The Social, the Socio, and the HPA: How peer rejection, peer acceptance, and socioeconomic status relate to children's HPA-axis activity

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The Social, the Socio, and the HPA: How peer rejection, peer acceptance, and socioeconomic status relate to children's HPA-axis activity

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Abstract

Understanding how socioeconomic status (SES) "gets under the skin" to impact health has puzzled researchers for decades. SES is a complex, multifaceted construct that can be examined using a variety of methodologies. Additionally, SES can impact diverse and interrelated spheres of development. This dissertation attempts to parse how SES and the peer group individually and additively impact children's cortisol levels. **Study 1** examines children's cortisol at the beginning and end of their first year of kindergarten and finds that rejected children have flatter morning cortisol slopes, while lower SES children have higher cortisol at the end of the school year compared to their higher SES peers. **Study 2** examines children's diurnal cortisol and cortisol response to positive and negative peer experiences and finds that girls from lower income families have a flatter diurnal cortisol slope. It also found that children not accepted by their peers had little cortisol response to positive or negative peer experiences, while accepted children had elevated cortisol following positive peer experiences. **Study 3** examines these same variables in relation to hair cortisol and found that children from a lower income background have higher hair cortisol, while low levels of parental education moderated the association between rejection and acceptance such that children from families with lower levels of parental education had low cortisol when they were rejected or not accepted and high cortisol when they were accepted or not rejected. Together, these sets of results highlight the importance of using multiple measures of SES to better understand its association with peer difficulties and cortisol. Understanding how social factors and SES impact the HPA-axis could inform intervention programs designed to help disadvantaged children.

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Contribution of Authors

Psychophysiological adjustment to formal education varies as a function of peer status and socioeconomic status in children beginning kindergarten

Leah Wright	Study conception, design, data organization, planned and executed analyses, writing, editing, proofing.
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Luz Stella Lopez	Facilitated Barranquilla data collection process
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Gender is Key: Girls' and Boys' Cortisol Differs as a Factor of Socioeconomic Status and Social Experiences During Early Adolescence

Leah Wright	Study conception, design, data organization, planned and executed analyses, writing, editing, proofing.
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Socioeconomic status, peer acceptance, and peer rejection predict hair cortisol concentrations in early adolescents in Colombia

Leah Wright	Study conception, design, data collection, data organization, planned and executed analyses, writing, editing, proofing.
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Luz Stella Lopez	Facilitated Barranquilla data collection process

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General Introduction

Social inequality and inequity are known to affect children from all cultures (Watkins, 2016). Disease morbidity and mortality, as well as the prevalence of anxiety, depression, externalizing behaviours, impaired cognitive performance, and decreased academic achievement occur at higher rates in children from lower socioeconomic status (SES) backgrounds (Bukowski et al., 2020). With the complexity of these multifaceted constructs, disentangling the mechanisms that underlie these associations is challenging. Researchers have hypothesized multiple causal factors to explain how SES impacts health: prenatal influences, parental care, cognitive stimulation, and critically, stress exposure (Evans, 2004; Hackman et al., 2012; Shonkoff et al., 2012). Children who grow up in low SES environments experience more stress (Evans, 2004; Goodman, McEwen, Dolan, et al., 2005). High stress levels alter the activation of the hypothalamic pituitary adrenal axis (HPA-axis) and the level of stress hormones to which the child is exposed (Blair et al., 2011; Lupien et al., 2001). Increased exposure to stress hormones in early life can cause later dysregulation of the HPA-axis (Gunnar & Quevedo, 2007). Interestingly, social factors also influence stress hormone levels (Sapolsky, 2005). While the peer system has been proposed to be an ideal context to explore the negative effects of low SES on children (Bukowski et al., 2020), the peer context has rarely been studied in tandem with stress hormone exposure, low SES backgrounds and rarely outside of a Western industrialized context.

In this dissertation three studies are used to examine the interplay between SES, peer relationships, and the stress hormone cortisol in children in Montreal, Quebec, and Baranquilla, Colombia. These studies examine how different types of environmental influences impact physiological responses by studying the predictive capacity of SES and social factors on the HPA-axis.

Socioeconomic Status

Aspects of the social environment affect development across the life span (Repetti et al., 2002). SES is a broad and complex construct that can be conceptualized as the extent to which individuals, families or groups have access to different types of capital: social (e.g., status), material (e.g., wealth), or human (e.g., education; Oakes & Rossi, 2004). SES has repeatedly been connected to health (Repetti et al., 2002), such that individuals from lower SES backgrounds are at greater risk of a number of negative health outcomes (Chen & Miller, 2013; Fitzsimons et al., 2017). One theory explaining why SES negatively impacts health is that low SES during childhood is a chronic stressor (Baum et al., 1999) and chronic stressors can “get under the skin” to impact health outcomes (Steptoe & Marmot, 2002). The consequences of stress exposure vary as a function of developmental timing (Lupien et al., 2009) Stress in childhood is likely to have stronger effects than similar experiences in adulthood (Luo & Waite, 2005). By examining the physiological underpinnings of the body’s response to stress, researchers can better understand the process by which SES impacts lifespan development.

Cortisol

Cortisol is the end product of the HPA-axis, which contributes to the maintenance of homeostasis. The HPA-axis is stimulated by exposure to stress, which initiates a sequential process that begins with the release of corticotrophin-releasing-hormone (CRH) from the paraventricular nucleus of the hypothalamus. CRH stimulates the pituitary to secrete adrenocorticotropic releasing hormone which, in turn, stimulates the adrenal glands to release the steroid hormone cortisol (Tsigos & Chrousos, 2002). Cortisol activates energy stores both in anticipation of the stressor or in response to the stressor (Herman et al., 2016). Cortisol takes almost an hour to effect neural functioning and these effects can last just a few hours or as long as days (Joels & Baram, 2009). Cortisol can be measured via saliva, hair, blood or urine (Stalder et al., 2017). Overexposure to cortisol during childhood may lead to alterations in downstream physiological activity, holistic health, and well-being (Herbert et al., 2006; Joels & Baram, 2009; McEwen & Gianaros, 2011).

Diurnal Cortisol

Cortisol is secreted diurnally in a circadian rhythm. It slowly increases during the night, peaks shortly after awakening, and decreases throughout the day, reaching nadir at bedtime (Steptoe, 2007). Diurnal cortisol levels are fairly stable across early childhood (Laurent et al., 2014; Zalewski et al., 2016). Diurnal cortisol secretion is typically examined in several ways, such as examining the total amount of cortisol secreted, diminishing slope of cortisol throughout the day, area-under-the-curve (AUC) of the diurnal slope, and single samples of cortisol. The diurnal cortisol profile is used to make inferences about the functioning of the HPA-axis and the body's long-term response to stress, and thus can be used to examine the impact of SES on the HPA-axis.

SES has been associated with altered diurnal cortisol profiles in children, both cross-sectionally and longitudinally. In children aged 6-10, but not aged 11-16, lower parent income was associated with higher morning cortisol (Lupien et al., 2001). Evans (2003) investigated the physiological accumulation of chronic and acute stress, or allostatic load, of 9-11 year-old children and found that increased allostatic load was positively associated with cumulative risk (which included factors such as poverty, single parenthood, maternal high school dropout status). SES has also been inversely associated with cortisol increases over a two-year period in adolescents aged 13-18, in that lower SES (as assessed by examining household chaos) predicted increases in AUC cortisol (Chen et al., 2010b). Miller and colleagues (2009) used genome-wide transcriptional profiling on adults and found that SES during childhood was associated with the downregulation of genes associated with glucocorticoid receptor function and the regulation of cortisol. These findings were independent of current SES and are consistent with a phenotype where glucocorticoid receptors are desensitized to cortisol expression, leading to increased cortisol responses and less effective regulation of physiological reactivity during adulthood. Income and cumulative risk have been found to impact the development of diurnal cortisol trajectories in children as young as 36 months old (Zalewski et al., 2016). To summarize, it appears that SES is associated with the secretion of diurnal cortisol, such that lower SES children have higher salivary cortisol levels.

Cortisol Reactivity

Cortisol is also secreted in response to real or perceived threats to an individual's well-being. By examining cortisol reactivity important information regarding the bodies response to acute stress can be gathered. Studies of cortisol reactivity are conducted using laboratory tasks such as the Trier Social Stress Task where an individual is exposed to various social stressors, such as completing a public speaking task or a mental arithmetic task in front of impassive judges, and cortisol is then assessed at multiple points before, during, and after the stress exposure (e.g., Hostinar et al., 2015). Few studies have examined the association between stress reactivity and socioeconomic status. In adults, some studies have found lower SES to be associated with increased cortisol reactivity in the face of laboratory stressors (e.g., Sripada et al., 2014), while other studies have found negative associations (e.g., Kraft & Luecken, 2009). In 5 and 9-year old's, lower SES, as measured by income-to-needs ratios, were associated with increased cortisol reactivity (Blair et al., 2005; Gump et al., 2009). Neighbourhood disadvantage is predictive of an increased cortisol response and steeper recovery in 13 to 18-year-old boys but not girls (Hackman et al., 2012). The extant literature examining the impact of SES on cortisol reactivity appears to indicate that children from lower SES backgrounds may have an increased cortisol response to stress. However, the ecological validity of laboratory studies is limited; thus, examining children's cortisol in relation to their moment-by-moment stressful experiences in their everyday life would benefit the literature.

Hair Cortisol

Studying the stress response system by examining cortisol content in hair offers yet another perspective on the secretion of cortisol. Proponents of hair cortisol argue that it is less vulnerable to intra and inter-individual differences and time-varying confounders than salivary cortisol (Staufenbiel et al., 2013), and that it is a plausible retrospective biomarker of cortisol exposure, and therefore stress exposure (Staufenbiel et al., 2013). Hair cortisol is arguably able to assess cortisol exposure over months at a time, although the length of time is still being debated (Wosu et al., 2013). Some studies have found that more distal hair segments (i.e. the older the hair) have lower cortisol levels regardless of stress exposure (Hamel et al., 2011; Steudte et al., 2011), while others found no difference (Manenschijn et al., 2011).

Hair grows at a rate of approximately 1cm per month (Loussouarn et al., 2005). Generally, a segment is collected as close to the scalp as possible, and is approximately three centimetres in length (Meyer et al., 2014), with each centimetre of hair collected representing one month of cortisol exposure. It is possible to compare cortisol in hair segments before and after a stressful event, therefore enabling researchers to consider cortisol reactions over months without a longitudinal study design. Hair cortisol as a marker of stress has been validated in primates by confirming that a prolonged stressful event, e.g., relocation, led to an increase in cortisol levels (Davenport et al., 2006). Thus, hair cortisol is a validated measure of cumulative stress exposure.

Hair cortisol may also be influenced by factors other than stress, although there is much debate in the literature. Some studies have found that the frequency of hair washing may decrease hair cortisol in distal hair segments (Dettenborn et al., 2012); and some, but not all, have found that the use of chemical hair treatments have been associated with decreased hair cortisol (Manenschijn et al., 2011; Sauvé et al., 2007). One study found that children have higher hair cortisol than adults and males have higher hair cortisol than females (Dettenborn et al., 2012), although this sex effect was not found elsewhere (Gow et al., 2010; Raul et al., 2004; Xie et al., 2013). BMI has also been found to have a positive association with hair cortisol (Manenschijn et al., 2011). The extant literature has typically found that lower SES has been associated with higher hair cortisol levels (Hollenbach et al., 2019; Ursache et al., 2017; Vaghri et al., 2013).

Peer Relationships

As children grow up, the social sphere steadily gains importance in its relation to their healthy development and well-being (Bukowski et al., 2015; Rubin et al., 2015). While some interactions with peers can be negative — e.g., bullying and victimization — positive interactions can be powerful contributors to children's well-being (Laursen et al., 2007). Having friends reduces the negative impact of parental conflict (Wasserstein & La Greca, 1996), protects against mental health problems (Laursen et al., 2007), bullying (Bukowski et al., 2015), and critically, diminishes the impact of stressors such as negative life events (Sandler et al., 1989). One's social status within the classroom is also a powerful predictor of well-being during childhood—being rejected (disliked by the peer group) or accepted (liked by the peer group) — can impact a child's well-being (Rubin et al., 2015). Socioeconomic status can also impact a child's position within the social group. Individuals in tenuous financial situations may be more likely to change schools (Alexander et al., 1996), which makes the development of a strong, stable and supportive social context more challenging (Dodge et al., 1994). Children from lower SES backgrounds have less stable peer relationships from kindergarten until grade 3 (Dodge et al., 1994). They are also significantly more likely to interact with aggressive peers (Sinclair et al., 1994). While the risks associated with peers and socioeconomic status have been studied (e.g., deviant peer influences mediate the association between poverty and anti-social behaviour; Eamon, 2002), the effects of social status, i.e., peer acceptance and rejection, in the context of SES have not been examined extensively. The literature on cortisol and the peer context will be discussed below.

Peer relationships and cortisol. In adults, the association between cortisol and peer rejection appears to indicate that peer rejection is associated with elevated cortisol levels (Blackhart et al., 2007; Ford & Collins, 2010; Stroud et al., 2002). These findings are fairly consistent with studies of children. Laboratory-based studies found that socially-rejected youth had elevated cortisol levels (Blackhart et al., 2007; Stroud et al., 2009). Leaving the laboratory, Gunnar and colleagues (2003), examined cortisol responses in preschoolers in the context of peer rejection. Children were assessed by their peers as being either "liked" or "disliked". Children who were disliked (rejected) had higher basal cortisol levels than their peers, indicating that children who are rejected may experience more stress. Children who have either experienced peer rejection or interpersonal problems with their peers tend to have higher cortisol levels and a flattened diurnal slope (Bai et al., 2017). Clearly, negative peer relationships impact cortisol secretion.

Few studies have examined the effects of positive peer relationships on cortisol. In laboratory studies having a friend present during a stressful task increased HPA-axis reactivity to the stressor (Doom et al., 2017). One study of exclusion found that if children were excluded by their peer group they had elevated cortisol levels at school and a flattened diurnal curve; interestingly, if children had more or better friendships, this effect was weakened (Peters et al., 2011). Adams and colleagues (2011) found that having a best friend present buffered the impact of negative social experiences on cortisol. Miller and colleagues (2016) found that the cortisol awakening response (CAR) was attenuated in response to previous stress. This attenuation was buffered when adolescents had strong attachments to their peers. Interestingly, the few studies that examined peer acceptance found that acceptance was associated with higher afternoon cortisol, indicating a flatter diurnal slope, which is an indicator of poorer health outcomes (Catherine et al., 2012; Oberle, 2018). In summary, some studies showed that positive peer relationships appear to reduce the impact of stressful experiences on the diurnal cortisol response, but not necessarily stress reactivity.

To the best of our knowledge, only one study has examined the moderating potential of SES on the association between social support and cortisol. Hooker and colleagues (2017), examined the association between subjective SES and cortisol reactivity as moderated by perceived social support in 115 adults. They found that those who reported a lower subjective SES had higher cortisol when they also had low perceived social support. Objective SES was measured via parental education and was not predictive of cortisol responses. This may indicate that subjective SES has a more powerful influence on the stress response system. Using multiple measures of objective SES may help to clarify the association between SES, peer influences, and cortisol reactivity. Thus, while social rejection appears to impact cortisol both in and outside of the laboratory, the moderating or mediating role of SES remains unclear. To the best of our knowledge, no studies of children's cortisol, SES, and peers have been conducted. Utilizing three similar but different methodologies, papers from this dissertation address this gap and expand the literature by including samples from both North and South America.

The Current Studies

Study one examines the cortisol secretion in 95 children from Barranquilla, Colombia when they begin kindergarten. Classroom based sociometric measures were collected and acceptance and rejection were assessed. Cortisol was collected twice a day for three consecutive days at the beginning and end of the school year, for a total of 12 cortisol samples per child. Acceptance and rejection were assessed throughout the school year. The change in cortisol trajectories across the school year is examined as a function of SES and peer acceptance and rejection. Longitudinally examining children's cortisol across the school year in the context of their social environments will elucidate if peer rejection, acceptance, and SES are predictive of children's cortisol across the school year.

Study two examines if SES, peer acceptance and rejection, and positive or negative peer experiences are predictive of cortisol secretion in 95 children in Montreal. Parents completed questionnaires on SES that assessed parental education, employment, and current income. Five samples of saliva were collected across four consecutive school days, for a total of 20 saliva samples per child. Additionally, daily diaries were used five times throughout the day to assess children's current mood and the quality of their peer experiences 20 minutes prior to providing a saliva sample. We examine if lower SES children have altered cortisol trajectories and if peer acceptance and rejection moderate the association between peer experiences and cortisol.

Study three returns to Colombia to collect data on SES, peer acceptance and rejection and to examine the impact of these variables on hair cortisol. Measures of objective and subjective SES were completed by parents. This study assesses if children from higher and lower SES backgrounds have different levels of cumulative stress, and if the impact of acceptance and rejection differentially predicts the cortisol of higher and lower SES children. Combined, these studies help clarify the important roles of SES, peer acceptance, and peer rejection in the functioning of the HPA-axis during childhood.

Psychophysiological adjustment to formal education varies as a function of peer status and socioeconomic status in children beginning kindergarten

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Abstract

The transition to kindergarten can be stressful as children adjust to novel separations from their caregivers and become accustomed to their peer group. A nine-month study of 96 children (Mage = 5.37 years, SD = 0.42) from Barranquilla, Colombia, assessed socioeconomic differences in hypothalamic–pituitary–adrenal (HPA) axis functioning across the kindergarten year. Children were from four different classrooms in one school. Saliva samples were collected twice a day across three consecutive days at the beginning and end of the school year. We examined whether change in HPA axis activation across the year varied as a function of a child's socioeconomic status (SES) and experience in the peer group. We found that rejected children and lower SES children had lower cortisol levels early in the morning. Rejected children had a flatter cortisol slope. Lower SES children had higher cortisol than their higher SES peers at the end of the school year and a flatter cortisol slope. Taken together, these findings suggest that diurnal cortisol in children beginning kindergarten is influenced by both peer rejection and SES.

Introduction

The transition into formal education is a significant milestone for children. It requires adaptation to a new social environment as children must learn to engage in complex social interactions, follow a structured routine, cope with prolonged separations from their caregivers, and learn in a classroom environment. As children adapt socially by beginning relationships within their new peer group, research has established that children are concurrently adapting physiologically, such that cortisol levels are different on daycare days compared to non-daycare days, e.g., a positive vs. a negative diurnal slope on daycare vs. non-daycare days, respectively (Dettling et al., 1999; Vermeer & van IJzendoorn, 2006; Watamura et al., 2003, 2009) or a flatter diurnal slope on daycare vs. non-daycare day (Ouellet-Morin et al., 2010). Changes also occur in response to beginning kindergarten, with studies finding evidence of elevated cortisol levels after beginning kindergarten (c.f., Parent et al., 2019). However, most studies examining the school transition have limited their assessments of cortisol to the beginning of the school year. Less is known about changes in cortisol throughout the first year of full-time education, and if social factors moderate the relation between cortisol and beginning school. Given that formal education is typically the first time children become fully enmeshed in the peer domain, peer factors, such as peer rejection, may also effect children's cortisol levels. Finally, given that children from lower socioeconomic status backgrounds are at higher risk of academic adversity (Janus & Duku, 2007), they may find school more stressful and thus have elevated cortisol levels when adjusting to kindergarten. This study expands the literature by examining if different types of risk (peer rejection and low socioeconomic status) moderate changes in cortisol levels throughout the first year of full-time education.

Cortisol

Beginning school for the first time is a natural stressor (Groeneveld et al., 2013; Rimm-Kaufman & Pianta, 2000; Russ et al., 2012). Therefore, physiological processes related to stress response, such as alterations in hypothalamic-pituitary-adrenal (HPA)-axis activity, should be measurable. The HPA-axis is a reactive part of the neuroendocrine system that is critical to the maintenance of stability through change, i.e., allostasis. Activation of the HPA-axis occurs when corticotrophin releasing hormone and arginine vasopressin in the paraventricular nucleus stimulate the anterior pituitary to secrete adrenocorticotrophic hormone, which then causes cortisol to be released from the adrenal glands (Herman et al., 2016; Tsigos & Chrousos, 2002). The HPA-axis is activated in response to stress and in response to circadian variations. Cortisol levels peak approximately 30 minutes after awakening and decrease during the remainder of the day reaching nadir at bedtime (Clow et al., 2004; Saxbe, 2008). Cortisol can be extracted from saliva, blood, urine, or hair (Stalder & Kirschbaum, 2012). A common means of assessing cortisol is the cortisol slope, the rate of decrease of cortisol level from morning to evening (Saxbe, 2008). Flatter cortisol slopes may indicate pathological deviations from normal circadian variation (Adam et al., 2017). Dysregulated cortisol release may be damaging to health as the continuous over-activation of these systems can decrease their capacity to respond effectively to arousal (by hyper- or hypo-secretion of cortisol; Gunnar & Quevedo, 2007). This may lead to maladaptive outcomes across the life course, such as psychopathology (Gunnar & Quevedo, 2007; Gunnar & Vazquez, 2006;

McEwen & Gianaros, 2010).

Cortisol at school entry

Multiple studies have examined children's cortisol levels as they begin a new elementary-school year (e.g., Bernard, Peloso, Laurenceau, Zhang, & Dozier, 2015; Bruce, 2002; Gutteling, de Weerth, & Buitelaar, 2005; Quas, Murowchick, Bensadoun, & Boyce, 2002) . There is evidence that at the beginning of the first year of formal education, the HPA-axis may be hyper-activated as children respond to the novelty and uncertainties of a new environment (Bernard et al., 2015; Parent et al., 2019; Russ et al., 2012). For example, hair cortisol was higher two months after beginning kindergarten when compared with hair cortisol two months before beginning kindergarten, indicating increased cortisol in response to the transition to school (Groeneveld et al., 2013). As demonstrated by meta-analyses, the association between cortisol and school entry is moderated by age: younger children have the most pronounced cortisol response at school entry (Geoffroy et al., 2006; Vermeer & van IJzendoorn, 2006). Structural equation modelling demonstrated that cortisol levels remain elevated during the first 10-weeks of school (Bernard et al., 2015), indicating that the HPA-axis may take longer than 10-weeks to adjust to the novelty of full-time schooling. A recent literature review supported the hypothesis that there is an increase in cortisol concentrations in response to transitioning to school (Parent et al., 2019). Currently, an important limitation in the literature is a lack of studies conducted to identify other factors that may moderate cortisol responses to novel environments throughout the school year.

Two longer-term longitudinal studies were located. One longitudinal study examined preschool aged children's cortisol across a two-year period (Ouellet-Morin et al., 2010). Saliva was collected in the morning and afternoon on two consecutive days when the children were age two and again at age three. They found that at age two, the cortisol of children who were in preschool had a flatter diurnal cortisol slope than their peers who stayed home. By age three, this effect had largely disappeared. Nevertheless, three-year-old children who had never before been to preschool had higher mean cortisol levels than 3-year-old children who had attended preschool at age 2. This finding suggests that transitioning into school may be a more stressful experience when it is a novel experience. Another longitudinal study examined four-year-old children's salivary cortisol before school began, during the second week of school, and six months into the school year (Turner-Cobb et al., 2008). Two samples of saliva were collected across two consecutive days at waking and in the evening when children returned home from school. While mean waking cortisol levels were not highly elevated in response to the immediate transition into school, mean waking cortisol decreased significantly as the year progressed. Temperament explained some end-of-year variability in cortisol levels, in that children with higher extraversion had higher mean levels of morning and evening cortisol across the school year. This effect was strongest in children who were both extraverted and socially isolated indicating that peer experiences effect cortisol secretion. Thus, by examining cortisol trajectories over longer periods of time, as well as the peer variables which moderate cortisol secretion, a nuanced understanding of children's hormonal response to beginning school becomes possible.

Peer Rejection

In kindergarten, children face new social challenges. They are immersed in the peer environment and engage in increasingly sophisticated interactions in an increasingly demanding environment as they develop from the late stages of toddlerhood into school-aged children. Despite the fact that from infancy, children recognize and appreciate prosocial actions (Hamlin, 2013), rejection of one's peers begins as early as preschool (Wood et al., 2002). Rejection represents how disliked one is within the peer group (Bukowski et al., 2000), and is predictive of kindergarteners' loneliness, social dissatisfaction, school adjustment, school liking, academic readiness, and classroom involvement (Johnson et al., 2000; Ladd et al., 1997). Rejection during kindergarten is also predictive of internalizing and externalizing problems during middle school (Keiley et al., 2000), and childhood rejection is stable over time (Salmivalli & Isaacs, 2005). Additionally, some children have reported feeling increased stress following exposure to rejection (Sandstrom & Cramer, 2003). Given the relevance of peer rejection to well-being, exploring if and how the body physiologically adapts to rejection across time and in a stressful condition (i.e., beginning kindergarten) is a crucial next step in the literature.

The effect of peer factors on diurnal cortisol secretion have been rarely studied. In school aged children, research has demonstrated that cortisol increases when children experience negative peer interactions; interestingly, the presence of a best friend was protective and reduced the amount of cortisol secreted (Adams et al., 2011). Children who are rejected by their peers have higher basal levels of cortisol both directly and indirectly (via temperament; Gunnar, Sebanc, Tout, Donzella, & Van Dulmen, 2003). Contextual factors, such as SES, have been associated with increased rates of rejection (Hjalmarsson, 2018) and may also effect cortisol secretion throughout the school year.

Socioeconomic status

SES is a multidimensional construct and an index of one's material, social, and human capital (Oakes & Rossi, 2003). Individuals who come from lower-SES backgrounds are at higher risk for a number of negative health outcomes (Adler & Snibbe, 2003); one way this association has been explained is via the stress-response system. Individuals from lower SES backgrounds are exposed to more stress, which alters the body's physiological response to stress and increases vulnerability to disease (Adler & Snibbe, 2003; Evans, 2004; Gallo & Matthews, 2003). Children from lower SES backgrounds are known to have trouble functioning in school when they are older and they are less likely than other children to have had preparatory experiences, such as early learning experiences due to lack of available parental time, that would prepare them to function well in school (Duncan & Magnusun, 2005; Janus & Duku, 2007). These children can have more difficulty transitioning into full-time schooling and exhibit higher levels of externalizing behaviour (Entwisle & Alexander, 1993; Silver et al., 2005), a temperamental factor which has been associated with higher mean cortisol in response to the beginning of the school year (Turner-Cobb et al., 2008). Cortisol levels have also been associated with SES. For example, children from low SES neighborhoods have heightened basal cortisol levels when transitioning into school (Lupien et al., 2001), although some studies have found that low SES is associated with lower basal cortisol (Chen & Paterson, 2006), while other studies have found no association at all between cortisol levels and SES (Goodman, McEwen, Huang, et al., 2005). A better understanding of how SES effects the psychophysiological and social adaptation of children to the school environment is critical when considering the adaptive functioning of children from low SES backgrounds in school.

The current study

The effects of stress at the level of SES and at the level of the classroom peer group have not been studied together in the context of school transition. Given that the transition to full-time schooling can be conceptualized as exposure to a collective stressor, examining the effect of SES as well as the effect of developing social relationships on morning cortisol would be valuable. The goal of the present study is to examine the effect of stress at two levels: that of the individual and that of the family in children from an urban setting in Colombia. Using cortisol collected at the beginning and end of the school day from children who are beginning kindergarten for the first time, this study replicates prior research that found that children's diurnal cortisol was elevated in response to the school transition. In Colombia, kindergarten takes place only in the morning, thus morning variability in cortisol levels was examined. This study expands the literature by assessing whether SES and peer rejection moderated the effect of time (time of year and time of day) on cortisol secretion. Based on the prior literature, we hypothesized that 1) all children would secrete more morning cortisol at the beginning of the school year; 2) children from lower SES families as well as children who were rejected by their peers would secrete more morning cortisol than their higher SES peers; and 3) children from lower SES families would have higher morning cortisol at the end of the school year. Thus, this study examined whether children who have been rejected by their peers and children from lower SES backgrounds experienced greater stress, as measured by morning cortisol, in response to beginning formal education.

Methods

Participants and Procedure

The sample consisted of 110 Spanish-speaking children (*N* = 47 girls), between the ages of 5 and 6 (M_{age} = 5.37, *SD* = 0.42), from lower SES and higher SES families in Barranquilla, Colombia, a moderately sized city on the Caribbean coast. All children attended the same school in four separate classrooms. Participation rates varied only slightly across the four classes. Based on a typical class size of 30 children the participation rates in the four classes were 80%, 97%, 93%, and 97%. A total of 94 children were included in the final analysis due to missing sociometric data. Participants were students in their first year of mandatory, formal education, called *grado cero* or *el grado de transición* (Saavedra et al., 2014). An active consent procedure was used to obtain parental permission. Assent from the children was also obtained. Assessments were conducted at three times to correspond with the Colombian school schedule as some schools begin the new school year in February. Time 1 (T1) was in week 3 of the school year in late February; T2 was in week 15; and T3 was in week 40 near the end of the school year in November. Two types of assessments were conducted with each child. At T1 and T3, saliva samples were collected from each child so that cortisol levels at the beginning and end of the school year could be assessed. Samples collected at the beginning of the school year were coded as 0 and samples collected at the end of the school year were coded as 1. At T1, T2, and T3, a sociometric measure was administered to assess the level of rejection experienced by each child. SES was assessed at T1. Descriptive statistics separated by classroom can be found in Table 1.

Cortisol measures

Saliva samples were collected twice a day at the beginning and end of the school day in the classroom for three consecutive days. Children refrained from eating or drinking 30 minutes prior to the collection of the samples. A cotton swab was placed in each child's mouth for 3 minutes, and children were instructed to chew the swab until it was full of saliva and a research assistant could extract the sample. Samples were stored in Salivette containers. This procedure occurred on three consecutive days at the beginning of the school year and again at the end of the year. On each day, one sample was collected just after the children arrived at school (M_{time} = 7:35am; $M_{cortisol}$ =19.57 nmol/L, SD = 11.78) and then three hours later just before the school day ended (M_{time} = 10:28am; $M_{cortisol}$ = 16.80 nmol/L, SD = 9.00). Samples collected at the beginning of the school day were coded as 0 and samples that were collected at the end of the school day were coded as 1. A total of 12 samples were collected from each child. Cortisol data was transformed using a Log10 transformation to normalize the distribution. The saliva samples were assayed for cortisol in Barranquilla using a dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) assay. Missing cortisol data was imputed using MPlus software to estimate individuals' missing cortisol levels based on other available samples at the same time of day and time of year; 13% of the cortisol data was missing. After imputation, there were 1,152 cortisol data points, and there were 12 data points for each child. The procedures of the study have been reviewed and approved by Concordia University.

Sociometric methods

An unlimited-choice positive and negative sociometric technique was used to calculate measures of rejection for each child. In individual interviews, a member of the research team sat opposite to the child. The interviewer showed the child individual pictures of each of their child's classroom peers who were participating in the study and told the child the names of each peer. The pictures were then placed in a random order on the table so that each one could be seen clearly by the child. The interviewer told the child that she wanted to know who the child's best friends in class were and asked to point to the picture of the child's best friend. After this choice was made, the interviewer then asked the child if they had another best friend. If the child said 'yes', the child was asked to point to the picture of this peer. The interviewer continued with these questions until the child indicated that they had no further best friends. The interviewer then asked the child whether the child disliked any of the peers in the class. If the child said 'no' the interview was terminated. If the child said 'yes', the interviewer asked the child to point to a peer whom the child disliked. After the child pointed to one peer, the interviewer asked if the child disliked any other peers. If the child said 'yes,' the child was asked to point to this peer. This process continued until the child replied 'no' to the question, "do you dislike any other peers." The information collected in these interviews was used to compute a rejection score for each child. The measure of rejection was the number of times a child was chosen as a disliked peer. These measures were adjusted for variations due to class size using a the procedure described in Velásquez, Bukowski, & Saldarriaga (2013).

Socioeconomic status

The measure of SES for each child was based on a governmental index of SES computed for each neighbourhood in the country. Known as *estrato*, this index is derived using data from the national census about the quality of housing (e.g., size, materials, outdoor space) and services (e.g., access to roads) within each neighbourhood (Departamento Administrativo Nacional de Estadística, n.d.). Scores range from 1 (lowest SES) to 6 (highest SES). Information about each child's estrato score was taken from the school's records. The number of children in each of the estrato levels 1 through 4 were as follows: estrato 1 consisted of 31 children; estrato 2 consisted of 21 children; estrato 3 consisted of 44 children; and estrato 4 consisted of 14 children. Due to the limitations of our sample size, children in estratos 1 and 2 were assigned to a lower SES group ($N = 52$) and those from estratos 3 and 4 were assigned to a higher SES group ($N = 58$). The classrooms did not differ by SES ($F = 0.408$, $p = 0.708$). The data that support the findings of this study are available from the corresponding author upon reasonable request to the corresponding author.

Data analysis

A growth curve analysis was conducted with hierarchical linear modeling (HLM; Raudenbush et al., 2000). Growth curve analyses assesses change at the level of the individual and identifies the degree to which between-person variability in change is associated with other person-related characteristics. These analyses increase statistical power in within-person, repeated-measures designs (Raudenbush & Liu, 2001). In addition, growth curve modelling adjusts the weight of participants with smaller standard errors thus reducing error, allowing multiple cortisol measures to be modelled simultaneously, and allowing time-varying covariates (time of day), and non-time varying covariates (SES, peer rejection) to be included in the same model (Adam, 2006). The effect of time of day and time of year on cortisol were modelled at Level-1, while the effects of stable individual variables, such as SES, were modeled at Level-2. Level-2 findings can be seen in Table 2.

Using Restricted Maximum Likelihood estimates (REML), an unconditional model was first tested, i.e., a model with no predictors, in order to examine if cortisol varies randomly. Then, at Level-1, each individual's cortisol levels were predicted by the time of day of each sample in order to estimate the diurnal cortisol rhythm across three days of sampling. Time of day was expressed as number of hours since awakening. Time of year and time of year by time of day were also modeled at Level-1. A total of 1,152 samples were included at Level-1.

Next, time-invariant variables were group-centered and introduced to the model. The associations between SES and peer rejection were examined as moderators of the association between the Level-1 coefficients and cortisol. Exploratory analyses were conducted by entering each of the time-invariant variables as a Level-2 predictor of the Level-1 coefficients in order to examine if the Level-2 predictor moderated the association between the Level-1 predictor and cortisol. Any Level-2 variable that was significantly associated with a Level-1 predictor was then entered simultaneously into the Level-2 model for that Level-1 predictor. In order to preserve degrees of freedom, variables that were not significantly associated with the Level-1 coefficient were not included in the final model. Thus, the final model, where *i* represents the individual, and *j* represents the repeated-measures data, is as follows:

Level 1: $\text{LogCortisol} = \beta_{0j} + \beta_{1j}\text{TimeDay} + \beta_{2j}\text{TimeYear} + \beta_{3j}\text{TimeYear*TimeDay} + r_{ij}$

Level 2: $\beta_{0j}\ \text{Intercept} = \gamma_{00} + \gamma_{01}\text{Rejection} + \gamma_{02}\text{SES} + U_{0j}$

$\beta_{1j}\text{TimeDay} = \gamma_{10} + \gamma_{11}\text{Rejection} + \gamma 1_2\text{SES} + U_{1j}$

$\beta_{2j}\text{TimeYear}^2 = \gamma_{20} + \gamma_{21}\text{SES} + U_{2j}$

$\beta_{3j}\text{TimeDay*TimeYear} = \gamma_{30} + \gamma_{31}\text{SES} + U_{3j}$

Results

Multilevel modeling was used to analyze a data structure where within-person variables (Level-1) were nested within between-person variables (Level-2). Of specific interest was the association between cortisol (the outcome variable) and three Level-1 predictors: (a) time of year, (b) time of day, and (c) the interaction of time of year by time of day. Two Level-2 variables were used to account for random variance in the effects observed at Level-1: (i) rejection at T2, (ii) SES group. The Level-2 variables were significantly correlated ($r = -0.259$, $p = 0.005$). An unconditional model was used in the first analysis. This model was run as a baseline model to which subsequent models could be compared, and to calculate an initial intra-class correlation (ICC), which describes the amount of variance both within and between individuals. In this model, the participants' cortisol level was the outcome variable with no predictors. Sigma-squared was observed to be 0.0551. The ICC was observed to be 0.029, indicating that 2.9% of the variance was from between-person variables, and 97.1% was from within-person variables. The reliability estimate of the intercept was observed to be 0.260.

The next analyses examined Level-1 effects. Three Level-1 predictors were observed to be significant predictors of the within-person variability in cortisol. They were time of year (coefficient = -0.378, t = -28.082, p <.001), time of day (coefficient = -0.086, t = -7.210, p <.001), and time of year by time of day (coefficient = 0.0673, t = 4.463, p <.001). Sigma-squared decreased to 0.020 which indicated that the Level-1 predictors accounted for 36.3% of the within-person variability.

In the next analyses, we assessed the effect of the Level-2 variables on the variability of the intercept and on the Level-1 effects of time of day, time of year, and time of year by time of day. Both Level-2 variables were group centered to aid in interpretation of the intercept. Thus, the intercept is representative of the first morning sample of cortisol at the beginning of the school year. Variation in the intercept (first morning sample at the beginning of the year) was accounted for by T2 rejection (Figure 1.; coefficient = -0.011, $t = -3.370$, $p = 0.001$); the difference in cortisol at the intercept between children who were rejected by their peers and non-rejected children was 0.287 of a standard deviation. The simple slopes for high and low rejection on the intercept were statistically significant (Low: $t = 115.725$, $p < 0.001$; High: $t = 111.738$, $p < 0.001$). Variation in the intercept was also accounted for by SES group (coefficient = -0.044, $t = -1.788$, $p = 0.046$), with a difference in cortisol at the intercept between lower and higher SES children 0.269 of a standard deviation. The simple slopes for higher and lower SES on the intercept were statistically significant (Low: $t = 115.495$, $p < 0.001$; High: $t = 111.968$, $p < 0.001$). Variation in the slope of time of day was accounted for by T2 rejection (coefficient = 0.008, $t = 2.060$, $p = 0.042$). This simple slopes for high and low rejection on time of day were statistically significant (Low: $t = -8.181$, $p < 0.001$; High: $t = -5.429$, $p < 0.001$). In the first morning sample, the difference between cortisol levels in children who were rejected by their peers vs. non-rejected children was 0.415 of a SD, while in the second morning sample, that difference shrunk to 0.304 of a SD, indicating that the morning cortisol slope of children who are rejected is flatter than the morning slope of children who are not rejected. SES group did not explain variation in the slope of time of day (coefficient = 0.025, $t = 1.065$, $p = 0.290$). Variation in the slope of time of year was accounted for by SES group (coefficient = 0.069, $t = 2.663$, $p = 0.010$), such that at the beginning of the school year, children from lower SES backgrounds had higher cortisol levels, but by the end of the school year, higher SES children had higher cortisol levels. This was unexpected. However, these analyses did not take into account the diurnal variations in cortisol secretion, thus the final set of analyses examined the effect of SES on diurnal variations across the school year. Variation in the slope of time of year by time of day was accounted for by SES group (Figure 2; coefficient = -0.071, t = -2.408, p = 0.018). All simple slopes were statistically significant at p <0.002, with the exception of children from lower SES backgrounds at the end of the school year, which was not statistically significant (p >0.50). Whereas the change in cortisol at the beginning of the school day from February to November was the same for the lower and higher SES participants, the change in cortisol at the end of the school day from February to November was positive for the children from lower SES backgrounds and negative for the higher SES participants. In other words, the children from lower SES backgrounds' cortisol at the end of the school day *increased* by 0.107 of a standard deviation from February to November, whereas the higher SES participants cortisol at the end of the school day *decreased* by 0.352 of a standard deviation. This was indicative of a flattened cortisol slope for the children from lower SES backgrounds, both in reference to their higher SES peers and in reference to their diurnal slope at the beginning of the school year.

Discussion

This study had two overarching goals: (a), to examine whether physiological changes occur during the first year of formal schooling, and (b), to assess if these changes vary as a function of socioeconomic status and peer rejection. Hierarchical modeling shows that time of day, time of year, and time of year by time of day predicted cortisol secretion. Our findings are in line with prior research and support our first hypothesis, which indicated that as children progress through the school year, their cortisol levels are lower at the end of the school year, presumably as they adjust to their school environments (e.g., Turner-Cobb et al., 2008). Additionally, we found that the interaction between time of year and time of day was significant and positive, indicating that cortisol slopes at the end of the year are flatter than cortisol slopes at the beginning of the year. Further analyses were conducted to understand the variability in these Level-1 results.

In contrast to our second hypothesis, variation at the intercept (i.e., in the morning at the beginning of the year) was accounted for by peer rejection early in the school year, indicating that children who were socially rejected had lower cortisol in the morning than their non-rejected peers. Experimental studies examining social rejection and cortisol reactivity in adults have produced mixed results: they have found null effects of rejection on the cortisol response to stress (Linnen et al., 2012), heightened cortisol responses to rejection (Blackhart et al., 2007), and significant gender differences in the cortisol response to stress in the context of peer rejection, with females exhibiting a greater cortisol response to a social rejection paradigm (Stroud et al., 2002). The association between rejection and diurnal cortisol has been less frequently studied. Studies have found heightened cortisol in pre-school and school-age children when rejected socially, as well as a flatter diurnal slope in school-age children (Behnsen et al., 2018; Gunnar et al., 2003). While our results support prior research in that children who have been rejected by their peers have a flatter morning cortisol slope than non-rejected children, in contrast to prior research, our findings indicate that cortisol in children rejected by their peers is lower throughout the morning. However, our study is unique in that we examine peer rejection in the context of school transition, thus it is plausible that children who are rejected not only secrete less morning cortisol but also respond differently to the transition into school than non-rejected children. Social stressors are widely recognized as the most poignant stressors in laboratory studies of cortisol (Dickerson & Kemeny, 2004). While it might be expected that children who experience more stress will have higher cortisol levels, research has demonstrated that exposure to prolonged, chronic, profound stress may eventually lead to the down regulation of the HPA-axis, otherwise known as hypocortisolism (Fries et al., 2005). Children who are socially rejected by their peers may experience the stress of that rejection so strongly that their HPA-axis begins to secrete less cortisol throughout the day.

The measure of SES explained variability of cortisol at the beginning of the school year in the morning, as well as across the school year. Children from lower SES backgrounds had significantly higher cortisol in the morning at the beginning of the school year than children from higher SES backgrounds. Prior research has demonstrated that in adults, diurnal cortisol levels are higher on the days of stressful events (e.g., dance competitions, exams; Preuß et al., 2010; Rohleder et al., 2007). Thus, the higher cortisol at the beginning of the school year in children from lower SES backgrounds could indicate that children from lower SES backgrounds find the beginning of the school year more acutely stressful than their peers.

The overall decrease in levels of cortisol was observed to vary as a function of SES. In support of our third hypothesis, a comparison of the morning cortisol at the beginning and end of the school year indicates that children from lower SES backgrounds have morning cortisol that is significantly higher than the cortisol of their higher SES background peers. Notably, at the end of the school year, the morning cortisol slope of children from lower SES backgrounds was not significant ($p > 0.50$); by the end of the year, the late morning cortisol in children from lower SES backgrounds had *increased* compared to their late morning cortisol at the beginning of the school year. This demonstrates that lower SES children's morning cortisol slopes became flatter as the school year progressed. The current state of the literature shows that a flatter diurnal slope is associated with negative health outcomes, such as depression, externalizing and internalizing problems, obesity, inflammation, cardiovascular disease, and various types of cancer (Adam et al., 2017). While the current study was limited to an examination of morning cortisol, diurnal cortisol secretion is steepest in the morning, thus it is reasonable to assume that significant flattening of the slope in the morning equates significant flattening throughout the day.

These findings indicate that both lower and higher SES children responded adaptively to the novel stressors at the beginning of the school year, with elevated cortisol levels and steep morning slopes. Additionally, both groups' morning cortisol decreased by the end of the school year. However, children from lower SES background's cortisol did not decrease to the same extent as higher SES children's cortisol and their slope flattened. Previous research has demonstrated that children from lower SES backgrounds' cortisol increases as they begin school (Lupien et al., 2001); our results indicate that lower SES is associated with higher cortisol at the end of the year. As the school year continues, the HPA-axes of children from lower SES backgrounds remain more activated throughout the school day. Theory, such as the biological sensitivity to context model, posits that some children from higher stress backgrounds are more sensitive or reactive to stressors (Boyce & Ellis, 2005). Exposure to too much stress without environmental supports (e.g., family resources) may lead to dysregulation of the stress-response system. Children from lower SES backgrounds have reduced access to resources and may experience more stress at home, which could explain why their cortisol levels remained elevated and with a flatter slope at the end of the school year, two signs of HPA-axis dysregulation which have been associated with various negative health outcomes (Adam et al., 2017).

Our findings suggest that, following a critical normative transition, peer rejection and SES differentially moderate children's physiology. Children who are rejected have a flatter morning cortisol slope than children who are not rejected. Additionally, while beginning school is stressful for all children, children from lower SES backgrounds have increased morning cortisol levels compared to their higher SES peers, yet a flatter slope at the end of the school year. This demonstrates that social status, be it in the context of friendship or the context of wealth and resources, effects physiology in different ways. This has important implications for understanding the differential impact of distinct chronic stressors, supporting the theory that different stressors elicit different stress responses (e.g., Miller et al., 2007); various stressful situations may require different adaptive responses. In a meta-analysis, Dickerson and Kemeny (2004) found that situations that involved social threat evoked the strongest cortisol responses; they hypothesized that safeguarding social status is a priority among humans, thus any threat to social status mobilizes the HPA-axis. Overactivation of the HPA-axis may lead to its eventual down-regulation, which could explain the lower cortisol levels in children who are rejected. It is possible that growing up in a lower-SES neighbourhood is less consistently stressful than experiencing peer rejection on a daily basis at school, thus the

HPA-axis adapts in a different manner. In older adolescents, lower neighbourhood SES has been associated with lower basal cortisol levels (Chen & Paterson, 2006); however, to our knowledge, no studies have examined these predictors in children beginning kindergarten. Perhaps over time and with increased exposure, the hyper secretion of cortisol we see in these children beginning kindergarten will downregulate to become hypo secretion.

Limitations

The current study has multiple strengths, such as the longitudinal and repeated measures design, the sample from Colombia in Latin America, and the detailed, unlimited choice method of sociometric measurement. Further, the sample size is comparative to other similar studies and the data analysis strategy is appropriate for the complexity of the data. This study also has limitations. Related to cortisol, while cortisol trajectories changed over the course of the school year, data preceding the beginning of kindergarten were not collected, thus it is difficult to determine how much of the initial elevation of cortisol at the beginning of the year was due to the transition into school. Other studies which examined cortisol before the beginning of school (Russ et al., 2012; Turner-Cobb et al., 2008) found that cortisol levels were elevated months before beginning school, which was hypothesized to be due to the anticipation of beginning school. Additionally, we did not have information related to time of awakening, medication, or if the children had prior childcare experience. Statistically, the between-person variance accounted for by this study was small (2.9%). Few meta-analyses have been conducted which examined the effect of stress on cortisol (e.g., Fogelman & Canli, 2018), but the effect sizes have been small and at times not statistically significant.

However, even though the effect size is small, the effect may still be physiologically significant as the body adjusts to these minor changes in cortisol secretion. Related to SES, SES is a complex variable to measure and can be assessed in multiple ways. This study used census designated labels to infer SES from the area in which children lived. While this is a valid measure of SES, it assumes that individuals live in areas that accurately represent their economic means which may be a flawed assumption. A final limitation is that temperament was not assessed in this study. Previous literature on transitioning into novel school environments emphasized the important role temperament can play in the adjustment to school (Davis et al., 1999; Gunnar et al., 2003; Quas et al., 2002; Russ et al., 2012).

Conclusion

In summary, longitudinal data on peer relations, SES, and cortisol across the school year in children beginning kindergarten were assessed. These findings, observed using a basic measure of social relations and a fundamental form of psychophysiology, demonstrate that cortisol decreases throughout the course of the school year, children who are rejected have a flatter cortisol slope, and children from lower SES backgrounds have higher cortisol and a flatter slope at the end of the school year than their higher SES peers, indicating that the HPA-axis both down-regulates and up-regulates in response to different stressors. This research replicates and extends previous research on school transitions and cortisol, indicating that external factors such as friendship and SES differentially predict cortisol levels.

Table 1. Descriptive statistics by class

	Class size	Sex (% female)	SES (% lower SES)	Rejection (Mean, SD)
Class 1	24	46%	45.8%	2.07, 2.06
Class 2	29	38%	41.3%	1.63, 0.99
Class 3	28	43%	53.5%	1.39, 0.83
Class 4	29	45%	48.2	2.59, 1.61

Note: Rejection is averaged across the school year.

Figure 1. Children who are rejected by their peers at the second data collection have a flatter morning cortisol slope then children who were not rejected by their peers. Predicted scores are plotted using log10 transformed cortisol values.

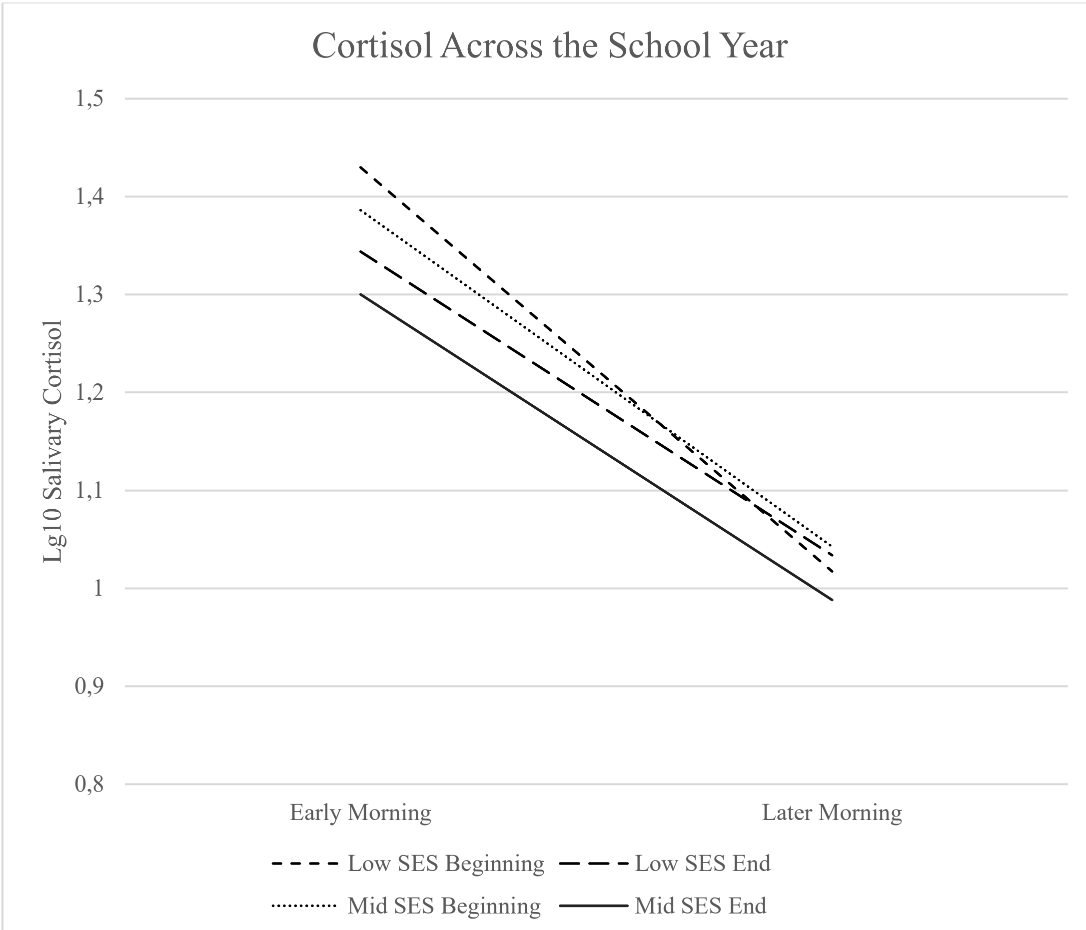

Figure 2. SES group is predictive of cortisol's change across the year, such that at the end of the year, lower SES children have higher cortisol at the end of the school day in comparison with higher SES children. This may indicate that lower SES children find school more stressful for a longer period of time than higher SES children. Predicted scores are plotted using log10 transformed cortisol values.

Bridging Studies

Results from Study 1 provided evidence that social and environmental factors influence the psychophysiological development of children as young as 5 years old. All children showed an elevated cortisol response to beginning kindergarten. By the end of the year, children who were rejected by their peers had a flatter cortisol slope in the morning while at school. While all children had decreases in cortisol levels by the end of the school year, children from lower SES backgrounds had higher cortisol levels at the end of the year than their higher SES peers.

While these findings provide new evidence regarding children's longitudinal psychophysiological adjustment to school, the importance of SES, as well as new evidence regarding the association between peer rejection and cortisol in young children, the focus was on morning cortisol in younger children. Thus, it is unclear if these social and environmental factors impact diurnal cortisol secretion, and if so, if they continue to effect children throughout their childhood.

Study 2 sought to expand the findings from Study 1 by examining the diurnal cortisol secretion in a population of Montreal-based early adolescents in the context of their SES and peer relationships. In addition, this study examined how stressful children's peer experiences were throughout the day and if those experiences were predictive of cortisol levels. Thus, using multiple measures of SES, early adolescent's diurnal cortisol levels were examined in the context of their peer experiences, their SES, and their levels of peer acceptance.

Gender is Key: Girls' and Boys' Cortisol Differs as a Factor of Socioeconomic Status and Social Experiences During Early Adolescence

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Abstract

The risks associated with negative peer relationships and low socioeconomic status (SES), and how they impact diurnal cortisol and the cortisol response to negative experiences, have never been studied together in early adolescents; this study aims to fill this gap in the literature. Saliva was collected from 95 early adolescents (M_{age} = 10.80, *SD* = 0.72) and daily diaries were completed 30 minutes after awakening, beginning of school, 15 minutes after first recess, 15 minutes after lunch, and end of school across four consecutive days. Hierarchical Linear Modelling was used to estimate the within- and between-person variances of diurnal cortisol and the cortisol response to stress in the context of SES and peer experiences. Cortisol secretion differed by gender and was predicted by SES and social status within the peer group. Low-SES early adolescents had higher morning cortisol. Girls who were from higher SES families had the steepest diurnal cortisol slope. Non-accepted early adolescents had low cortisol in response to both positive and negative social experiences. The findings from this study clarify the impact of both SES and peer relations on early adolescent psychophysiological development.

Introduction

Over the last decades, understanding how stress *gets under the skin* to negatively impact well-being has garnered increasing attention (e.g., Miller et al., 2011). Allostatic load theory hypothesizes that as people experience stress, their ability to cope with that stress changes (McEwen & Wingfield, 2003); thus, bodies sustain "wear and tear", which leads to the alteration of the stress response system and leaves exposed individuals at an increased risk of negative health outcomes (Felitti et al., 1998). Stress exposure during childhood can have severe long-term effects on health (Miller et al., 2011). However, the ways in which childhood stress increases vulnerability to disease are not well understood. The secretion of cortisol, a hormone that is emitted diurnally and in response to stress, can be altered following stress exposure (Gunnar & Quevedo, 2007). Intriguingly, some studies have found that following exposure to high and/or frequent stress cortisol over-secretes (hypercortisolism), while other studies have found that cortisol under-secretes (hypocortisolism; c.f., Fries, Hesse, Hellhammer, & Hellhammer, 2005). The type of stressor experienced may explain some of this heterogeneity (G. E. Miller et al., 2007). By examining two different types of stressors, low socioeconomic status (SES) and problems in peer relationships, the present study investigates how stress at distinct levels of social complexity differentially predict diurnal cortisol and the cortisol response to momentary social experiences in early adolescents.

Cortisol

A cascade of physiological activity is triggered following exposure to stress. The HPA-axis is activated, beginning with the release of corticotrophin-releasing-hormone (CRH) from the paraventricular nucleus of the hypothalamus (Tsigos & Chrousos, 2002).

This release of CRH causes the pituitary to secrete adrenocorticotropic-releasing hormone, which stimulates the adrenal glands to release the steroid hormone cortisol, a type of glucocorticoid (Tsigos & Chrousos, 2002). Cortisol is also secreted diurnally, following a circadian rhythm. Cortisol increases during the night, peaks shortly after awakening (i.e., the cortisol awakening response [CAR]; Stalder et al., 2016) and decreases during the day, reaching nadir at bedtime (i.e., the diurnal slope; Adam et al., 2017). The cortisol response to stress and the diurnal pattern of cortisol secretion may be altered following repeated stress exposure (Lupien et al., 2009). In general, steeper diurnal slopes (Adam et al., 2017) and increased cortisol levels in response to laboratory stress tasks (MacMillan et al., 2009) are associated with healthy HPA-axis activity. By experiencing chronic stress during childhood the functioning of the HPA-axis may be altered in ways that are ultimately maladaptive (Bunea et al., 2017).

Socioeconomic Status

SES has been widely acknowledged to be a social determinant of health. Coming from a low SES background has been associated with a number of health disparities (c.f., Chen & Miller, 2013). One possible explanation is that growing up in a lower SES background is more stressful and leads to stress-related disease (Cundiff et al., 2020). SES can be categorized by types of capital; for example, material capital (i.e., income), human capital (i.e., education), and social capital (i.e., employment; Oakes & Rossi, 2003). By examining different types of capital to assess SES, studies can find different results (Lahelma et al., 2006), and may help explain why the association between cortisol and SES is mixed. This literature is discussed in greater detail below.

Socioeconomic status and diurnal cortisol. Chronic stressors, such as being raised in a low-SES environment, can predict cortisol secretion throughout the lifespan. During childhood, lower SES, as measured by neighborhood, parental income, and parental education level, has been associated with higher single-sample salivary cortisol (Lupien, King, Meaney, & McEwen, 2001). Income has been used to predict the development of diurnal cortisol trajectories in a curvilinear fashion in children from age 36-months, indicating that children in both lower income families and higher income families may have more sensitive and responsive HPA-axes (Zalewski et al., 2016). During adolescence, research on the association between cortisol and SES is mixed. Some studies have found a positive association, with lower neighborhood SES related to lower baseline cortisol (Chen & Paterson, 2006), while others found a negative association, with lower SES, as measured by family assets or poverty, associated with higher baseline (Evans & English, 2002) and area under the curve (AUC) cortisol (Chen, Cohen, & Miller, 2010). Some studies found no effect of SES as measured by lower parent education on cortisol (Goodman, McEwen, Huang, et al., 2005). Thus, there is a heterogenous association between diurnal cortisol and SES. The association between cortisol reactivity and SES is less varied.

Socioeconomic status and cortisol reactivity. Cortisol reactivity is the secretion of cortisol in response to real or perceived threats to an individual's well-being. Studies of cortisol reactivity are conducted using laboratory tasks wherein cortisol is assessed before, during, and after exposure to a stressor (e.g., a negative social experience, or mental math in front of impassive judges). Few studies have examined the association between cortisol reactivity and socioeconomic status in children and adolescents. Lower income-to-needs ratios, parental education, and neighbourhood disadvantage have been associated with increased cortisol reactivity (Gump et al., 2009). Gender has been found to moderate the association between SES and cortisol reactivity, such that lower SES as measured by parental education and neighborhood disadvantage, predicted a heightened cortisol response in boys, but not girls (Hackman et al., 2012). Thus, children and adolescent's cortisol reactivity are typically heightened when they come from lower SES backgrounds, and gender has been found to moderate this effect.

Peer Relationships

Social relationships can serve to regulate well-being or buffer the impact of stress throughout the lifespan (Rubin, Bukowski, & Bowker, 2015). As children age parental relationships become less important (Hostinar et al., 2015), and social relationships become more salient and influential (Allen et al., 2003). In adults and children, negative social evaluation is one of the most consistent ways to elicit a stress response in the laboratory (Dickerson & Kemeny, 2004). Given how stressful negative evaluation by one's peers can be, examining social relationships in day-to-day life is an ecologically valid way to investigate the stressors of peer evaluation on the cortisol response outside the laboratory (Dickerson & Zoccola, 2013). The majority of studies of social relationships and cortisol have examined early childhood and relationships with caregivers; comparatively few studies have examined the association between cortisol and school-aged children and their peers (Hostinar & Gunnar, 2013). The extant literature is discussed below.

Peer relationships and diurnal cortisol. Being rejected by one's peer group, i.e., being disliked in the peer group (Rubin et al., 2015), can be an emotionally powerful experience (Murray-Close, 2013), yet has rarely been studied in the context of the HPA-axis. Only two studies have been located that examined the relation between children's diurnal cortisol levels and social rejection. Preschoolers who were rejected had higher basal cortisol levels than their non-rejected peers (Gunnar et al., 2003). One study of school-aged children found no relation between peer rejection and basal cortisol levels (Behnsen et al., 2018). During middle childhood, peer exclusion (i.e., being left out/ignored by one's peer group; Buhs, Ladd, & Herald, 2006) was predictive of diurnal cortisol levels such that children who were excluded by their peer groups had elevated cortisol levels at school and flattened diurnal curves (Peters et al., 2011). Taken together, this nascent research is mixed, yet it is beginning to indicate that being rejected and excluded by one's peer group may be predictive of the HPA-axis.

Only three studies have been located that examined the association between peer acceptance (being "liked" by one's peers; Rubin et al., 2015) and cortisol. Greater acceptance by peers plays a mediational role between higher afternoon cortisol levels and higher levels of prosocial behaviour; while lower levels of peer acceptance mediate the association between lower afternoon cortisol levels and higher social and relational aggression in adolescents (Catherine et al., 2012). Another study found that the association between afternoon cortisol and higher levels of peer acceptance was mediated by prosocial behaviour and perspective-taking skills (Oberle, 2018). Interestingly, both these studies found that afternoon cortisol was positively associated with peer acceptance, indicating a flatter diurnal slope. This contradicts the findings in the peer rejection literature where the flatter slope was associated with the subjectively negative variable (i.e., rejection), not the subjectively positive variable (i.e., acceptance). A final study found that low levels of peer acceptance were associated with higher AUC cortisol, a flatter cortisol slope, and a heightened CAR (Behnsen et al., 2018). This contradictory literature merits further investigation.

Peer relationships and cortisol reactivity. Only one study was located that examined cortisol reactivity and peer relationships in children and adolescents (Stroud et al., 2009). This study examined developmental differences in children and adolescents stress responses. During a social exclusion task children and adolescent's cortisol reactivity were assessed. While adolescents showed a greater cortisol response to the social exclusion than the children, there was no statistically significant difference between adolescents and children.

Cortisol, Socioeconomic Status and Peer Relationships

It appears that only one study has examined how SES may interact with cortisol and social support. This study examines the association between subjective SES and cortisol reactivity, as moderated by perceived social support in 115 adults, and found that for those who reported lower perceived support, lower subjective SES predicted a higher cortisol response to stress (Hooker et al., 2017). Objective SES was measured via parental education and did not predict the cortisol response (Hooker et al., 2017). This may indicate that subjective SES is a more powerful predictor of the stress response system. Alternatively, it may indicate that those who are more subjectively negative about their SES are also more physiologically sensitive to negative events. Using multiple measures of objective SES may help to clarify the association between SES, peer influences, and cortisol reactivity.

Cortisol and Gender

There is evidence of gender differences in diurnal cortisol secretion and cortisol reactivity (Hollanders et al., 2017). A systematic review indicated that girls have a more variable diurnal profile, a higher cortisol awakening response (CAR), as well as a stronger cortisol response to social stress in laboratories (Hollanders et al., 2017). Also, girls' cortisol levels are influenced by pubertal stage such that, after controlling for age, puberty is predictive of a flatter diurnal cortisol slope (Shirtcliff et al., 2012). However, gender differences in cortisol levels are not consistently reported (Kudielka et al., 2004). It is unclear if other risk factors influence gender differences in cortisol secretion during childhood. This study will address this gap by examining if gender moderates the effect of peer relations and SES on children's cortisol.

Current Study

The current study utilizes sophisticated data collection and analytical techniques to examine within- and between-person associations between two types of peer stress (peer rejection and low peer acceptance) and SES, and two types of cortisol secretion, diurnal cortisol and cortisol responses to moment-by-moment social experiences in 95 children between the ages of 9 and 11 years. Daily diaries are used to measure social experiences throughout the day. Utilizing a similar methodology to several prior studies on adults (e.g., Doane & Adam, 2010) and one study on children (Adam, 2006), the cortisol response to these social experiences is then assessed. This study hypothesizes the following: First, diurnal cortisol trajectories will be predicted by SES, such that lower SES children will have a flatter cortisol slope. Second, there will be within-person associations between early adolescent social interactions and cortisol levels, after controlling for the effects of time of day. Furthermore, after controlling for time of day, adolescent cortisol levels will be higher following negative social interactions. Third, children's social status (i.e., peer acceptance and rejection) within the classroom will moderate the association between quality of social experience and cortisol. Finally, these effects may differ based on the gender of the child. By examining the effect of multiple levels of risk on cortisol secretion this novel study aims to fill broad gaps in the literature by exploring if SES and peer variables predict cortisol levels and if gender may moderate these effects.

Methods

A total of 116 children from an English-speaking school in the greater Montréal area were recruited. Of this sample, 95 children provided saliva, were not taking a steroid-based asthma medication or methylphenidate and returned a completed parent questionnaire; only children with both cortisol and the parent questionnaire were included in this study (49% female; M_{age} = 10.80, SD = 0.72). Participation was between 80%-100% per class.

Procedure

School consent, parental consent and child assent were obtained. Children were given an SES questionnaire for their parents to complete. Children provided saliva samples five times per day for four consecutive school days (Tuesday-Friday). Saliva was collected 30 minutes after awakening, at the beginning of the school day, 15 minutes after first recess, 15 minutes after lunch, and at the end of the school day. Research staff were present during each in-class assessment and provided help with saliva collection if necessary. Mean times of cortisol collection, as well as mean cortisol levels, can be found in Table 1. At each time point, the participants also completed a daily diary in which they described their social interactions 20 minutes prior to providing the saliva sample. When the data was collected at school (i.e., all time points excluding the first), research staff were present to respond to questions and provide reference points for the previous 20 minutes. Vials containing saliva were coded for time and day, temporarily stored in a container with dry ice and then placed in long-term storage in a "-80C" freezer. For the first sample of the day, participants were given a plastic bag and saliva vial to transport their saliva to school.

Measures

Peer relationships. *Social experiences.* Students completed daily diary booklets five times per day across four consecutive days. Only daily diary entries that were completed at school were included in this study. Students were asked to report how they felt regarding the quality of their social experiences that occurred 20 minutes prior to data collection (i.e., the valence of their peer experience). For each social experience, the student was asked "How did you feel about it?" and "How was the interaction with your friend/classmate?" Participants' answers ranged from 1 (very negative) to 7 (very positive). These items were combined, and the mean was calculated (M = 4.80, SD = 1.75).

Peer rejection and acceptance. Peer rejection and acceptance were assessed via peer nomination. Children were given questionnaires with lists of their classmates and were asked, "How much do you like each person" on a scale of 1 (do not like the person; M = 1.17, SD = 1.53) to 5 (like the person very much; M = 2.99, SD = 1.93). The number of 1s and 5s each child received were then tallied and averaged to indicate the extent to which each child was rejected or accepted by their peer group. Data was adjusted for class size using the method described in Velásquez, Bukowski, and Saldarriaga, 2013.

Socioeconomic status. Each child was given a questionnaire for their parent to complete. Multiple aspects of SES were assessed: maternal and paternal income, family size, family education (maternal years of education combined with paternal years of education). Income-to-needs ratios were calculated by dividing income by family size; thus, a higher income to needs ratio indicated a higher income relative to family size. The analyses in this study focused on the income to needs ratio and family education, which are significantly correlated ($r = 0.434$, $p < 0.001$).

Cortisol. Salivary cortisol was assayed with procedures modified to increase assay sensitivity (DSL, Webster, Texas). In short, 50 µL of saliva, 50 µL of ^{125}I-cortisol, and 50 µL of primary antibody were incubated and placed in a water bath at 37 °C for 2 hr. A 500 µL PBS wash was added to the tubes prior to centrifugation. The pellet, representing the bound cortisol, was counted in a gamma counter, and cortisol concentrations were calculated from a standard curve. The limit of detection for cortisol was 0.01 µg/dl, and the intra- and inter-assay variabilities were 4.0% and 4.6%, respectively. Six outliers (cortisol > 2.5 standard deviations from the mean) were located in over 1,900 samples; they were brought towards the mean. Raw cortisol values were log transformed normalize the distribution after one was added to each score (M= 1.271, SD= 0.407). Means of raw cortisol values can be found in Table 1.

Data Analysis

Growth curve analyses were conducted using hierarchical linear modeling (HLM; Raudenbush et al., 2000). Growth curve analysis is a regression-based procedure that creates indices of change at the level of the individual participant. It is an ideal approach for assessing data structures in which data are nested within individuals. Multilevel growth curve modelling is employed in order to increase statistical power in the within-person, repeated-measures design (Raudenbush & Liu, 2001). Additionally, growth curve modelling utilizes adjusted weighting of participants with smaller standard errors, permits the modelling of multiple cortisol measures simultaneously, and permits the measurement of time-varying covariates (time of day, quality of social experience), and non-time varying covariates (SES, peer acceptance, peer rejection) on cortisol levels (Adam, 2006). The effect of time of day and quality of social experience on cortisol were modelled at Level-1, while the effects of stable individual variables, such as SES, were modeled at Level-2.

First, an unconditional model was tested, i.e., a model with no predictors, in order to examine if cortisol varies randomly. Then, at Level-1, each individual's cortisol levels were predicted by the time of day of each sample in order to estimate the diurnal cortisol rhythm across four days of sampling. Time of day was expressed as number of hours since awakening. A curvilinear model was used because diurnal cortisol levels are not typically linear. Quality of social experience was also modeled at Level-1, as it varied throughout the day.

Next, time-invariant variables were group-centered and introduced to the model. The associations between gender, SES, peer acceptance, and peer rejection were examined as moderators of the association between the Level-1 coefficients and cortisol. Exploratory analyses were conducted by entering each of the time-invariant variables as a Level-2 predictor of the Level-1 coefficients in order to examine if the Level-2 predictor moderated the association between the Level-1 predictor and cortisol. These analyses included examinations of both acceptance and rejection, as well as acceptance by SES interactions and rejection by SES interactions. Any Level-2 variable that was significantly associated with a Level-1 predictor was then entered simultaneously into the Level-2 model for that Level-1 predictor. Significant Level-2 predictors were then examined for gender differences by examining their interaction with gender. In order to preserve degrees of freedom, variables that were not significantly associated with the Level-1 coefficient were not included in the final model. Thus, the final model, where *i* represents the individual, and *j* represents the repeated-measures data, is as follows:

Level 1: $\text{LogCortisol} = \beta_{0j} + \beta_{1j}\text{Time} + \beta_{2j}\text{Time}^2 + \beta_{3j}\text{SocialExperience} + r_{ij}$

Level 2: $\beta_{0j}\ \text{Intercept} = \gamma_{00} + \gamma_{01}\text{Gender} + \gamma_{02}\text{Acceptance} + \gamma_{03}\text{Rejection} + \gamma_{04}\text{IncomeNeeds} + \gamma_{05}\text{FamilyEducation} + U_{0j}$

$\beta_{1j}\text{Time} = \gamma_{10} + U_{1j}$

$\beta_{2j}\text{Time}^2 = \gamma_{20} + \gamma_{21}\text{Gender} + \gamma_{12}\text{IncomeNeeds} + \gamma_{12}\text{Gender*IncomeNeeds} + U_{2j}$

$\beta_{3j}\text{SocExp} = \gamma_{30} + \gamma_{31}\text{Gender} + \gamma_{32}\text{Acceptance} + \gamma_{33}\text{Gender*Acceptance} + U_{3j}$

Reductions in sigma and tau were examined and coefficients were assessed for statistical significance. For the interactions of interest, simple slopes of the Level-1 variables were calculated in order to examine how they varied with the Level-2 variables. Then the standard error of the relevant Level-1 effect was used to compute a t-score and a corresponding p-value for each of the simple slopes.

Missing data was handled differently depending on the variable. For example, for obvious reasons, there was no data related to social experiences corresponding to cortisol at awakening, therefore in order to avoid large amounts of missing data the mean value of social experiences was used to replace the missing data points (n = 335). Data points involving missing daily diary entries for all other time points, and data points for missing cortisol samples, were deleted (n = 198). MPlus was used to impute missing SES data for partially completed parental questionnaires (8% of total sample).

Each of the 95 participants provided data at up to 20 separate occasions. After accounting for missing cortisol samples, this study includes 1,702 cortisol samples, or nearly18 samples per participant. Given this large number of samples, the degrees of freedom at Level-1 are sufficient to model the multiple time of day and time-invariant variables, as well as the modeling of the seven Level-2 variables simultaneously. The number of samples in this study are comparable to similar studies (Adam, 2006; Doane & Adam, 2010).

Results

Hierarchical Linear Modelling was used to analyze a data structure wherein within-person variables (Level-1) were nested within between-person variables (Level-2). Of specific interest were the relationships between cortisol (the Level-1 outcome variable), and four Level-1 predictors; specifically, time, time squared, social experience, and social experience squared. An unconditional model was assessed in the first model. This model was conducted as a baseline model to which subsequent models could be compared and to calculate an initial intra-class correlation (ICC). In this model, participants' log transformed cortisol scores were the outcome variables and there were no predictors. The ICC was 0.24 indicating that 24% of the variance was between participants and 76% was within. The value of sigma squared was 0.127. The value of the intercept was 1.263. This effect was random (X (94) 634.345, $p < .001$).

The next model focused on Level-1 effects. Four Level-1 variables were statistically significant predictors of the within-person variability in the cortisol scores. They were the intercept (coefficient = 1.439, $t = 39.06$, $p < 0.001$), time of day (coefficient = -0.119, $t = -13.20$, $p < 0.001$), time of day^2 (coefficient = 0.009, $t = 7.78$, $p < 0.001$) and quality of social experience (coefficient = 0.014, $t = 1.79$ $p = 0.038$). Sigma squared decreased to 0.101, indicating that the Level-1 predictors accounted for 20% of the within-person variance. With the addition of these predictors, the value of the intercept increased to 1.439.

The final model assessed whether Level-2 variables moderated the association between cortisol and the Level-1 intercept, time of day, time of day^2, and quality of social experience. Variation in the intercept was accounted for by gender (i.e., boys had higher cortisol at the intercept; coefficient = -0.173, $t = -2.79$, $p = 0.007$), acceptance (i.e., accepted children had higher cortisol at the intercept; coefficient = 0.028, $t = 2.29$, $p = 0.024$), rejection (i.e., rejected children had lower cortisol at the intercept; coefficient = -0.037, $t = -2.23$, $p = 0.028$), and family education (i.e., lower education levels was associated with higher cortisol at the intercept; coefficient = -0.007, $t = -1.99$, $p = 0.049$). Variation in the slope of time of day was not accounted for by any of the moderator variables in the final model. Variation in the slope of time of day^2 was accounted for by income to needs (coefficient = 0.004, $t = 2.38$, $p = 0.019$). However, this effect was moderated by gender; once the gender by income to needs interaction was included in the model, the direction of the effect reversed (coefficient = -0.002, $t = -2.03$, $p = 0.044$; see Figure 1). When the simple slopes of boys and girls of higher and lower incomes' cortisol on time of day^2 were examined, all four slopes were statistically significant ($p < 0.001$). Quality of social experience was explained by acceptance (coefficient = -0.020, $t = -3.69$, $p = 0.001$). Gender moderated this association when a gender by acceptance interaction was added to the model (coefficient = 0.008, $t = 2.31$, $p = 0.023$; see Figure 2). When the simple slopes of boys and girls with high and low acceptance were examined, all four slopes were statistically significant ($p < 0.001$).

Discussion

The impact of social stress on cortisol levels in children, adolescents, and adults is clear from laboratory studies and the few studies that examine cortisol and social stress in naturalistic settings. Individuals do not live in a vacuum and given that SES is an environmental construct that is predictive of general health outcomes, understanding how SES and social stress differentially predict cortisol is a key step in the literature. This study examined how social stressors such as peer rejection, acceptance and environmental stressors such as SES, may explain variability in diurnal cortisol levels, as well as moment-by-moment cortisol responses to social experiences. More specifically, the goals of this study were three-fold: first, to examine if children from lower SES backgrounds or with less peer support had a flatter diurnal cortisol slope or an elevated cortisol response to social situations; second, to examine if early adolescents cortisol varied in response to social experiences, and if this variance could be explained by peer status within the classroom; and third, to examine if any of these results differ by gender. Results indicate partial support for these hypotheses. Lower SES early adolescents had a flatter cortisol slope, and this effect was moderated by gender, such that higher SES girls had the steepest diurnal cortisol slope. Cortisol varied as a function of social experience. Children who were accepted had increases in cortisol as their social experiences became more positive, while children who were not accepted had flatter cortisol responses to positive or negative social experiences; this was particularly true of girls. These findings may have implications regarding gender differences in the development of depression during adolescence. The results from this study will be discussed in greater detail below.

Cortisol was found to vary randomly throughout the day and in response to SES and peer experiences. Cortisol at the intercept, (i.e., 30 minutes post wakening) was negatively predicted by gender, such that boys had higher cortisol levels at the beginning of the day. Thus, boys may have experienced greater levels of arousal in anticipation of the school day. Additionally, cortisol levels at awakening were predicted by SES, such that children whose families were of lower levels of education had higher cortisol levels when they awaken. These findings lend credence to the results of a study which found that low-SES (as measured by paternal profession) across the lifespan leads to a doubling of the proportion of extreme high morning cortisol values (Li et al., 2007). In this study, lower SES as measured by family education levels predicted heightened cortisol upon awakening, which may indicate that children whose families have less education experience greater arousal in anticipation of the coming school day. Children from lower income households, but not necessarily less parental education, did not experience similar levels of arousal. Different SES measures predict cortisol differently.

Cortisol at the intercept was lower for rejected children and higher for accepted children. It has been well-established in the literature that social stressors are the most potent predictors of a stress response in the laboratory (Dickerson & Kemeny, 2004). This response to rejection may be an expression of socially-rooted learned helplessness, wherein an individual does not believe in their ability to overcome an obstacle and thus gives up (Goetz & Dweck, 1980). While animal studies of learned helplessness have shown an increased cortisol response to stressors (e.g., Dess, Linwick, Patterson, Overmier, & Levine, 1983), these studies are typically short term (e.g., two days). Perhaps when examined over longer periods of time learned helplessness may lead to blunted cortisol expression in some individuals. Hypocortisolism occurs in some children following prolonged exposure to stressors and has been associated with an increased risk of depression. For example, youth with lower average cortisol levels who also reported more stressors had elevated symptoms of depression (Badanes et al., 2011). Learned helplessness has been used as an explanatory hypothesis in depression, in that people who suffer from depression do not believe they have control within their lives, and thus suffer from cognitive and motivational deficits that then prolongs their depressive states (Abramson et al., 1981). The association between learned helplessness and cortisol secretion has not been studied in children. One study involving adults found that, unlike in control patients, depressed inpatients' cortisol response did not differ by controllability of the stressor; their cortisol decreased in response to both controllable and uncontrollable stressors, indicating hypoactivity of the HPA-axis (Croes et al., 1993). Perhaps lower morning cortisol in rejected children is indicative of learned helplessness in the social context, while higher levels of cortisol for accepted children may be indicative of a healthy awakening response (Fries et al., 2009).

In the full model, time of day effects were not predicted by any moderating variables. However, curvilinear time of day was predicted by the income to needs ratio, that was in turn moderated by gender. Higher SES girls have the steepest diurnal slope, while lower SES girls, and upper and lower SES boys, have a flatter diurnal slope (see Figure 1). A flatter diurnal slope has been associated with negative health outcomes (Adam & Kumari, 2009). These findings may indicate that girls are more vulnerable to the stressors of lower SES backgrounds than boys, such that higher SES girls had significantly steeper slopes than their same-gendered low-SES peers. While pubertal development has not been consistently associated with cortisol (e.g., El-Sheikh, Buckhalt, Keller, & Granger, 2008), some studies have found increased cortisol levels (e.g., Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009), or a steeper diurnal slope (Adam, 2006) to be associated with a later pubertal stage. Interestingly, pubertal status has been found to predict the direction of the cortisol response to stress, in that pre-pubescent children at risk for depression had a hyporeactive cortisol response to stress while post-pubertal children at risk for depression had a hyperreactive cortisol response to stress (Hankin et al., 2010). While pubertal stage was not assessed in this study, it is possible that the boys' flatter diurnal slope is attributable to the fact that they reach puberty later and may not have established a typical diurnal cortisol rhythm (Marceau et al., 2011).

The effect of the quality of social experiences (i.e., positive or negative) on cortisol is predicted by acceptance. Gender moderated the effect of acceptance on social experiences in that both boys and girls who are high in acceptance have high cortisol levels in response to positive social experiences; this is particularly true of girls (see Figure 2). This finding was not in line with the hypothesis that *lower* standing within the peer group (higher levels of rejection or lower levels of acceptance) would be associated with *higher* levels of cortisol in relation to negative peer experiences. Previous research on loneliness in adults found that higher levels of loneliness were associated with momentary increases in cortisol throughout the day (Doane & Adam, 2010). However, in this study, boys and girls who are *low* in acceptance have a *low*, or blunted, cortisol response to both negative and positive social experiences; again, this was particularly true in girls. When analyses included rejection in the model, rejection was not a statistically significant predictor of the cortisol response to the quality of social experience, suggesting that the variance between rejection and acceptance is overlapping. While this study did not assess the duration of low acceptance, social status is typically a stable construct; thus low acceptance is more likely to be a chronic stressor than an acute stressor (Cillessen et al., 2000). This finding is similar to prior research in which children who reported more peer problems had flatter diurnal cortisol slopes throughout the day (Bai et al., 2017). The findings in this study may indicate that the cortisol response to both positive and negative social experiences is particularly blunted for girls who are not accepted. Hypocortisolism has been posited to be an indicator of allostatic load in prepubescent children (Badanes et al., 2011). Perhaps girls are more sensitive to the psychological impact of low acceptance than boys, which could make low acceptance one of the pathways through which girls become more vulnerable to the development of depression. Studies on cortisol reactivity in adolescence may support these findings. For example, a study that compared depressed adolescents to non-depressed adolescents found that girls, not boys, had blunted cortisol reactivity to a laboratory stress test (Mazurka et al., 2018). In addition, research has found that girls who are victimized, not boys, exhibit blunted cortisol (Vaillancourt et al., 2008). The blunted cortisol levels in girls who are less accepted may be indicative of learned helplessness and suggests that they are giving up socially, even in the face of positive peer interactions.

Throughout this study, the multiple ways SES was measured were differentially predictive of cortisol levels. Going to school appeared to be more arousing for children whose families had less education, while time at school appeared to be more arousing for children whose families had proportionately less income. This may be because children whose families are less educated experience more anticipation or stress before attending school, while children who have less income may find themselves in positions throughout the day when their lack of material wealth impacts their stress levels. In exploratory analyses, these SES measures were combined, but they were not statistically significant predictors of cortisol levels, possibly because they are measuring different types of capital (Oakes & Rossi, 2003). Education level assesses human capital while income-to-needs assesses material capital, which separately account for variance in health-related outcomes, such as depression and other chronic conditions (Oakes & Rossi, 2003). These effects highlight the importance of multifaceted measurements of SES.

While this study has many strengths, such as the repeated measures design and the use of daily diaries to assess children's varying social interactions throughout the day, there are also limitations that must be considered. By using a unique study design, this study was able to assess children's social experiences as they changed throughout the day. However, adding additional questions related to the quality of the peer relationships may have further nuanced these findings. Regarding cortisol sampling, although five cortisol samples were collected throughout the day, samples were not collected to enable measurement of the CAR. In addition, while the sample size of this study enabled us to examine certain interaction effects, a larger sample would allow us to further explore differences within the individual child, such as gender by age, that would increase the generalizability of these findings. Another limitation is the lack of data on pubertal development. Puberty has been found to impact diurnal cortisol (Adam, 2006) and cortisol reactivity to stress (Hankin et al., 2010). Thus, future studies of this age group should include measures of pubertal status. Given this studies' implications on the development of depression in adolescent girls, data on depressed mood would have added further context to these findings. Finally, a longitudinal study design would enable researchers to better understand the stability and long-term impact of children's peer rejection, acceptance, moment-to-moment peer experiences, and SES.

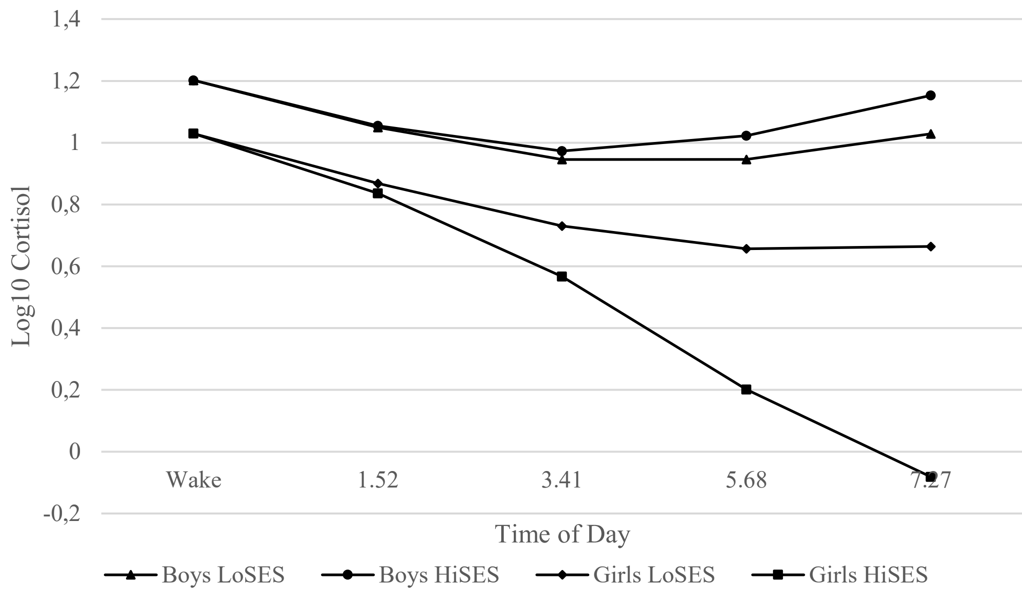
Conclusion

There is a growing body of evidence that demonstrates that social experiences *get under the skin* to impact one's physiological functioning. This study expands on this literature by examining peer relationships and SES, and how they predict cortisol levels during early adolescence. The findings from this study indicate that gender is key to understanding how peer and SES variables moderate cortisol levels. Girls' cortisol levels generally appear more vulnerable to the effects of social stressors, while low-SES girls appear to be most vulnerable to stress across the day. By examining change in the stress-response system in tandem with change in social experiences during the day, the importance of SES and the peer context on a child's holistic development were highlighted.

Table 1. Mean and standard deviation of untransformed cortisol levels by gender

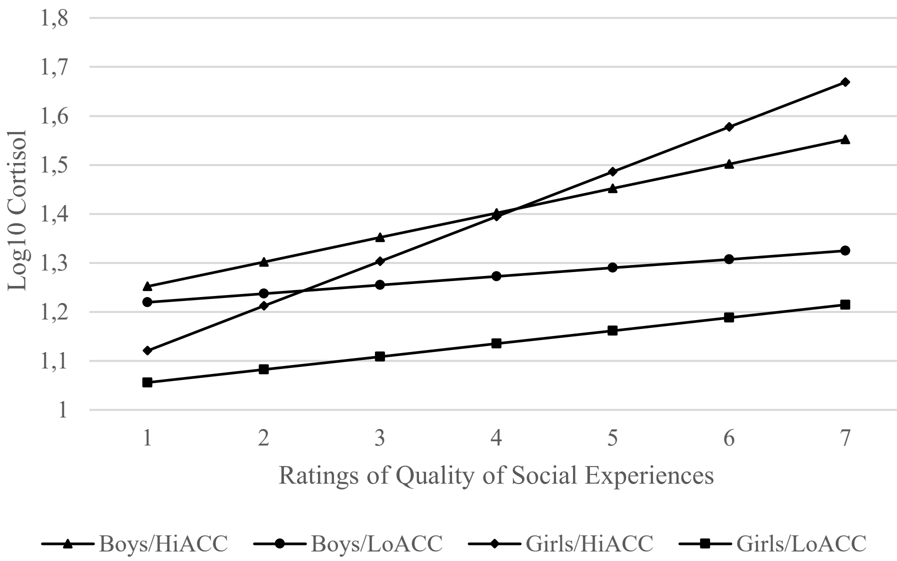
Cortisol Mean, *SD*				
Sample (Mean time of sample)	Day 1	Day 2	Day 3	Day 4
Girls				
Sample 1 (6:29)	0.565, *0.871*	0.270, *0.178*	0.270, *0.166*	0.203, *0.114*
Sample 2 (8:07)	0.344, *0.647*	0.167, *0.116*	0.136, *0.134*	0.102, *0.065*
Sample 3 (9:41)	0.380, *0.796*	0.127, *0.096*	0.102, *0.079*	0.065, *0.034*
Sample 4 (12:07)	0.357, *0.721*	0.134, *0.091*	0.132, *0.109*	0.091, *0.059*
Sample 5 (13:55)	0.284, *0.477*	0.128, *0.143*	0.081, *0.071*	0.052, *0.031*
Boys				
Sample 1 (6:28)	0.327, *0.562*	0.261, *0.198*	0.286, *0.276*	0.160, *0.070*
Sample 2 (8:10)	0.312, *0.676*	0.118, *0.084*	0.153, *0.167*	0.082, *0.045*
Sample 3 (9:46)	0.234, *0.468*	0.098, *0.089*	0.098, *0.089*	0.060, *0.026*
Sample 4 (11:46)	0.266, *0.620*	0.120, *0.096*	0.113, *0.093*	0.098, *0.078*
Sample 5 (13:52)	0.280, *0.575*	0.092, *0.128*	0.081, *0.062*	0.078, *0.162*

Figure 1. Gender by Income to Needs across Time[2]



Note: Boy's cortisol is higher during the day than girl's cortisol. Girls from higher SES backgrounds, as measured by an income to needs ratio, had the steepest cortisol slopes, while girls from lower SES backgrounds had flatter diurnal cortisol slopes.

Figure 2. Acceptance by Gender on Quality of Social Experience



Note: Children who are more accepted are more physiologically aroused when they have positive peer experiences. This is particularly true of girls. Unaccepted girls have the lowest cortisol regardless of the valence of their peer experiences. Both unaccepted girls and boys have the lowest cortisol in response to positive peer experiences. This may indicate social withdrawal or a type of learned helplessness.

Bridging Studies

Results from Study 2 confirmed results from Study 1, in that children's cortisol levels are associated with SES and peer status. Specifically, it was observed that in early adolescents from Montreal, different measures of SES are differentially predictive of cortisol levels; it was observed that girls from lower income families have a flatter diurnal cortisol slope than girls from higher income families; and it was observed that early adolescents who were accepted had high cortisol secretion following positive social interactions, while early adolescents who were not accepted had low cortisol secretion, regardless of the quality of their peer interaction.

While these findings are strong contributions to the literature, they are not longitudinal. Additionally, while peer rejection and SES have been predictors of salivary cortisol, it remains unclear if other methods used to assess cortisol levels are equally impacted by these variables. Thus, the final study of this dissertation examines the hair cortisol of early adolescents and whether it can be predicted by SES and peer variables collected 3 months earlier. This study examines if children's hair cortisol is predicted by distinct measures of SES and by peer acceptance and rejection.

Socioeconomic status, peer acceptance, and peer rejection predict hair cortisol concentrations in early adolescents in Colombia

Leah Wright

Luz Stella Lopez

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Abstract

Socioeconomic status (SES) is known to have profound impacts across different levels of development. This study examines the association of multiple measures of SES, measures of social status, peer acceptance and peer rejection, and hair cortisol. A sample of 123 early adolescents (M_{age} = 12.40) in Colombia provided sociometric peer ratings, data on family SES, and hair cortisol. Multiple linear regressions were conducted in order to determine if sociometric measures and different measures of SES are predictive of cortisol. Income-to-needs ratios were predictive of cortisol, such that families with lower income have higher hair cortisol levels. Parental education moderated the association between peer acceptance and hair cortisol, and peer rejection and hair cortisol. Peer rejection and acceptance did not predict the hair cortisol of children with parents with high levels of education. However, low peer acceptance and high peer rejection were associated with low hair cortisol when parents had low levels of education. The findings from this study indicate that distinct measures of SES are differentially predictive of cortisol, and parental education is protective against social risk factors.

Introduction

Children exposed to greater levels of adversity, such as living in lower socioeconomic status environments, are at risk of adverse health effects (Oh et al., 2018). The stress hormone cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA)-axis, has been proposed as a pathway through which adversity impacts health outcomes due to its role in activating energy to maintain homeostasis in the face of stress (Koss & Gunnar, 2018). Children from lower SES backgrounds are at higher risk of adverse peer experiences, such as bullying/victimization (Arseneault, 2018). In studies of North American children, those who experience victimization have altered patterns of salivary cortisol secretion (Ouellet-Morin et al., 2011), as well as hair cortisol concentrations (HCC; Ouellet-Morin et al., 2020). Using a sample of early adolescents from an urban setting in Colombia, this study expands on this literature by examining if another adverse peer experience, specifically, high peer rejection and low peer acceptance, are predictive of HCC and if SES moderates that association.

Cortisol is secreted in a circadian rhythm as well as in response to stress. While cortisol is typically examined by studying its presence in saliva, recent research has established that, over time, secreted cortisol accumulates in ones' hair at the rate of approximately 1cm per month (Manenschijn et al., 2011; Stalder et al., 2017). HCC can be utilized to examine stable amounts of secreted cortisol (Bryson et al., 2019), and it is less likely to be impacted by time-varying confounders, something salivary cortisol is particularly vulnerable to. While some research has found there is a dose-response relationship between HCC and adversity (higher levels of stress lead to higher levels of HCC; Staufenbiel et al., 2013), other research has painted a more complex picture.

Similar to the research on salivary cortisol, findings have been mixed, such that systematic reviews and meta-analyses have found both heightened and reduced levels of HCC in the face of adversity (Khoury et al., 2019; Stalder et al., 2017). A possible explanation of these mixed findings is that in a well-functioning HPA-axis, when cortisol levels reach their optimal level, a negative feedback loop is triggered that results in the cessation of cortisol production (Sapolsky et al., 2000). However, in the face of chronic stressors, the initial activation of the HPA-axis does not appropriately trigger the negative feedback loop, which over the long-term leads to physiological changes to the HPA-axis that can include hypo-activation (Fries, 2010, Giulliams & Edwards, 2010). These physiological changes, which wear down the body's ability to maintain homeostasis due to stress exposure, have been described as "allostatic load" and the accumulation of allostatic load impacts the bodies response to stress (McEwen & Gianaros, 2011). The type and timing of the stressor experienced can alter the allostatic load accumulated (Ganzel et al., 2010). In relation to HCC, the type of adversity experienced is an important meta-analytic moderator in the direction of the association between HCC and adversity (Khoury, 2019). Thus, assessing types of adversity separately is critical to understanding the association between adversity and HCC.

SES is a multifaceted construct that is conceptualized as being comprised of different types of capital: social (e.g., social status), material (e.g., wealth), and human (e.g., education; Oakes & Rossi, 2003). A systematic review has found there is some evidence of a negative association between HCC and SES (Gray et al., 2018). Some studies have combined the different types of capital to calculate a cumulative measure of SES and found that lower SES predicted higher HCC levels (Bhopal et al., 2019). Others examined distinct levels of capital and found slightly varying results. Growing up with less educated parents has been associated with higher HCC (Merz et al., 2019; Tarullo et al., 2020; Ursache et al., 2017; Vaghri et al., 2013; Vliegenthart et al., 2016), lower HCC (Lu et al., 2018; White et al., 2017) or was unrelated to HCC (Gerber et al., 2017). Regarding family income, lower income was associated with higher HCC levels (Rippe et al., 2016), or no differences were detected (Merz et al., 2019).

Research on SES has typically focused on adversity within the family unit (Bukowski et al., 2020). However, as children age, the social sphere becomes increasingly relevant. Research has found that children from lower SES backgrounds have fewer friends, feel more socially isolated in class (Hjalmarsson & Mood, 2015), and are more likely to be rejected by their peers (Hjalmarsson, 2018). Rejection and social isolation are social stressors. Social stress is a powerful trigger of the HPA-axis in children and adults (Dickerson & Kemeny, 2004). Outside of the laboratory, the impact of social stress on cortisol has been rarely studied. The extant research has shown that peer problems effect diurnal salivary cortisol levels. Low peer acceptance was associated with higher total cortisol levels and less steep diurnal slopes (Behnsen et al., 2018), and children who reported more peer problems had flatter diurnal slopes the day of the peer problems and higher cortisol levels at awakening the day following the peer problems (Bai et al., 2017). Additionally, children who were accepted by their peers had higher diurnal cortisol in response to positive peer interactions, while children not accepted by their peers had lower cortisol levels, regardless of how positive or negative their peer interactions had been (Wright & Bukowski, 2021). The association between HCC and peer relationships has rarely been explored. One study found a curvilinear association, such that boys with moderate levels of victimization had the lowest cortisol, while boys with higher and lower levels of victimization had heightened HCC (Ouellet-Morin et al., 2020). Children who experienced a larger increase or decrease in victimization over childhood and adolescence had a decrease in HCC, while children with a moderate decrease in victimization had an increase in HCC. When there is no increasing peer victimization, youth with higher HCC had lower depressive symptoms (Ouellet-Morin et al., 2020). This study helps explain why both higher and lower levels of cortisol have been associated with adversity-- the social context in which a child has higher or lower cortisol is critically important. Thus, high cortisol on its own is not necessarily predictive of negative outcomes.

As demonstrated by the above study (Ouellet-Morin et al., 2020), gender differences have been found in relation to the association between adversity and HCC, specifically that males have higher HCC (Gray et al., 2018; Ouellet-Morin et al., 2020; Rippe et al., 2016). For example, male adolescents who experienced a person-related adversity, such as the loss of a relationship, had higher HCC, while girls did not (Van Dammen et al., 2020). It is also possible that girls who experience adversity have lower cortisol levels in response to stressors—for example, girls less accepted by their peers had the smallest cortisol activation to positive peer interactions (Wright & Bukowski, 2021). Gender differences in cortisol secretion may be explained by differences in coping mechanisms, stress sensitivity, or pubertal factors (Del Guidice et al., 2011; Shirtcliff et al., 2012). A recent meta-analysis did not find sex to be a statistically significant moderator (Khoury et al., 2019), yet most studies of HCC in children have not conducted gender-moderation analyses. Further studies of the impact of gender on HCC levels are needed.

Hypotheses

This study uses an innovative approach to study the association between HPA activity and experiences with peers. Working with a sample of early adolescents from middle class and lower middle-class neighbourhoods in a Caribbean city in Colombia, a multidimensional measure of SES and classroom-based sociometric measures were used to examine the additive and interactive effects of SES and peer acceptance and rejection on HPA activity as manifested in measures of HCC. This study addresses four questions: (a) are diverse measures of SES predictive of hair cortisol in early adolescence (b) are peer acceptance and peer rejection predictive of HCC; (c) does SES moderate the effect of peer variables on HCC; and (d) does gender further moderates these associations?

Methods

Participants and Procedures

The participants were 123 children (71 girls) from four, eighth-grade school classrooms in Barranquilla, Colombia. Permission for the study was initially obtained from the school administration and teachers. An active consent procedure was used to obtain parental permission for their children to participate in the study. Each child's verbal assent was obtained. All children were given a small reward for returning the parental consent form, regardless of their participation in the study. All children were from low-SES or middle-SES backgrounds. Participation rate was 86% in classroom A, 95% in classroom B, 79% in classroom C, and 75% in classroom D. From this sample, 70 children provided hair samples and 64 had their parents complete a parent questionnaire;

only children with both cortisol hair samples and the completed parent questionnaire were included in this study (58% female; $M_{age} = 12.40$, $SD = 1.96$). Children were given an SES questionnaire for their parents to complete. Children were given a small reward for returning this questionnaire. Data included in this study were collected at two different waves. Sociometric data and self- and peer-report data were collected at Time 1 (T1) and hair samples, body weight and height were collected at Time 2 (T2), approximately three months later. Data collection took place during the final three months of the school year.

Measures

Socioeconomic status. Each child was asked to have a parent fill out a questionnaire in which the parent indicated maternal and paternal income, family size, and maternal and paternal education. Income-to-needs ratios were calculated by dividing income by family size; thus, a higher income-to-needs ratio indicated a higher income relative to family size. Family education was calculated by combining both parent's education levels and calculating the mean. Subjective SES was assessed using Adler's Ladder, in which parents are presented with a "social ladder" and asked to place an "X" on the rung on which they feel they stand (Singh-Manoux et al., 2003).

Peer rejection and acceptance. Peer rejection and acceptance were assessed via peer nomination. Using tablet computers children were shown lists of their classmates and asked "How much do you like each person" on a scale of 1 (do not like the person) to 5 (like the person very much). The number of 1's and 5's each child received was then tallied and averaged to indicate the extent to which each child was rejected or accepted by their peer group. The greater the numbers of "1's" received, the more rejected the child was. The greater the number of "5's" the child received, the more accepted the child was. Thus, on both scales, a higher number indicates that the child was more rejected or more accepted. Class size was controlled using the method described in Velásquez et al., (2013).

Cortisol. Hair strands were carefully cut with fine scissors as close as possible to the scalp from a posterior vertex position. The number of strands obtained differed in accordance with the subject's permission to cut more, or less, hair. In the laboratory, the strands were lined up and cut into 3 cm segments.

The protocol of Davenport et al., (2006) was used for washing of hair and steroid extraction. In brief, each hair segment was immersed in 2.5 ml of isopropanol in a 10 ml glass tube, which was then gently mixed on an overhead rotator for three minutes. After decanting, the wash cycle was repeated two times before drying the hair samples for at least 12 hours.

Next, 7.5 mg of hair segments were transferred into a 2 ml cryovial, 1.5 ml of pure methanol was added and steroid extraction was performed for 18 hours. Samples were then spun in a microcentrifuge at 10.000 rpm for 2 min, after which 1 ml of the clear supernatant was transferred into a new 2 ml glass vial. The alcohol was evaporated at 50 degrees Celsius under a constant stream of nitrogen until the samples were completely dried. Finally, 0.4 ml of water was added and the tube vortexed for 15 sec. Fifty microliters were removed from the vial and used for cortisol determination with a commercially available immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Germany). The intraassay and interassay coefficient of variance of this assay is below 8%.

Data Analysis

Prior to conducting statistical analyses, data were screened to assess the normality of each variable's distribution. Outliers were identified and converted to the next most extreme value in the dataset that was not an outlier (Tabachnik & Fidell, 2007). After correcting for outliers, none of the variables showed any significant kurtosis or skewness, save for cortisol. Hair cortisol samples that were 3SD above or below the mean were considered outliers and were windsorized; one sample was adjusted. Cortisol data had significant skew (statistic= 5.172, standard error = 2.99) and kurtosis (statistic = 33.39, standard error = 0.59), thus a Log10 transformation was utilized to normalize the distribution (skew statistic = -.643, SE = .299; kurtosis statistic = 1.226, SE = .590). Descriptive statistics are reported in Table 1. Correlations of relevant variables are reported in Table 2.

Hierarchical multiple regressions were tested to examine the relationship between SES, peer acceptance and rejection, and hair cortisol. Given the recent literature, both body mass index and pubertal status were examined as control variables, however, only puberty was at times a significant predictor. Therefore, in order to preserve degrees of freedom, the final analyses are presented with only puberty as a control variable. Control variables were entered in Step 1. In Step 2, predictor variables were entered (i.e., SES variables, peer rejection). In Step 3, interaction variables were entered (i.e., SES variables multiplied by peer rejection). In Step 4, the interaction variables were examined by gender. The outcome variable in each analysis was child HCC. Results for significant interaction models are shown in Tables 3 and 4.

Results

Family Education

These analyses examined how peer rejection and family education predicted HCC. The dependent variable (DV) was child HCC, the predictor variables were family education and gender. In the regression model predicting rejection, family education, and a rejection by family education interaction, at Step 2, none of the predictor variables were significant. However, at Step 3, gender ($\beta = .264$, $t = 2.155$, $p = 0.036$), rejection ($\beta = -1.453$, $t = -4.358$, $p < 0.001$), family education ($\beta = -.539$, $t = -3.157$, $p = 0.003$), and rejection by family education ($\beta = 1.413$, $t = 3.945$, $p <0.001$) were significant (Figure 1). In Step 4, a rejection by family education by gender interaction was examined and it was not statistically significant ($p = 0.775$). Regarding the simple slopes, the cortisol of children from low family education backgrounds was significantly predicted by rejection ($p < 0.001$), while the cortisol of children from high family education backgrounds was not ($p = 0.947$).

These analyses examined how acceptance and family education predicted HCC. In the regression model, the DV was children's HCC and the predictor variables were acceptance, family education and gender. At Step 2, none of the predictor variables were significant. However, in Step 3, acceptance ($\beta = 1.490$, $t = 3.771$, $p < 0.001$), family education ($\beta = .834$, $t = 2.906$, $p =0.005$), and acceptance by family education ($\beta = -1.648$, $t = -3.513$, $p =0.001$) were significant (Figure 2). Gender was borderline significant ($p =0.076$). In Step 4, an acceptance by family education by gender interaction was examined, and it was not statistically significant ($p = .283$). Regarding the simple slopes, the cortisol of children from low family education backgrounds was significantly predicted by acceptance ($p < 0.001$), while the cortisol of children from high family education was not ($p = 0.119$).

Income-to-needs

The next set of analyses examined the how income-to-needs and rejection predicted HCC. In the regression model, the DV was HCC, and the predictor variables were rejection, income-to-needs, and gender. At Step 2, income-to-needs was a significant predictor of HCC ($\beta = -.309$, $t = -2.652$, $p = 0.010$). At Step 3, gender ($p = 0.074$), rejection ($p = 0.106$), income-to-needs ($p = 0.307$), and a rejection by income to needs interaction ($p = 0.298$) were not statistically significant. The income-to-needs by rejection by gender interaction was not assessed.

The next set of analyses examined how acceptance and income-to-needs predicted HCC. In the regression model, the DV was HCC and the predictor variables were acceptance, income-to-needs, and gender. At Step 2, income to needs was a significant predictor of HCC ($\beta = -.319$, $t = -2.736$, $p = 0.008$). At Step 3, while income-to-needs remained significant ($p = 0.038$), an income-to-needs by acceptance interaction was not statistically significant ($p = 0.298$). The income-to-needs by acceptance by gender interaction was not assessed.

Subjective socioeconomic status

The next set of analyses examined how rejection and subjective SES predicted HCC. In the regression model, the DV was HCC and the predictor variables were rejection, subjective SES, and gender. At Step 2, gender ($p = 0.080$), rejection ($p = 0.098$), and subjective SES ($p = 0.805$) were not statistically significant. At Step 3, gender ($p = 0.096$), rejection ($p = 0.661$), subjective SES ($p = 0.815$) and a rejection by subjective SES interaction ($p = 0.924$) were not statistically significant.

The final set of analyses examined how acceptance and subjective SES predicted HCC. In the regression model, the DV was HCC and the predictor variables were acceptance, subjective SES, and gender. At Step 2, gender (p = 0.120), acceptance (p =0.133), and subjective SES (p =0.713) were not statistically significant. At Step 3, gender (p =0.079), acceptance (p =0.525), subjective SES (p =0.291) and an acceptance by subjective SES interaction (p =0.321) were not statistically significant.

Discussion

This study examined how SES and social context predict HCC in a group of Colombian early adolescent school children over a 3-month period. Classroom based measures of peer rejection and acceptance were utilized and SES was measured; then, 3-months later, hair cortisol was assessed. Results indicated that parental education was protective such that the cortisol of children whose parents had more education did not vary in relation to peer acceptance or rejection. However, children whose parents had lower levels of education had cortisol levels that varied as a function of peer acceptance and rejection. Additionally, independent from social status, income-to-needs ratios were predictive of HCC such that children from lower income families had higher HCC.

The most compelling results from this study are that rejection and acceptance are predictive of HCC when this effect is moderated by parental education. The social status of children from families with higher levels of parental education is not a predictor of their cortisol levels. However, when children are from families with less parental education, social status is an important predictor of cumulative cortisol levels, such that children who are rejected or not accepted have low levels of HCC, while children who are accepted or not rejected have high levels of HCC. This appears to indicate that children from higher SES households may be protected from the emotional distress caused by peer rejection or a lack of acceptance, while children from lower SES backgrounds are more vulnerable to the downstream physiological effects of social problems.

But the question remains, what does this higher cortisol mean in the face of the objectively positive contexts of low rejection and high acceptance? While the classic conceptualization of cortisol posits that higher amounts of cortisol is indicative of higher levels of stress, the association between stress and cortisol is variable (Hellhammer et al., 2009). Some research has supported that theory and found higher cortisol levels to be associated with stress (Lupien et al., 2001), while other research has found lower cortisol levels to be associated with stress, resulting in what is called hyposecretion, or a "blunting" or "suppression" of the stress response due to compensation of the HPA-axis following over-activation (Badanes et al., 2011). To further complicate matters, other research has found elevations of cortisol in relation to positive stressors, not only negative stressors (Het et al., 2012; Hoyt et al., 2016). When cortisol was examined in response to positive or negative social interactions in a sample of Canadian pre-adolescents, similar results were found; children who were more accepted had higher cortisol in response to positive peer interactions (Wright & Bukowski, 2021). Another study examined victimized and unvictimized children and found that when boys were unvictimized, higher levels of HCC was protective against the development of depressive symptoms (Ouellet-Morin et al., 2020). While these authors hypothesized that higher levels of HCC may undermine healthy development in highly vulnerable individuals, they were examining higher cortisol levels in response to negative stimuli. The current study found higher cortisol levels in response to positive stimuli in vulnerable children, which may indicate that higher cortisol in vulnerable children can be protective, depending on the context.

The biological sensitivity to context theory may explain these seemingly contradictory findings (Ellis & Boyce, 2008). This theory suggests that stressful childhood environments lead to an increase in biological sensitivity, thereby increasing an individual's ability to detect and respond to environmental threats. When one's environment is not extreme, one becomes less biologically sensitive to their environments. Therefore, children whose parents have higher levels of education are likely less exposed to early life adversity and their HPA-axes are therefore less responsive to stressful social interactions in their environments. On the other hand, children who have been exposed to more adversity have an increased biological sensitivity to their environments. Therefore, children whose parents have lower levels of education may hyposecrete cortisol in response to the additional stress of peer rejection and their cortisol may be heightened in response to the positive stress of high peer acceptance or low peer rejection. The seemingly contradictory findings between the association of income-to-needs ratios with higher cortisol and the association of parental education with lower cortisol may be attributable to different levels of stability, and therefore chronicity, of these stressors. It is possible that income-to-needs ratios vary more during a child's life as siblings are born and parents change jobs, while educational levels likely remain fairly stable given the many barriers that may prevent parents from returning to school (Hovdhaugen & Opheim, 2018); thus these differentiating results may be a factor of the difference between more acute vs. more chronic stressors (G. E. Miller et al., 2007). While laboratory studies have found that acute peer rejection is associated with increased levels of salivary cortisol (Blackhart et al., 2007), acute rejection may be distinct from chronic rejection as measured in this study, in that chronic stressors have been found to lead to the hyposecretion of cortisol (Fries et al., 2005; Heim et al., 2000).

While the vast majority of research has examined how higher levels of cortisol are associated with negative outcomes, some studies have examined cortisol levels in response to positive stimuli/stressors. Within-person increases in cortisol secretion were associated with subsequent rises in positive features (activeness, alertness, relaxedness) and lower levels of stress, indicating that increases in cortisol may help regulate mood and affect (Hoyt et al., 2016). Another study found that administering cortisol to a study group was associated with lower levels of negative affect following a stress task (Het & Wolf, 2007). Thus, higher levels of cortisol have been associated with positive mood and affect—this may explain why children from low-SES backgrounds who are accepted have significantly higher levels of cortisol—biologically, they may have been programmed to be more reactive and they are responding to the positive stress of acceptance.

Partially replicating an earlier study (Wright & Bukowski, 2021), children from families with low parental education who are rejected or not accepted have the lowest levels of HCC, which may be an indication of such chronic stress that their HPA-axes are hyposecreting cortisol. These rejected, lower SES children may be at higher risk of developing depression. Hypocortisolism has been posited to be a biomarker of internalizing disorders in children (Badanes et al., 2011). In fact, youth with low cortisol and more stress had elevations in depressive symptoms (Badanes et al., 2011).

Additionally, lower HCC in boys was associated with higher depressive symptoms in the absence of peer victimization (Ouellet-Morin et al., 2020). The fact that gender was also a significant predictor in these models indicates that one gender, likely girls, may be more vulnerable to the negative effects of interpersonal stressors, as has been found in the literature (Hankin et al., 2007; Rudolph, 2002).

Additionally, while multiple measures of SES were utilized, parental education was the only pertinent measure of SES in combination with social factors. This may be because education levels are a type of social capital (Oakes & Rossi, 2003), and we examined how social capital interacts with a social stressor to predict cortisol. Lower income-to-needs ratios, a type of material capital, were predictive of higher cortisol levels, independent of social factors. Thus, this study further emphasizes the importance of utilizing multiple measures of SES to assess the impacts of low-SES environments.

Limitations

While this study has multiple strengths, including a longitudinal design, the use of sociometric classroom-based measures, a sample from outside of North America, and novel research questions, there are also some limitations. The sample size in this study was small. Given that no other studies have examined peer acceptance or rejection in relation to HCC, this study was a preliminary exploration designed to explore if these variables were related. Larger sample sizes may increase power and enable researchers to further investigate gender differences. Additionally, data on employment stability and educational stability was not collected, therefore we could not explore if stability of SES impacted the direction of our findings. Future studies with larger sample sizes are necessary to confirm and further develop the theory underlying the relationship between HCC and peer variables.

Conclusion

This study found that the physiology of children whose parents have lower levels of education is effected by negative peer variables, such that they might be more vulnerable to the development of internalizing disorders. While further study is needed, these findings are relevant to intervention as children from families with lower education levels may benefit more from the implementation of social supports at school.

Table 1. Descriptive statistics

Variable name	Mean	Standard Deviation
HCC	.7003	.31393
BMI	19.5245	3.30690
Puberty	1.7031	.30928
Rejection	2.0868	1.89498
Acceptance	7.9504	4.33814
Income to needs ratio	-.0071	.36252
Perceived Stress Scale	3.3500	.89354
Adler’s Ladder	6.0469	1.75869
Family education	2.0789	.74576

Table 2. Bivariate Correlations between primary variables of interest

Variable	1	2	3	4	5	6	7	8	9	10
1. HCC	1	.176	-.174	-.157	.172	-.045	.059	.184	-.136	-.312*
2. Gender		1	.149	.000	-.070	.150	.002	.044	-.215	-.030
3. Rejection			1	-.599**	.007	-.041	.073	.417**	.031	.019
4. Acceptance				1	-.134	.179	-.039	-.351**	-.010	.025
5. Puberty					1	.305*	.012	.303*	-.154	.035
6. AL						1	.009	-.167	.088	.336**
7. PSS							1	.143	.153	.040
8. BMI								1	-.091	-.215
9. Family Ed									1	.161
10. Income: need										1

Note. HCC = Hair cortisol concentration; AL = Adler's Ladder; PSS = Perceived stress scale; BMI = Body Mass Index; Family Ed = Family Education; Income: need = Income to needs ratio
**$p < 0.01$.
*$p < 0.05$.

Table 3. Rejection interacts with family education to predict HCC

Variable	**B**	**β**	***t***
Model 1			
Puberty	0.048	0.150	1.125
Model 2			
Puberty	0.056	0.174	1.213
Gender	0.124	0.193	1.412
Family Education	-0.020	-0.063	-0.460
Rejection	-0.068	-0.220	-1.674
Model 3			
Puberty	0.132	0.413	2.562**
Gender	0.169	0.264	2.155*
Family education	-0.171	-0.539	-3.157**
Rejection	-0.451	-1/453	-4.358***
Rejection*Family Education	0.448	1.413	3.945***

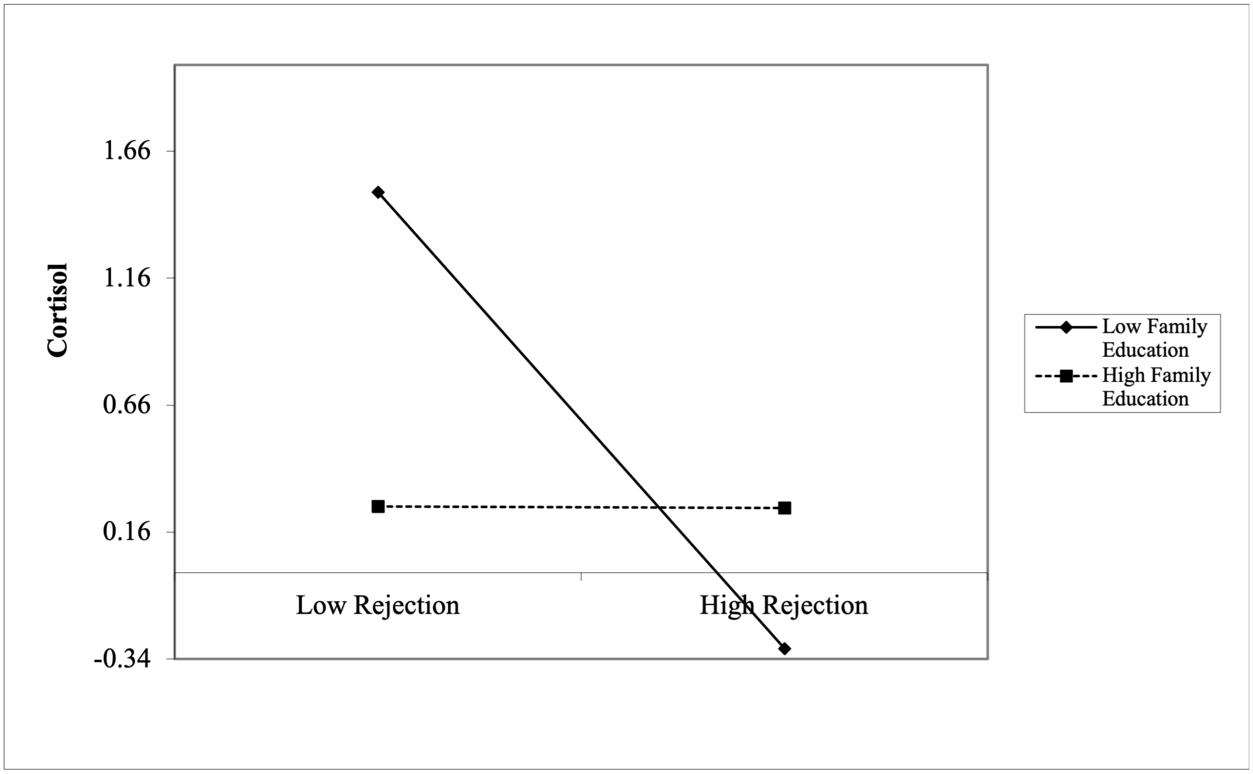
Model 2: $R^2 = 0.088$, F Change = 1.712, $p = 0.176$
Model 3: $R^2 = 0.208$, F Change = 15.567, $p < 0.001$
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 4. Acceptance interacts with family education to predict HCC

Variable	**B**	**β**	***t***
Model 1			
Puberty	0.048	0.150	1.125
Model 2			
Puberty	0.058	0.182	1.327
Gender	0.100	0.155	1.123
Family Education	-0.023	-0.073	-0.532
Acceptance	0.054	0.169	1.263
Model 3			
Puberty	0.061	0.191	1.537
Gender	0.148	0.231	1.813
Family education	0.265	0.834	2.906**
Acceptance	0.473	1.490	3.771***
Acceptance*Family Education	-0.523	-1.648	-3.513**

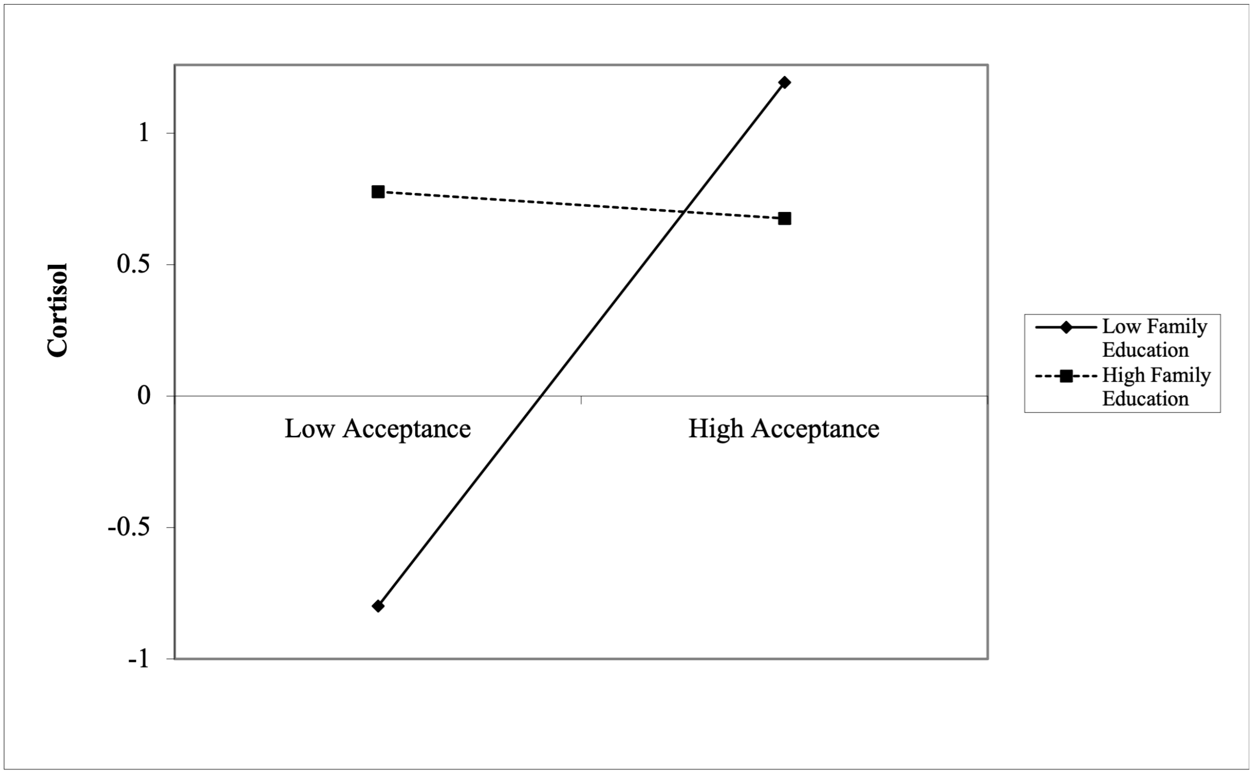
Model 2: $R^2 = 0.068$, F Change = 1.293, $p = 0.287$
Model 3: $R^2 = 0.177$, F Change = 12.338, $p = 0.001$
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure 1. Rejection by family education predicts HCC



Note. Family education moderates the effect of rejection on children's HCC.

Figure 2. Acceptance by family education predicts HCC



Note. Acceptance predicts cortisol in children from families with lower levels of education.

General Discussion

The three studies in this dissertation were designed to examine the degree to which the association between socioeconomic status (SES) and the functioning of the HPA-axis is moderated by other social risk factors. These studies examined risk at different levels of social complexity and how these different types of risk functioned together in an additive and interactive way to affect the secretion of cortisol by the HPA-axis. Together, these studies show that the functioning of the HPA-axis needs to be assessed according to the effects of multiple forms of social risk.

Summary of findings

Study 1 used a two-wave longitudinal design to examine three forms of change. It assessed whether recorded decreases in cortisol levels during a three-hour school day varied from the beginning of the school year to the end and whether this change fluctuated as a function of SES. Peer rejection and SES, as measured by neighbourhood census data, were examined. At the beginning of the school year, all children had heightened levels of salivary cortisol, indicating increased arousal in response to this new social environment. Ten months later, peer rejection and SES independently, but not additively, predicted cortisol levels. Children rejected by their peers, regardless of their SES, had flatter cortisol slopes in the morning. Children from lower SES backgrounds had higher cortisol levels than their peers from higher SES backgrounds. This indicates that children raised in less materially wealthy homes, as measured by neighbourhood wealth, found school more arousing at the end of the school year. This study concluded that children as young as 5 years of age are differentially impacted by these distinct levels of their social environments. The next study expanded on these findings by improving the measurement of SES and cortisol in a group of early adolescents in Montreal.

Using a more nuanced approach, Study 2 (Wright & Bukowski, 2021) examined the diurnal cortisol slope across the day, the cortisol response to peer experiences, multiple measures of SES and peer rejection and acceptance. Study 2 found that early adolescents from families with less parental education had higher cortisol at awakening than early adolescents from families with more parental education, indicating higher levels of arousal in response to the school day. The study also found that girls from families with higher incomes had what appeared to be a healthy diurnal slope, while girls from families with lower incomes had a flatter diurnal slope, a finding which may be indicative of worse health outcomes later in life (Adam et al., 2017). In addition, early adolescents who were accepted by their peers experienced greater arousal and higher cortisol levels in response to positive peer interactions, while children not accepted by their peers exhibited less arousal in response to positive or negative peer interactions. This finding was especially true of girls. Study results also revealed that different measures of SES based in human capital and material capital predicted cortisol in different ways. SES did not moderate arousal in response to the quality of peer interactions, and peer acceptance and rejection were not predictive of the diurnal cortisol slope, indicating that these different types of risks elicit different cortisol responses in early adolescents. Study 2 posited that girls who are not accepted may be experiencing learned helplessness, and thus may be more vulnerable to the development of depression.

The final study of this dissertation made methodological improvements by examining the longitudinal and cumulative impact of SES and peer variables on the acquisition of cortisol in hair (hair cortisol concentrations; HCC). Returning to Colombia, Study 3 used HCC to measure cumulative cortisol secretion over a three-month period. Our research found that the ability of SES to predict HCC differed on the type of capital measured; being lower in material capital (i.e., coming from a lower income family) predicted higher HCC, while being lower in human capital (i.e., having lower parental education levels) only predicted HCC in combination with social variables (acceptance and rejection). This study found that peer variables were not predictive of HCC in early adolescents raised in families with higher parental education levels. But, if parents had lower levels of education, peer acceptance and rejection were important predictors of HCC, such that children rejected or not accepted had low levels of HCC, while children who were accepted or not rejected had high levels of HCC. These results indicate that higher levels of parental education protect children from being vulnerable to the negative effects of social risk. Taken together, these studies found that 1) the measurement of SES is critically important to understand its effects on development; 2) peer acceptance and rejection are differentially predictive of the HPA-axis; 3) peer difficulties are associated with blunted cortisol levels; and 4) high cortisol levels can be a sign of positive arousal. These issues will be discussed below.

SES measurement

As has been discussed extensively throughout this dissertation, SES is a complex, multifaceted construct. The literature examining the association between SES and cortisol has largely focused on types of material capital (e.g., income, neighbourhood disadvantage) and found that lower SES is associated with higher cortisol (morning cortisol; Lupien et al., 2001; reactivity, Blair et al., 2005). This study confirms those findings—in all three studies, lower levels of material capital was associated with higher cortisol—kindergartners had higher cortisol at the end of the school year; diurnal cortisol did not decrease as much in early adolescents; and children from low-income families had higher HCC. However, the association between human capital (e.g., education levels) and cortisol was different. Early adolescents had higher cortisol at awakening if their parents had less education, but it was not predictive of cortisol throughout the rest of the day. When cortisol was looked at cumulatively and longitudinally, children whose parents had less education were more vulnerable to the negative and positive effects of social factors. Independent of employment, mothers with higher education spend more time with their children (Guryan et al., 2008) and therefore children have more time to learn social skills from their parents. Conversely, children whose parents have lower levels of education tend to display more emotional problems (Hoglund & Leadbeater, 2004). One explanation for this may be that lower parental education has been associated with reduced executive functioning (Conway et al., 2018), a critical set of skills for emotion regulation (Zelazo & Cunningham, 2007). Thus, children living with parents with lower levels of education may be less prepared to handle social adversity and therefore become more reactive as they experience frequent social stressors that eventually leads to the downregulation of the HPA-axis and blunted cortisol levels.

Peer acceptance and rejection are distinct

An interesting aspect of these studies is that acceptance and rejection were differentially associated with levels of cortisol. In kindergarteners in Colombia only rejection was predictive of cortisol levels recorded at the end of the school year. In early adolescents in Montreal, only acceptance was predictive of children's cortisol response to peer experiences. In early adolescents in Colombia, both rejection and acceptance were predictive of HCC in the context of SES. These similar yet different associations between peer variables and cortisol may be explained by the association between acceptance and rejection. Acceptance and rejection are not opposites of one another. Acceptance is akin to being liked, and rejection is akin to being disliked. Liking is not the opposite of disliking (Bukowski et al., 2000). Thus, the findings from this study further support Bukowski's (2000) assertion that acceptance and rejection are dimensional variables that must be examined as such.

Peer difficulties and cortisol blunting

A consistent finding across studies is that social rejection or low acceptance predicted lower cortisol, either in the form of a blunted cortisol slope in kindergartners, blunted cortisol response to positive peer interactions, or blunted HCC when parents were of low education backgrounds. Social stressors are some of the most powerful predictors of cortisol responses (Dickerson & Kemeny, 2004), and it is difficult to conceive of a social stressor more challenging than rejection. Both peer rejection and acceptance are stable over time (Hardy et al., 2002), thus peer rejection and low acceptance can be conceptualized as chronic stressors that require frequent response from the HPA-axis (Murray-Close, 2013). However, frequent activation of the HPA-axis can be damaging to the body, thus the HPA-axis begins to downregulate, which leads to the blunting of cortisol responses (Fries et al., 2005; Koss & Gunnar, 2018). In the context of this dissertation, children rejected or low in acceptance by their peers are vulnerable, and their cortisol blunting may indicate they are detached from their social environments.

High cortisol and positive arousal

While cortisol is colloquially known as a “stress hormone”, the studies in this dissertation demonstrated that higher levels of cortisol do not necessarily mean higher levels of stress. In fact, it is possible that higher cortisol levels are protective and improve moods. Studies found that after receiving cortisol, participants reported more positive moods compared to controls (Het & Wolf, 2007). In addition, increased reactivity to laboratory stressors was associated with less negative affect (Het et al., 2012; Kazén et al., 2012). Cortisol predicted participants feeling alert and relaxed one hour later (Hoyt et al., 2016). However, positive events during the day were associated with marginally lower area-under-the-curve (AUC) cortisol levels (Sin et al., 2017). The studies from this dissertation contribute to this body of work. Early adolescents accepted by their peers had elevated cortisol in response to positive peer interactions. Early adolescents whose parents had low levels of education and who were accepted or low in rejection had higher hair cortisol levels. Perhaps, as the biological sensitivity to context (BSC) theory posits (Boyce & Ellis, 2005), children from low parental education backgrounds are “orchids”; they are more sensitive to their social environments, and more able to benefit from positive social contexts, yet they suffer more when in negative social contexts. Children with parents of higher levels of education are “dandelions”; they are less sensitive to both the positive and negative effects of their social environments.

Theoretical contributions

As demonstrated by this dissertation, children are differentially susceptible to different risks. In Study 1, non-rejected children developed a healthier morning cortisol slope; in Study 2, higher SES girls had a steep diurnal cortisol slope, while accepted girls had steep elevations in cortisol in relation to their positive peer experiences; in Study 3, children with parents with more education had HCC levels that were unaffected by social stressors. Other children were more sensitive to the stressors experienced, perhaps because of the combination of stressors—e.g., being a girl and having a lower-income family, or being rejected and having parents with less education. These differentially susceptible children fit into the "biological sensitivity to context" theory (Boyce & Ellis, 2005), which posits that evolution has led some individuals to develop highly reactive phenotypes which can be advantageous or disadvantageous depending on the context. In Study 2, girls were more sensitive to their social environments than boys. In Study 3, early adolescents whose parents had less education were more sensitive to their social environments than children whose parents were more educated. Vulnerability, or sensitivity, may be impacted by gender, by parental education level, or both, which can be used to inform future research and intervention planning.

Limitations and future directions

This dissertation examined the same question using three distinct but related methodologies to generate a nuanced view of the complex interrelationship between diurnal, reactive, and hair cortisol, peer relationships, and SES. While this dissertation further developed its methodology with each study, there were methodological limitations throughout. Study 1 collected two cortisol samples at the beginning and end of the school day, approximately 3 hours apart. Thus, these findings must be interpreted with caution. Study 2 was cross-sectional; thus, a longitudinal follow-up study would be beneficial to confirm the results. Study 3 had a small sample size; thus, the generalizability of findings is limited.

While this research answered many questions, it left many unanswered. The role of friendship was not explicitly examined. Literature has shown that having friends can buffer cortisol responses to stress (Adams et al., 2011) and examining the stress-buffering effect of positive peer relationships will be critical to further developing this nascent field (Gunnar, 2017). Additionally, further exploration of the association between cortisol secretion and positive events would help clarify exactly when arousal becomes stress and how to differentiate the two. From a theoretical perspective, the application of a theoretical framework that incorporates multiple levels of social complexity, such as Bronfenbrenner's model of bioecological development, would serve to further the field. Finally, from a physiological perspective, studying how cortisol varies in relation to other biomarkers (e.g., heart rate variability, inflammatory markers) would further contribute to our understanding of how SES and peer variables impact psychophysiological development.

Practical implications

While the findings of this dissertation must be interpreted with caution given the preliminary nature of the studies and their limitations, they can inform future research with the goal of eventually informing future intervention. What is clear is that rejection and low acceptance are risk factors, and their impact can be seen in the development of the HPA-axis. This research indicates that young children rejected by their peers, early adolescent girls rejected or not accepted, and early adolescents with parents with low education levels who are rejected or not accepted may benefit the most from intervention. Based on this research schools could collect SES data on their students to determine which students are most vulnerable to peer difficulties. Both SES and peer difficulties are important predictors in a child's social success at school, and a critical component of their healthy development. With more research, appropriate interventions could be planned to improve the lives of these at-risk children.

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