events over the past year and CAR in healthy children and adolescents, previous research on female college students found no relationship between high perceived stress in the past two weeks and high trait anxiety on the CAR AUC_G (Suh, 2018). Differences in sample characteristics and time frame of stressor assessment likely account for discrepant findings. Research on childhood maltreatment has observed a relationship between trait anxiety, childhood maltreatment, and CAR AUC_G,

however, this varied based on serotonin transporter polymorphism (5-HTTLPR) (Ergin et al., 2016). Contrary to the present study's findings, literature on anxiety and life events on the stress response has found no impact of life stress over the past year on the relationship between cortisol and anxiety disorders (Doane et al., 2013). Research has found a relationship between anxious arousal in youth (Doane et al., 2013), trait anxiety in young adults (Walker et al., 2011), and trait anxiety in adult women (Therrien et al., 2008) on the CAR. While this lends some support to the current findings of a stronger relationship between high exposure to recent life events and total morning cortisol output in participants with high levels of trait anxiety, other studies have observed no association between trait anxiety and CAR (Melia et al., 2019; Oskis et al., 2009), and morning cortisol levels (Van den Bergh et al., 2008).

As only the association between high exposure to recent life events (0 – 3 months) and CAR was moderated by trait anxiety, it is plausible that participants with high levels of trait anxiety were still actively coping with these stressors at the time of cortisol sampling in comparison to participants with moderate or low levels of trait anxiety. These findings parallel those that have shown that when the stressor is still present at the time of sampling, morning cortisol levels are significantly higher (see meta-analysis conducted by Miller et al., 2007). Further, anxious individuals have been shown to be sensitive to potential threats, with unclear events interpreted as threating (Mathews, 1990). Fear and anxiety have also been associated with the anticipation of stressful events, which has been hypothesized to result in allostatic load (Schulkin et al., 1994). Interestingly, research on trait anxiety and life events has shown a significant positive correlation between negative life events in the past year and trait anxiety (Pluess et al., 2010). It could be suggested that high trait anxiety influences how the stress response system interprets and copes with stressors, with increased exposure to stressors associated with greater morning cortisol as a way to protect the body from threats. Indeed, an increase in the CAR has been shown to provide an individual with resources to cope with demanding events they are anticipating (Clow et al., 2010; Wetherall et al., 2015).

The present study found that trait anxiety moderated the relationship between life events and diurnal cortisol output. However, findings were inconsistent and dependent upon the time points of the life event scale. This suggests that timing and cumulative exposure of life events have a complex association with trait anxiety. Indeed, the current study found that high levels of trait anxiety moderated the relationship between recent life events and diurnal cortisol AUC_G,

whereas medium levels of trait anxiety moderated the association between exposure to life events within the past six months and diurnal cortisol AUC_G. These findings concur with those of Suh (2018), who found that high levels of trait anxiety and high perceived stress from life events over the past two weeks was associated with a greater total diurnal cortisol secretion in adults (Suh, 2018). In contrast, a relationship between blunted afternoon cortisol levels and elevated negative life events during high school has been observed in anxious adolescents (Ruttle et al., 2014). Novel life events have been associated with a greater cortisol response, whereas recurrent life events have been shown to have a reduced impact on the stress response, referred to as a process of habituation (van Eck et al., 1996). However, van Eck et al. (1996) found that individuals with high trait anxiety were less likely to demonstrate habituation as evidenced by their diurnal cortisol response. In line with this research, it could be suggested that when participants with high trait anxiety are exposed to recurrent life events, they continue to respond as if the event was novel, as revealed by a strong relationship between life events and diurnal cortisol secretion. As stated previously, it is also possible that participants with high levels of trait anxiety were still coping with life events at the time of cortisol sampling in comparison to those with medium or low levels of trait anxiety. However, based on the literature, it would be expected that high trait anxiety would moderate the relationship between diurnal cortisol and all the life event time points. Further, while the influence of high levels of trait anxiety has emerged within the literature, an explanation for the current study's findings on medium levels of trait anxiety being a significant moderator of the relationship between life events at 0 – 6 months and diurnal cortisol AUC_G remains unclear.

The study also found that the association between the high stress exposed group and total diurnal cortisol output was moderated by high and medium levels of trait anxiety. However, findings were inconsistent and varied based on when the HSE occurred. As stated previously, heightened levels of recent stress and trait anxiety have been associated with a greater diurnal cortisol secretion in adults (Suh, 2018). Further, it has been suggested that habituation to recurrent stressors is less likely to occur in individuals with high levels of trait anxiety (van Eck et al., 1996). Taken together, this lends support to this study's findings that the relationship between high exposure to recent life events and diurnal cortisol AUC_G was strongest in participants with high levels of trait anxiety relative to those with moderate and low levels of trait anxiety. Nevertheless, this explanation does not account for the finding that medium levels

of trait anxiety was a strong predictor of the relationship between high exposure to cumulative life events and the diurnal cortisol response. Based on these inconsistent findings, further research is needed to better understand the moderating role of trait anxiety on the association between high stress exposure and diurnal cortisol AUC_G in healthy children and adolescents.

Parental Anxiety

Parental anxiety was found to be a moderator of the relationship between life events and the diurnal cortisol response. Specifically, the association between life events and total diurnal cortisol output was significant in participants with no parental history of anxiety, whereas no association was found for participants with a parental history of anxiety. The literature on parental anxiety and the diurnal cortisol response is mixed, with an increased diurnal AUC_G observed in female offspring with maternal social phobia (Goldstein et al., 2017) and no relationship observed between lifetime or current maternal anxiety and diurnal cortisol in children (Smith, 2016). While these studies do not account for the relationship between life events and daily cortisol, it suggests that the influence of having a parent with anxiety on the stress response system is complex. Indeed, in a study of children beginning school, maternal social anxiety was associated with increased evening cortisol levels (Russ et al., 2012). Differences in the stress response was also detected among children with maternal generalized anxiety disorder versus those with maternal social anxiety, suggesting that specific parental anxiety disorders impact offspring stress response (Russ et al., 2012). In the present study parental anxiety included mothers and fathers with panic disorder, generalized anxiety disorder, and social anxiety disorder. The anxiety disorder diagnoses were not differentiated from one another in the analysis and thus may have confounded results. Nevertheless, based on the previous findings, it would be expected that having a parent with anxiety would have a greater impact on the association between life events and diurnal cortisol than not having a family history of anxiety.

The current study also found that the relationship between the high stress exposed group at 0 - 3 months and diurnal AUC_G was stronger for participants who did not have a parental history of anxiety. It is interesting to note that the association between HSE based on the other life event time points and diurnal AUC_G was not moderated by parental anxiety. This suggests that only high exposure to recent life events and diurnal cortisol output was strongly associated in participants with no parental history of anxiety. As suggested previously, participants without

a parental history of anxiety and who were exposed to increased recent stressors may have still been actively coping with these events at the time of cortisol sampling. However, further research is needed before conclusions can be drawn.

Parental Bonding

The current study found that the association between life events at 0 – 3 months and CAR AUC_G was significantly stronger for participants who reported high levels of paternal care, followed by low levels of care, with moderate levels of care being insignificant. In contrast to these findings, a study on stressors in children and adolescents found that parental warmth did not moderate the relationship between daily stressors and the CAR (Lippold et al., 2016). However, unlike the current study, Lippold et al. (2016) did not differentiate between maternal and paternal warmth, which may explain discrepant findings. While the literature on parental bonding as a moderator of the association between life events and the CAR is limited, research on parenting styles has found a relationship between high perceived emotional warmth and the CAR in adolescents (Marsman et al., 2012), as well as low levels of parental care related to a higher CAR in a sample of adults (Engert et al., 2010). Further, Kawai et al. (2017) observed that among young adults, high levels of parental care and low overprotection, as well as low care and high overprotection, impacted the CAR. While these studies support a moderating role of parental bonding on the relationship between life events and the stress response, they did not differentiate between maternal and paternal care. In a study on father and daughter relationships, no association between a warm relationship and morning cortisol was observed, however negative aspects of the relationship were related to morning cortisol (Byrd-Craven et al., 2012). While the study by Byrd-Craven et al. (2012) only included females and used one morning cortisol sample, their findings suggest that low levels of paternal care rather than high levels of care is a potential moderator of the relationship between life events and the CAR. It should be noted that none of the other associations between the life event time points and the CAR was moderated by paternal care. This suggests that only recent life events (0 – 3 months) and total morning cortisol output are influenced by high and low levels of paternal caring. Based on the limited literature, further research is needed to confirm paternal caring as a moderator of life events and CAR AUC_G.

The present study was unable to replicate previous findings of a moderating role of parental bonding on the relationship between life events and diurnal cortisol profiles (Hanson &

Chen, 2010; Pendry & Adams, 2007). Similarly, although parenting style has been found to predict cortisol reactivity to a laboratory stressor in youth (Vergara-Lopez et al., 2016), this study failed to support a moderating role of parental bonding on the relationship between life events and cortisol reactivity to a laboratory stressor. Conversely, other investigators have detected an association between cortisol reactivity AUC_G and negative life events over the past year in adolescents and young adults with low levels of positive parenting (Hagan et al., 2010). Jaffee et al. (2014) similarly observed that the relationship between recent trauma exposure and cortisol reactivity to a laboratory task in children varied based on levels of non-responsive parenting. As the current study relied on child self-report of parenting, it is possible that different results would have emerged if detailed observations of parent-child interactions were used to assess parenting style. Additional research using different measures of parent-child bonds would help clarify if parenting is an important moderator of the association between life events and HPA axis functioning in children and adolescents.

Study Strengths and Limitations

A strength of the present study was that the sample consisted of healthy children and adolescents with no history of psychopathology, which improves generalizability to child populations and limits confounding influences of psychological disorders on the HPA axis. Additionally, this study focused on a range of life events within the last year, which extends the research beyond abuse and maltreatment and lends support to the potential influence of common life events on the stress response system in children and adolescents. Further, this study measured the stress response using AUC_G and AUC_I, accounting for both total cortisol output and sensitivity of the stress response system (Fekedulegn et al., 2007).

Despite the numerous strengths of the study and statistically significant findings, limitations should be considered. First, research has shown a relationship between early life stress and current stress on the diurnal cortisol response (Young et al., 2019), as well as an interaction between childhood adversity and life events within the past year predicting the CAR AUC_I (Starr et al., 2017). Although the focus of the present study was on the impact of recent life events on the HPA axis, life events occurring before the previous year were not accounted and may have impacted the results of the study. Second, perceived stress has been shown to be more closely associated with cortisol secretion than life events over the past year (Allwood et al., 2017), with current feelings of stress found to significantly predict the CAR (Pruessner,

Hellhammer, et al., 2003). The current study did not measure participants' perception of stress in relation to the life events they experienced, thus future research would benefit from controlling for this variable. Third, this study is limited by the cortisol sampling procedure and compliance. Salivary cortisol was collected on two consecutive days at home for the determination of the CAR and diurnal cortisol response. Hellhammer et al. (2006) have suggested that six sampling days are necessary to reliably calculate CAR AUC_I, as situational factors may bias results. However, increasing the number of sampling days may be unfeasible within a child population. Compliance with cortisol sampling has also been shown to impact findings, specifically with non-compliance for the CAR (Kudielka et al., 2003). Although participants in the current study were provided with specific instructions for collecting the saliva samples and were asked to record the time of sampling on a log, it was not feasible to verify the accuracy of sample collection times. To account for this limitation, use of electronic monitoring devices is suggested for future research.

Implications and Future Directions

The present study builds on the literature examining the association between life events and the CAR, diurnal cortisol response, and cortisol reactivity to a laboratory stressor in healthy children and adolescents. Specifically, the study demonstrated that life events within the last year influences HPA axis function biomarkers within a healthy sample of children and adolescents. Further, the study provided evidence of a distinction between cortisol profiles (CAR, diurnal, stress reactivity) and highlights the significance of researching these different cortisol responses. While increased cortisol secretion was observed in response to greater life events, it remains unclear whether this relationship represents a healthy and adaptive response to stress or a dysregulated stress response. As previous research has found an association between heighted cortisol and negative physiological and psychological effects (e.g., Kaufman et al., 1997; Lupien et al., 1998), the finding of greater cortisol secretion in relation to increased life events is significant. A future direction for research could involve a longitudinal study to assess how life events influences the HPA axis over the period of development and possible physiological and psychological consequences. As a complex relationship between the life event time points and the HPA axis was observed, a longitudinal study could also help elucidate the impact of distal and recent life events on different cortisol profiles and the influence of potential moderators identified in the study.

Considering that exposure to life events is normative in youth, support for preventative interventions aimed at regulating the HPA axis in those with high exposure to life events is warranted. Psychosocial interventions have been shown to improve healthy cortisol regulation in children (see systematic review by Slopen et al., 2014) and evaluating the effects of these interventions on HPA axis regulation in children and adolescents is an important research endeavor. In addition, while some significant moderators in the present study are not modifiable (e.g., parental history of anxiety) or inappropriate to modify (e.g., high levels of paternal caring and low levels of behavioural inhibition), trait anxiety has the possibility to be altered. For example, mindfulness-based cognitive therapy has been associated with reductions in anxiety in children who scored high on the STAIC (Semple et al., 2009). However, given the conflicting findings with the moderator variables (e.g., high and medium levels of trait anxiety and low and high levels of behavioural inhibition) further research is needed to clarify the significance of the moderator variables and whether diminishing the strength of the relationship between life events and cortisol profiles improves regulation of the stress response system. In conclusion, this study provides insights for health professionals on the impact of life events and moderator variables on HPA axis function and suggests a need for implementing interventions to assist in healthy development of the stress response in children and adolescents.

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Appendix A

Pruessner et al. (2003) formula used to calculate AUC_G and AUC_I.

Cortisol awakening response

$$AUC_G = ((m_2 + m_1)/2) * 30) + ((m_3 + m_2) / 2) * 30).$$

$$AUCI = (AUC_G) – (m_1 * 60)$$

Diurnal Cortisol Response

$$AUC_G = (m_2 + m_1) / 2) * (8) + ((m_3 + m_2) / 2) * (4).$$

$$AUC_I = (AUC_G) - (m_1 * 8 + 4).$$

Cortisol Reactivity

$$AUC_G = (((m_2 + m_1) / 2) * (15)) + (((m_3 + m_2) / 2) * (15)) + (((m_4 + m_3) / 2 * (15)) + (((m_5 + m_4) / 2 * (15))$$

$$AUC_I = (AUC_G) - (m_1 * 60).$$

Appendix B

Table B1

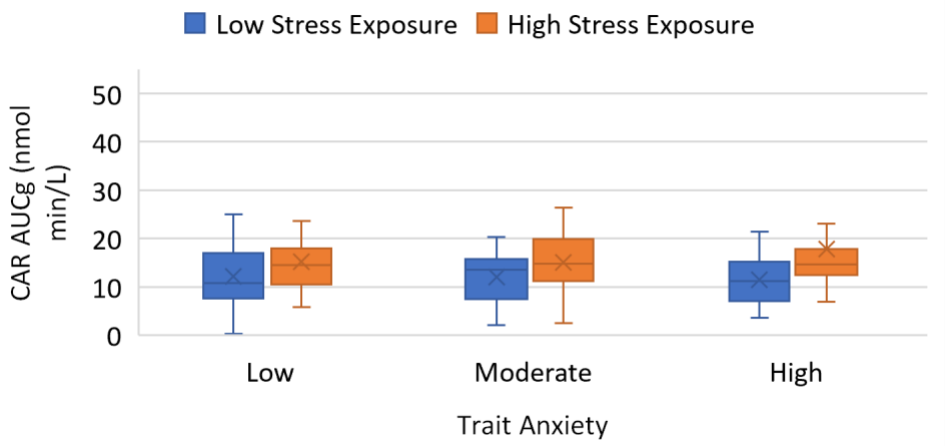
Multiple Linear Regression for the High Stress Exposed Group Predicting AUC_G for Cortisol Awakening Response

		Model 1				Model 2			
Time Point	Predictor	*B*	*β*	*t*	*p*	*B*	*β*	*t*	*p*
0 – 3 Months	HSE A	3.73	.25	3.04	.00	2.87	.19	1.44	.15
	HSE A x STAIC					.58	.43	2.29	.02
	HSE A x CSRI					-7.51	-.30	-1.79	.08
	HSE A x CASI					-.26	-.16	-.83	.41
	HSE A x PA					-4.26	-.23	-1.63	.11
	HSE A x Age					.05	.02	.11	.91
	HSE A x Sex					2.38	.14	1.10	.31
0 – 9 Months	HSE C	3.83	.26	3.12	.002	2.01	.13	.98	.33
	HSE C x STAIC					.37	.20	1.43	.16
	HSE C x CSRI					-9.60	-.30	-2.23	.03
	HSE C x CASI					.13	.05	.40	.69
	HSE C x PA					-1.28	-.06	-.48	.64
	HSE C x Age					-.27	-.06	-.59	.56
	HSE C x Sex					1.81	.10	.74	.46
0 – 12 Months	HSE D	3.78	.25	3.05	.00	1.76	.12	.88	.38
	HSE D x STAIC					.46	.27	1.82	.07
	HSE D x CSRI					-10.75	-.37	-2.51	.01
	HSE D x CASI					.01	.01	.04	.97
	HSE D x PA					-1.14	-.05	-.43	.67
	HSE D x Age					-.40	.43	-.10	-.92
	HSE D x Sex					1.66	.09	.69	.49

Note. N = 141. Life events at 0 – 6 months were excluded as no significant interactions were found. HSE = High stress exposure; STAIC = State-Trait Anxiety Inventory for Children Trait Form; CSRI = Childhood Self-Report of Inhibition; CASI = Childhood Anxiety Sensitivity Index; PA = Parental history of anxiety.

Figure B1

Interaction Between Stress Exposure at 0 – 3 Months and Levels of Trait Anxiety Predicting AUC_G for Cortisol Awakening Response

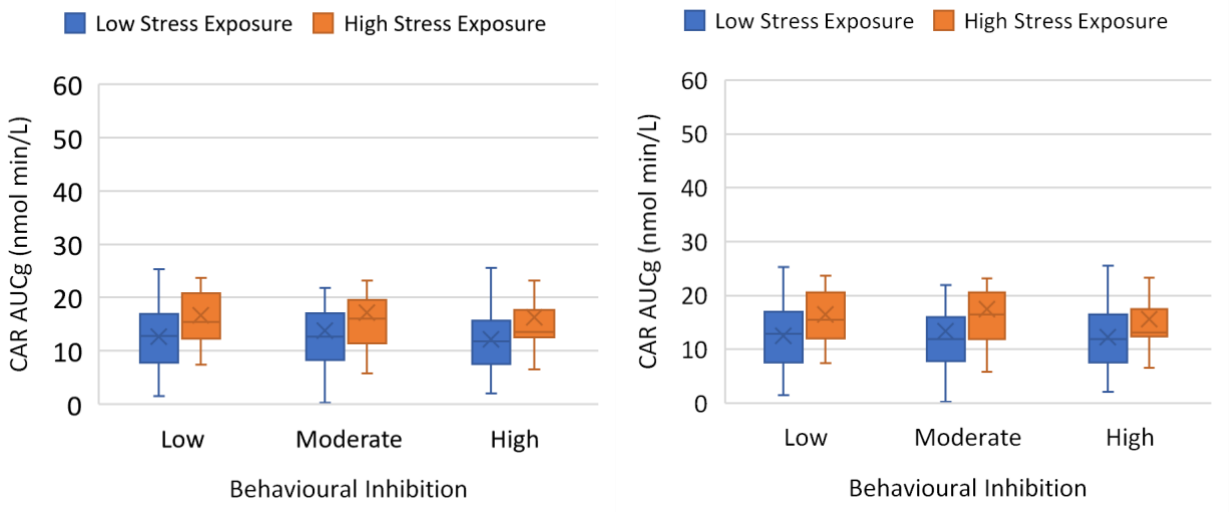


Note. N = 141.

Figure B2

Interaction Between Stress Exposure and Behavioural Inhibition Predicting AUC_G for Cortisol Awakening Response

(a) *(b)*



Note. N = 141. (a) Stress exposure 0 – 9 months; (b) Stress exposure 0 – 12 months.

Table B2

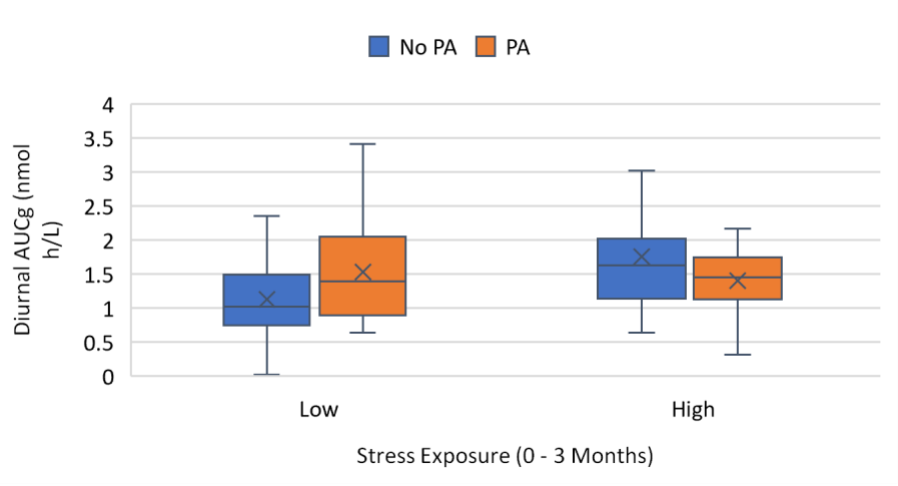
Multiple Linear Regression for the High Stress Exposed Group Predicting AUC_G for Diurnal Cortisol Response

		Model 1				Model 2			
Time Point	Predictor	*B*	*β*	*t*	*p*	*B*	*β*	*t*	*p*
0 – 3 Months	HSE A	.38	.26	3.20	.00	.35	.25	1.92	.06
	HSE A x STAIC					.05	.38	2.09	.04
	HSE A x CSRI					-.60	-.24	-1.54	.13
	HSE A x CASI					-.02	-.13	-.70	.48
	HSE A x PA					-.52	-.30	-2.17	.03
	HSE A x Age					-.01	-.03	-.20	.84
	HSE A x Sex					.16	.10	.73	.47
0 – 6 Months	HSE B	.38	.27	3.24	.00	.27	.19	1.45	.15
	HSE B x STAIC					.05	.32	1.98	.05
	HSE B x CSRI					-.46	-.16	-1.18	.24
	HSE B x CASI					-.02	-.12	-.77	.45
	HSE B x PA					-.38	-.19	-1.56	.12
	HSE B x Age					-.02	-.06	-.52	.61
	HSE B x Sex					.14	.08	.63	.53
0 – 12 Months	HSE D	.38	.26	3.21	.00	.29	.20	1.55	.13
	HSE D x STAIC					.05	.31	2.17	.03
	HSE D x CSRI					-.72	-.26	-1.82	.07
	HSE D x CASI					-.01	-.04	-.29	.78
	HSE D x PA					-.31	-.15	-1.29	.20
	HSE D x Age					-.03	-.08	-.77	.44
	HSE D x Sex					.01	.01	.06	.95

Note. N = 141. Life events at 0 – 9 months was excluded as no significant interactions were found. HSE = high stress exposure; STAIC = State-Trait Anxiety Inventory for Children Trait Form; CSRI = Childhood Self-Report of Inhibition; CASI = Childhood Anxiety Sensitivity Index; PA = Parental history of anxiety.

Figure B3

Interaction Between Stress Exposure and Parental Anxiety Predicting AUC_G for Diurnal Cortisol Response

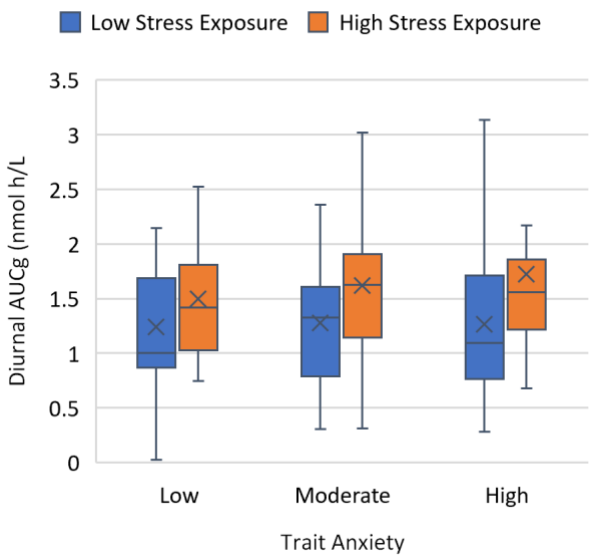


Note. N = 141.

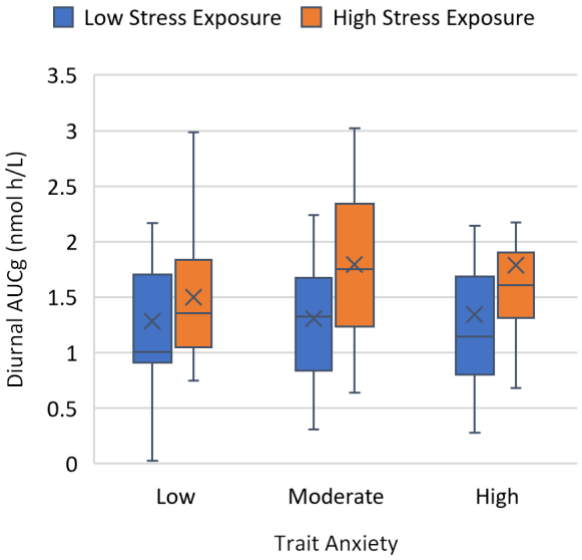
Figure B4

Interaction Between Stress Exposure and Trait Anxiety Predicting AUC_G for Diurnal Cortisol Response

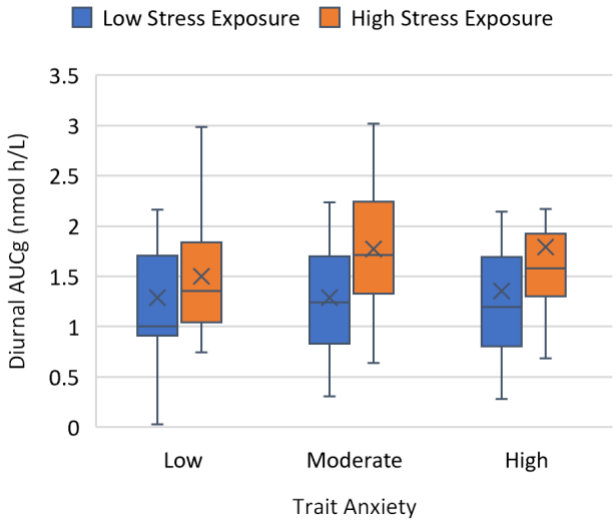
(a)



(b)



(c)



Note. N = 141. (a) Stress exposure 0 – 3 months; (b) Stress exposure 0 – 6 months; (c) Stress exposure 0 – 12 months.