

UCLA

UCLA Electronic Theses and Dissertations

Title

Children's Diurnal HPA-axis Activity: Assessment of Temporal Stability and Associations with Parent-child Relationship Qualities and Stress-induced HPA-axis Reactivity and Recovery

Permalink

<https://escholarship.org/uc/item/6057x9ns>

Author

Dickenson, Leah L.

Publication Date

2015

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Children's Diurnal HPA-axis Activity:
Assessment of Temporal Stability and Associations with Parent-child Relationship
Qualities and Stress-induced HPA-axis Reactivity and Recovery

A dissertation submitted in partial satisfaction
of the requirements for the degree
Doctor of Philosophy in Psychology

by

Leah L. Dickenson

2015

ABSTRACT OF THE DISSERTATION

Children's Diurnal HPA-Axis Activity:
Assessment of Temporal Stability and Associations with Parent-Child Relationship
Qualities and Stress-Induced HPA-Axis Reactivity and Recovery

by

Leah L. Dickenson

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2015

Professor Rena L. Repetti, Co-Chair

Professor Theodore Francisco Robles, Co-Chair

While a robust literature links childhood exposure to stressful family environments, hypothalamic pituitary adrenal axis (HPA-axis) functioning, and mental and physical health outcomes, there is a paucity of research examining associations between parent-child relationship qualities and HPA-axis activity during middle childhood and adolescence. In addition, despite assumptions implicit in the allostatic load literature, little is known about the temporal stability of children's diurnal cortisol and how children's cortisol responses to acute stressors are concurrently related to their diurnal cortisol profiles. To address these gaps in the literature, two studies using multi-method, repeated-measures designs were carried out in an ethnically diverse community sample of 47 children aged 8 to 13. Study 1 examined naturalistic associations

between two dimensions of the parent-child relationship (parent-child attachment and daily parent-child conflict) and two indicators of diurnal HPA-axis activity (children's diurnal cortisol slopes and end-of-day cortisol levels) at the between- and within-person levels of analysis. Children's reports of secure attachment to their fathers moderated the association between fathers' reports of daily father-child conflict and children's daily bedtime cortisol levels: Children who reported lower levels of secure attachment to their fathers had higher bedtime cortisol levels on higher conflict days compared to children who reported higher levels of attachment to fathers. Children's reports of secure attachment to their mothers predicted their diurnal cortisol slopes such that higher levels of secure attachment were associated with flatter slopes. Study 2 used multilevel-model derived intraclass correlation coefficients (*ICCs*) to assess short-term temporal stability in four metrics of diurnal cortisol -- waking cortisol levels, the cortisol awakening response (*CAR*), diurnal slope, and bedtime cortisol levels -- over a range of 2 to 8 sample days. Associations between each diurnal cortisol metric and children's reactivity to and recovery from the Trier Social Stress Task for Children (*TSST-C*) were also explored. Overall, children's diurnal cortisol metrics were moderately stable, with highest stability estimates observed in bedtime cortisol levels and lowest estimates observed in the *CAR*. Overall, increasing the number of sample days did not improve stability. Better cortisol recovery from the *TSST-C* was significantly correlated with higher waking cortisol levels, while cortisol reactivity to the laboratory tasks was not associated with any of the diurnal cortisol variables.

The dissertation of Leah L. Dickenson is approved.

Andrew J. Fuligni

Teresa Ellen Seeman

Rena L. Repetti, Committee Co-Chair

Theodore Francisco Robles, Committee Co-Chair

University of California, Los Angeles

2015

DEDICATION

I am incredibly grateful for the support, guidance, and inspiration I have received from so many people over the course of my graduate career: My dedicated advisor and mentor, Dr. Rena Repetti, whose passion and enthusiasm for science are contagious, and who invests so much in guiding her students and fostering their growth and development; I am truly grateful for all you have taught me and honored to have been your student. My dissertation co-chair, Dr. Ted Robles, for all his support, guidance, and mentorship over the years, and for including me in the larger study upon which this dissertation is based, from its inception. It was a privilege to work with two such talented, knowledgeable, and inspirational mentors over the course of my graduate career. My devoted husband, who has walked alongside me during this journey, and who has shown his support in countless ways -- I cannot find the words to thank you for your love, support, devotion, and strength over the years. My beautiful son, Gabriel, who inspires and teaches me every day, and who reminds me to live in the moment and cherish the important things in life. My family, to whom I owe a debt of gratitude for all the ways they have shown their support, especially since having a family of my own; I could not be more proud and grateful to have had you in my corner. And, finally, I am grateful for all of the faculty members, supervisors, and staff who have shared their guidance and support over the years, and thankful for the amazing labmates, classmates, and friends with whom I had the privilege of sharing this journey.

TABLE OF CONTENTS

Acknowledgments.....	vii
Introduction.....	1
STUDY 1: Associations Between Children’s HPA-Axis Activity and Parent-Child Relationships during Middle Childhood and Early Adolescence.....	4
Introduction.....	6
Methods.....	15
Results.....	22
Discussion.....	27
Figures & Tables.....	35
Appendix A	40
References.....	44
STUDY 2: Stability in Children’s Diurnal Cortisol and Connections with Cortisol Reactivity to and Recovery from a Laboratory Stressor.....	52
Introduction.....	54
Methods.....	64
Results.....	69
Discussion.....	74
Figures & Tables.....	86
Appendix B.....	91
References.....	96

ACKNOWLEDGMENTS

Ms. Dickenson's work on this dissertation was supported by a fellowship through The National Science Foundation (NSF) Graduate Research Fellowship Program and a Eugene V. Cota-Robles Fellowship. Any opinions, findings, conclusions and/or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation or the Eugene V. Cota-Robles fellowship program. Data used in both studies of this dissertation were collected as part of the UCLA Families and Health Study (Principal Investigators: Theodore Robles and Rena Repetti), which was supported by a generous Research Grant (9333) from the William T. Grant Foundation. The author would like to thank Rena Repetti and Theodore Robles for their guidance and thoughtful comments on earlier drafts of this dissertation, as well as their contributions toward co-authorship of the manuscripts in preparation connected with the studies making up this dissertation.

VITA

Education

C. Phil in Clinical Psychology, University of California, Los Angeles	2011
M.A. in Clinical Psychology, University of California, Los Angeles	2008
B.A. with Honors in Cognitive Science, <i>Summa Cum Laude</i> , University of California, Irvine	2007
B.A. in Psychology & Social Behavior, <i>Summa Cum Laude</i> , University of California, Irvine	2007

Selected Honors and Awards

Graduate Research Mentorship, UCLA	2013 - 2014
Graduate Research Fellowship, National Science Foundation	2008 - 2011
Graduate Summer Research Mentorship Fellowship, UCLA	2008
Eugene V. Cota-Robles Graduate Fellowship, UCLA	2007 - 2008
Phi Beta Kappa Honors Society	2007
Order of Merit, UC, Irvine	2007
Undergraduate Research Opportunities Program Grant, UC, Irvine	2006
NIH-funded Interdisciplinary Summer Undergraduate Research Experience, UC, Irvine	2005

Selected Clinical and Research Experience

Predoctoral Psychology Intern , The Guidance Center	2014 - 2015
Psychotherapy Supervisor , UCLA Psychology Clinic	2011 - 2012
Advanced Student Therapist , UCLA Psychology Clinic	2011 - 2012
Research Assistant , UCLA Relationships and Health Lab	2008 - 2012
Psychology Extern , Ventura County Behavioral Health	2010 - 2011
Intake Team Supervisor , UCLA Psychology Clinic	2010
Psychology Extern , Harbor-UCLA Medical Center	2009 - 2010
Statistical Consultant , Los Angeles, CA	2009
Psychology Extern , UCLA TIES for Families	2008 - 2009

Therapist , UCLA Psychology Clinic	2008 - 2009
Psychotherapy Intake Interviewer , UCLA Psychology Clinic	2008 - 2009
Psychological Assessor , UCLA Psychology Clinic	2008

Selected Papers and Presentations

Dickenson, L.L. & Repetti, R.L. (2015). Home is a haven: Children’s HPA-Axis activity following a stressful day at school. Manuscript in preparation.

Dickenson, L.L., Repetti, R.L. and Robles, T.F. (2012). *Methodological Considerations in the Inclusion of Naturalistic Biological Measures in Family Research.* Paper presented at the May 2012 UCLA Developmental Psychology Brown Bag, Los Angeles, CA

Dickenson, L.L., Repetti, R.L. and Robles, T.F. (2012). *The Inclusion of Naturalistic Biological Measures in Family Research: Implications and Methodological Considerations.* Paper presented in the symposium “Practical and Conceptual Issues in Naturalistic Research on Families: Integrating Biological, Observational, and Diary Methods” at the 2012 Society for Research in Adolescence Biennial Meeting, Vancouver, British Columbia, Canada.

Dickenson, L.L. & Repetti, R.L. (2011). *Home is a haven: Exaggerated HPA-axis activity after a bad day at school.* Poster presented at the 2011 Society for Research in Child Development Conference, Quebec, Canada.

Dickenson, L.L. & Repetti, R.L. (2010). *Parental depression associated with children’s diurnal cortisol.* Poster presented at the 2010 annual American Psychosomatic Society Conference, Portland, OR.

Dickenson, L.L., Repetti, R.L. & Robles, T.F. (2009). Children’s socialization experiences and HPA-axis activity: Family environment, mood, and school experiences. Paper presented at the 2009 annual UCLA Clinical Psychology Psychfest Symposium, Los Angeles, CA.

Dickenson, L.L. & Repetti, R. L. (2009). *The Association between trait mood and diurnal cortisol in middle childhood.* Poster presented at the 2009 Society for Research in Child Development Conference, Denver, CO

INTRODUCTION

While none of us are immune from stress, some children grow up in chronically more stressful home environments than others, with familial relationships and interactions serving as regular sources of strain. Parent-child relationship qualities and stressful childhood family climates, however, have been shown to influence psychological and biological health into adulthood. Evidence suggests that supportive parent-child relationships, and nurturing childhood home environments confer positive long-term mental and physical health benefits, while, conversely, interpersonal stressors within the family, such as non-nurturant parent-child relationships and/or family conflict, predict deleterious long-term health outcomes, including increased risk of depression, cardiovascular disease, and autoimmune disorders (Repetti, Robles, & Reynolds, 2011; Repetti, Taylor, & Seeman, 2002).

These observations have prompted researchers to identify biological pathways underlying the connections between stressful childhood family experiences and adverse health outcomes, with evidence suggesting that alterations in the hypothalamic-pituitary-adrenocortical (HPA) axis -- an essential component of the stress-response system and regulator of homeostatic functions responsible for the release of the hormone cortisol (Kirschbaum & Hellhammer, 1989) -- may represent one such mechanistic link. However, despite a rich and growing body of evidence linking early stress, HPA-axis functioning, and mental and physical health outcomes, there are relatively few studies examining associations between parent-child relationship qualities and HPA-axis activity, particularly during middle childhood and adolescence. What's more, studies examining daily processes within the family, such as daily parent-child interactions, and their connections with daily fluctuations in children's HPA-axis functioning are lacking in the extant literature. Relatedly, although individual differences in children's diurnal

cortisol profiles are assumed in the allostatic load literature -- which posits that HPA-axis dysregulation reflects wear and tear resulting from repeated and/or prolonged activation under stressful environmental conditions (McEwen, 1998) -- there is surprisingly little known about the temporal stability of children's diurnal cortisol or how children's reactivity to acute stressors is concurrently related to their diurnal cortisol profiles. The two studies composing this dissertation, therefore, are aimed at addressing these gaps in the literature and furthering our understanding of the pathogenesis of stress-mediated disease, particularly within the context of the family.

Both studies in this dissertation use multi-method and repeated measures study designs in an ethnically diverse community sample of children representing understudied periods of development: middle childhood and early adolescence. Study 1 explores whether two aspects of parent-child relationships -- parent-child attachment, and daily parent-child conflict -- are related to children's diurnal salivary cortisol activity. In addition to considering whether individual differences in children's security of attachment to parents are associated with between-person differences in diurnal cortisol activity, this study also uses a process-oriented approach, examining whether daily family processes, such as fluctuations in parent-child conflict, are related to same-day fluctuations in children's diurnal cortisol. Within-person designs like this may give us a window into how day-to-day experiences during childhood may, over time, shape individual differences in diurnal HPA-axis functioning and related long-term health outcomes.

Broadly, study 2 empirically investigates theoretical assumptions shaping study designs and methodologies in the allostatic load and child cortisol literatures. More specifically, one objective of study 2 is to assess short-term temporal stability in four metrics of diurnal cortisol: waking cortisol levels, the cortisol awakening response (CAR), diurnal slope, and bedtime

cortisol levels. In addition to investigating relative stability across these metrics, this study also examines whether diurnal cortisol stability is improved by increasing the number of sample days, a strategy recommended to improve accuracy when estimating individuals' typical diurnal cortisol profiles (e.g. Adam & Kumari, 2009). Study 2 also explores associations between each diurnal cortisol metric and children's reactivity to and recovery from an acute laboratory social stress task, with the goals of assessing the ecological validity of the laboratory stressor and determining whether links between alterations in stress-responsive HPA-axis activity and diurnal HPA-axis activity, implicated in the Allostatic load literature and models linking childhood family stress with later negative health outcomes (e.g. Repetti et al, 2002), are observable during middle childhood and early adolescence.

STUDY 1

Associations Between Children's HPA-Axis Activity and Parent-Child
Relationships during Middle Childhood and Early Adolescence

Abstract

We examined naturalistic associations between two dimensions of the parent-child relationship (parent-child attachment and daily parent-child conflict) and two indicators of diurnal HPA-axis activity (children's diurnal cortisol slopes and end-of-day cortisol levels) at the between- and within-person levels of analysis in a small community sample of children aged 8 to 13. At the within-person level of analysis, neither children's nor parents' reports of daily parent-child conflict were associated with either of the day-level cortisol variables in the overall sample. However, children's reports of secure attachment to their fathers moderated the association between fathers' reports of daily father-child conflict and children's daily bedtime cortisol levels. Children who reported lower levels of secure attachment to their fathers had higher bedtime cortisol levels on higher conflict days compared to children who reported higher levels of attachment to fathers. At the between-person level of analysis, children's reports of secure attachment to their mothers predicted their diurnal cortisol slopes such that higher levels of secure attachment were associated with flatter slopes. Results highlight the importance of studying children's relationships and interactions with their mothers and fathers, separately. Results also point to the importance of considering different indicators of HPA-axis functioning and moderators of within-person associations in order to facilitate progress toward understanding the ways in which short-term processes may be involved in linking the family environment and long-term health outcomes.

An abundance of research has linked social relationship factors with long-term physical health outcomes, with evidence indicating that changes in neuroendocrine, immune, and cardiovascular systems may mediate these associations (see Uchino, B. N., Uno, D., & Holt-Lunstad, J., 1999, for a review). Among adults, close social relationships have been linked with better immunological functioning and healthier neuroendocrine activity, whereas poor social relationships have been associated with deleterious physiological profiles (Dickerson & Zoccola, 2009; Seeman, 1996). Numerous studies indicate that supportive parent-child relationships, and a nurturing family environment during childhood are associated with salubrious physical and mental health outcomes, while, conversely, non-nurturant and/or conflict-ridden family environments are associated with negative long-term health outcomes (see Repetti, Taylor, & Seeman, 2002, for a review). One mechanism believed to link the early family environment with long-term health outcomes is alterations in hypothalamic-pituitary-adrenocortical (HPA) axis activity, measured through collection of the hormone cortisol. There are relatively few studies, however, examining associations between parent-child relationship qualities and HPA-axis activity, particularly during middle childhood and adolescence. Therefore, this article explores whether two aspects of parent-child relationships -- parent-child attachment, and daily parent-child conflict -- are related to children's diurnal salivary cortisol activity in a community sample of two-parent families with children aged 8 to 13 years.

A Brief Overview of the HPA-Axis

As the HPA-axis is both a regulatory and stress-responsive system (Kirschbaum & Hellhammer, 1989; McEwan, 1998), its end product -- the hormone cortisol -- is released in both a diurnal rhythm and in response to stressors. The basal activity of the HPA-axis follows a diurnal rhythm, with levels peaking in the early morning, declining steeply early in the day and

then continuing to decline slowly until reaching their lowest point during the night (Tsigos & Chrousos, 2002). Higher waking values, lower evening values, and steeper diurnal decline are all reflective of “healthier” HPA-axis functioning in adults, with deviations associated with chronic stress and adverse health outcomes (e.g. Sapolsky, Krey & McEwen, 1986).

The HPA-axis is responsive to environmental conditions, including physical, psychological, and social factors (e.g. Dickerson & Kemeny, 2004) and fluctuations in cortisol can be measured through the collection of saliva (Vining, McGinley, & Maksvytis, 1983). Cortisol plays a critical role in adaptive adjustments to environmental challenges with widespread influences on behavioral, cognitive, and physiological functions (e.g. McEwen, 2007). Chronic or prolonged activation of the HPA-axis, however, can result in cumulative strain, or “wear and tear” on these systems, a phenomenon known as Allostatic load (McEwen, 1998) and is believed to result in disruptions in healthy cortisol regulation, and, ultimately, even disease (McEwen and Seeman, 1999).

Family Environment and the HPA-Axis

The *risky families model* (Repetti et al, 2002) is based, in part, on the notion that, early in life, “risky” families – or those that are cold, non-nurturant, neglectful, and/or that exhibit overt conflict or aggression -- begin to exert their influence on long-term physical and mental health outcomes through disturbances in social competence, emotion regulation, and biological processes. In particular, changes in HPA-axis functioning have been implicated as a mechanism by which characteristics of early home environments may lead to deleterious mental and physical health outcomes in adulthood (Gunnar & Donzella, 2002; Gunnar & Vasquez, 2001; Luecken, 1998; Repetti et al., 2002; Taylor et al., 2004). Specifically, stressful family environments may lead to allostatic wear and tear of the HPA-axis through frequent and/or prolonged activation of

the stress response (Repetti et al., 2002). Much of the extant research, however, has either employed retrospective self-report methodologies to assess childhood home environment (e.g. Taylor et al, 2004; Leuken, 1998), increasing the likelihood of recall bias and measurement error, or has examined the effects of severe family risk, such as child maltreatment (e.g. Cicchetti & Rogosch, 2001; Gunnar & Vazquez, 2001; Edwards, Holden, Felitti, & Anda, 2003), leaving out a large segment of the population exposed to more subtle and normative forms of family risk. This study explores concurrent associations between parent-child relationship qualities and children's naturalistic diurnal cortisol activity in a community sample.

Attachment. Secure parent-child attachment is dependent on the extent to which a child can rely on his/her caregiver to be responsive and available (Bowlby, 1973; Ainsworth, Blehar, Waters, & Wall, 1978). As a construct initially developed within the context of infant-caregiver relationships, early attachment behaviors promote the infant's proximity and access to attachment figures whereas, as children get older, their perceptions of caregiver availability and responsiveness, particularly during times of stress and distress, become more important than the physical proximity of attachment figures (Bowlby, 1988). Consequently, attachment behaviors are not as frequent or easily observable past infancy, yet Bowlby (1979) asserts that the attachment bond remains throughout childhood, with children turning to attachment figures for comfort in the face of stressors such as illness or emotional distress during middle childhood. Therefore, attachment theory is applicable to the parent-child relationship during middle childhood and children's perceptions of caregiver availability and responsiveness during middle childhood represent a measureable construct relevant to our understanding of children's psychosocial development (Kerns et al., 1996; Kerns, Tomich, Aspelmeier, & Contreras, 2000).

Parents of securely attached children tend to respond more sensitively and responsively to signs of child distress compared to parents of insecurely attached children (Ainsworth et al., 1978). Thus, parental attachment behaviors capture parenting factors implicated in the *risky families model* -- warm, nurturant, supportive and responsive parenting -- and promote a child's tendency to view his or her caregiver as a source of security (Ainsworth et al., 1978). Securely attached children internalize these experiences, increasing their confidence in access to coping resources compared to insecurely attached children (Bowlby, 1973). In turn, threats in the environment are perceived to be less menacing and, therefore, we might expect that the stress response system, including the HPA-axis, might be activated less frequently and/or less intensely in securely attached individuals. Moreover, because cold and/or hostile parenting behaviors hinder feelings of security, parent-child conflict (discussed in more detail below) might also be indirectly related to HPA-axis function through attachment security.

Nurturant parent-child relationship qualities and children's HPA-axis activity. Despite the theoretical implications of attachment theory and the risky families model in connecting social relationships with stress response systems, there are relatively few studies examining associations between parent-child relationship quality and HPA-axis activity during middle childhood or adolescence. The majority of such studies have focused on infant-caregiver attachment, leaving middle childhood and adolescence almost entirely unrepresented in the literature (Ahnert, Gunnar, Lamb, & Barthel, 2004; Kerns, 2008).

The limited number of studies examining links between attachment or nurturant parent-child relationship qualities, and HPA-axis activity during middle childhood and adolescence suggest that warm and supportive parenting qualities are associated with children's diurnal cortisol levels. Higher ratings of parental acceptance and involvement were associated with

higher morning cortisol levels among adolescent girls (Booth, Granger, & Shirtcliff, 2008), and greater attachment security was associated with lower afternoon cortisol levels among boys and girls ranging in age from eight to 12 years (Borelli et al., 2010). Similarly, among adolescents, higher maternal warmth and involvement was associated with steeper diurnal cortisol slopes (Pendry & Adam, 2007). Broadening our search to include samples of younger children (exclusive of infants), non-nurturant and unsupportive maternal parenting characteristics were associated with flatter diurnal cortisol rhythms among preschoolers and kindergarteners (Ben-Dat Fisher et al., 2007; Pendry & Adam, 2007). The pattern of results in this small, but growing, body of literature indicates that close, supportive, and nurturant parenting behaviors and parent-child relationships are associated with healthy diurnal cortisol functioning: higher waking cortisol levels, steeper diurnal slopes, and lower afternoon levels.

While the above studies made important first steps in exploring linkages between parent-child relationship quality and HPA-axis activity during middle childhood and adolescence, the saliva sampling methodologies in two out of the three studies of children in middle childhood and adolescence precluded tests of diurnal cortisol slope, and none of these studies considered day-to-day fluctuations in cortisol production associated with concurrent fluctuations in parent-child relationship processes. Moreover, researchers either did not differentiate between children's relationships with their mothers and fathers or, when distinctions were made, often only maternal relationships were assessed, leaving father-child relationships drastically under-represented in the literature. These limitations and the dearth of research on associations between parent-child relationship factors and HPA-axis activity during this developmental period, in general, hinder our ability to understand the developmental trajectory and physiological mechanisms linking childhood attachment and later health outcomes. This paper seeks to address

this gap in the literature by studying links between parent-child attachment and children's diurnal cortisol profiles among 8- to 13-year-olds.

Parent-child conflict and children's HPA-axis activity. In addition to parent-child attachment and related nurturant parent-child relationship factors (or lack thereof) such as parental warmth, support, and responsiveness, relationship factors on the other end of the spectrum, such as parent-child conflict, represent another important area of family life that may be linked with children's diurnal HPA-axis activity and may represent a pathway linking childhood familial stress with deleterious health outcomes. The presence of overt family conflict represents a characteristic of risky families as defined by Repetti and colleagues (2002), and growing up in families in which anger, hostility, and conflict are commonplace has been shown to increase one's risk of mental and physical health disparities in adulthood (Repetti et al., 2002; Miller, Chen, & Parker, 2011). Disruptions in emotion regulation and biological regulatory systems have been posited as mechanisms linking childhood family conflict with long-term health risks (Repetti et al., 2002; 2011). Although research has demonstrated links between daily experiences of conflict within the home and emotional experiences, little work has focused on biological pathways, such as dysregulation of the HPA-axis, that might underlie developmental sequelae tied to conflictual childhood home environments.

From an allostatic load perspective, conflictual family environments exert their influence on biological pathways through the accumulation of "repeated hits" to regulatory systems (Repetti et al., 2011). Therefore, examining naturalistic, daily experiences in relation to emotional and physiological functioning provides the opportunity to understand how daily family processes may, over time, shape long-term health outcomes (Repetti et al., 2011; Repetti, Reynolds, & Sears, 2015). Previous within-persons research designs have demonstrated that

daily negative parent-child interactions are associated with higher same-day child negative affect and emotional distress (Almeida, Wethington, & McDonald, 2001; Chung, Flook, & Fuligni, 2009; Kiang & Buchanan, 2014). Similarly, researchers have observed that, despite its low frequency, naturalistic parent-child and sibling conflict remain significantly associated with daily psychological well-being and distress into young adulthood (Fuligni and Masten, 2010).

Complimentary research on connections between daily parent-child conflict and diurnal cortisol, however, is sparse. To our knowledge, only one study has demonstrated links between naturally occurring daily family conflict and children's diurnal cortisol (Slatcher and Robles, 2012). Using an Electronically Activated Recorder (EAR) device to assess for the occurrence of conflict between preschoolers and their family members over the course of one day, Slatcher and Robles (2012) found that preschoolers who were involved in more conflict at home had lower waking cortisol levels and flatter diurnal cortisol slopes compared to preschoolers engaged in less familial conflict. While the intensive EAR sampling methodology limited their ability to assess day-to-day fluctuations in conflict levels associated with daily diurnal cortisol patterns, this study was the first to demonstrate that naturally occurring interpersonal conflicts within the family are related to children's diurnal cortisol profiles. A recent within-subject design using data from a national sample of adults demonstrated that increases in adults' daily reports of arguments and pressures at home were associated with same-day increases in their cortisol output over the day (as indexed by Area Under the Curve (AUC); Stawski, Cichy, Piazza, and Almeida, 2013). Moreover, outside of familial conflict and daily stressors, researchers have observed that increases in momentary cortisol levels were associated with momentary increases in naturally-occurring daily stressors in adults (e.g. Smyth et al, 1998) and negative mood states in adolescents (Adam, 2006), supporting the notion that the experience of daily stressors and

emotional upsets have observable effects on daily HPA-axis activity. We seek to extend the previous scant research on the association between naturalistic parent-child conflict and children's HPA-axis regulation by exploring within-person effects of daily parent-child conflict on children's diurnal cortisol profiles with the aim of identifying daily processes that may elucidate biological pathways linking childhood family environments and later health outcomes.

Taken together, the limited empirical evidence linking nurturant parent-child relationship qualities, parent-child conflict, and children's diurnal cortisol profiles suggests that children less secure in their attachment to parents, reared by less nurturing parents, or engaged in more interpersonal conflict within the home display less healthy diurnal cortisol profiles. These preliminary findings warrant further investigation, with particular attention devoted to studying children's relationships and daily interactions with their mothers and their fathers separately. Moreover, to the best of our knowledge, studies investigating linkages between day-to-day fluctuations in parent-child interactions and children's cortisol during middle childhood and early adolescence are absent from the literature. Additional research in this area may shed light on neuroendocrine mechanisms believed to at least partially mediate the link between childhood family environments and long-term mental and physical health outcomes.

The Present Study

The present study is aimed at addressing the knowledge gap in the literature on the associations between two dimensions of the parent-child relationship (parent-child attachment and daily parent-child conflict) and two indicators of diurnal HPA-axis activity (children's diurnal cortisol slopes and end-of-day cortisol levels) in middle-childhood and early adolescence. To accomplish this, we pose three questions at the within- and between- persons levels of analysis:

First, “*Are day-to-day fluctuations in children’s conflict interactions with their mothers and fathers associated with day-to-day fluctuations in children’s diurnal cortisol?*” To our knowledge, no published studies have examined within-family variation in parent-child relationship factors in conjunction with within-person variation in children’s diurnal cortisol profiles. Such a design will shed light on short-term processes involved in one of the proposed mechanistic links between family risk and long-term health outcomes: alterations in the HPA-axis. While individual differences in HPA-axis activity have been linked with parent-child relationship qualities in a limited number of studies (reviewed above), to our knowledge, no published studies have investigated the ways in which these differences may come about through changes in short-term (daily) processes. For example, might children’s evening cortisol levels be higher or their diurnal cortisol slopes be flatter on days characterized by more parent-child conflict? Importantly, we will consider children’s daily diary reports of negative interactions with their mothers and fathers, separately, in addition to mother’s and father’s reports as indicators of conflict in mother-child and father-child dyads.

Second, “*Are individual differences in parent-child attachment related to individual differences in children’s diurnal cortisol?*” We seek to add to the limited findings suggesting that more secure parent-child attachment is related to a steeper diurnal cortisol rhythm during middle childhood and early adolescence. Our study design offers methodological advantages over previous designs in the literature: Multiple cortisol measures were taken over the course of the day, and this procedure was repeated over several days, providing the opportunity to map individual cortisol rhythms (due to repeated measures within days) as well as increasing accuracy in estimating an individual’s “typical” diurnal cortisol rhythm and bedtime cortisol levels (due to repeated measures over days). In addition, we assessed children’s security of

attachment to mothers and fathers separately, allowing us to investigate the unique effects of each on children's typical cortisol levels. Finally, our sample of children in middle childhood and adolescence allows us to explore the role of attachment on physiological functioning beyond infancy and early childhood, in an age range underrepresented in the literature.

And, third, "*Do individual differences in parent-child attachment moderate within-person associations between day-to-day parent-child conflict and children's diurnal cortisol explored in research question 1?*" In other words, will the link between children's diurnal cortisol and daily mother-child and father-child conflict depend on the child's security of attachment to the parent? Perhaps a day characterized by more parent-child conflict (compared to the child's usual level of conflict) is particularly stressful for children who are insecurely attached to their parents, increasing nighttime cortisol levels, compared to children who are securely attached to their parents. This line of investigation will potentially provide insight about how both between-family variables (such as secure versus insecure attachment) and within-family variables (such as daily levels of parent-child conflict) operate in concert in shaping children's diurnal cortisol activity.

Methods

The present study uses data collected as part of a larger study of family life and health.

Participants

Participants included 47 target children (28 girls) and their parents from the greater Los Angeles area. Children ranged in age from eight to 13 years ($M = 11.8$, $SD = 1.5$) and grades third through ninth (8.5% third, 10.6% fourth, 14.9% fifth, 14.9% sixth, 25.5% seventh, 23.4% eighth, and 2.1% ninth grades). The sample was ethnically diverse, with parents identifying their children as Caucasian (38%), African-American (15%), Hispanic (15%), Asian (11%), and other

or mixed ethnicity (21%). 47 mothers and 40 fathers participated; even when fathers did not participate, children completed questionnaires and daily diary reports assessing their interactions and relationships with their fathers.

Procedures

Recruitment, inclusionary/exclusionary criteria, and compensation. Participation was timed to coincide with cold and flu season, as the occurrence of upper respiratory infections (URIs) was an outcome assessed in the larger study. Families were recruited from the community using various means including flyers at local schools, medical clinics, and libraries, advertisements in school newsletters, and a direct mailing to middle class families in the Los Angeles area. Criteria for inclusion were: a) at least one child between the ages of 8 and 13¹ who agreed to participate (the target child), b) two cohabitating adults who had both lived with the target child for the prior five years or more, at least one of whom agreed to participate, c) ability to read and speak English at a 3rd grade level or better, and d) all participating family members were in normal health. Families were excluded if either parent or target child had medical conditions or reported behaviors that can affect neuroendocrine function.

When two children from the same family were in the target age range, the older child was the target child. Both parents were encouraged to participate, but families in which only one parent was willing and/or able to participate in the study were permitted to enroll. Families were compensated up to \$1,000 in cash and gift cards for their participation in the larger study. Participants were provided a base honorarium and earned weekly bonus honoraria contingent on thorough completion of measures.

¹ The age range of 8-12 years was used in cohort 1 and then advanced one year at each end of the range to 9-13 years for cohort 2 and 3 in order to increase participants' ability to read, understand, and respond to written questionnaire and daily diary measures. This age range balances children's ability to complete self-report measures with base rates of recurrent URIs.

Data collection

Data were collected using a variety of methods including questionnaires, daily diaries, and repeated sampling of child salivary cortisol. After informed consent and assent was obtained, families were instructed on procedures for completing online questionnaires, online and paper daily diaries, and sampling saliva.

Daily diaries. Participants were asked to complete daily diary reports assessing their daily experiences, including social interactions with other family members, before going to bed every night for eight weeks. Online diaries were collected using SurveyMonkey. The instant data transmission and date/time stamp accompanying online diaries allowed researchers to closely monitor participant compliance and communicate with participants, accordingly. When they chose to use them, participants were asked to mail date/time-stamped paper diaries to the lab in pre-addressed, postage-paid envelopes. Only daily diary reports that overlapped with the eight days of diurnal cortisol sampling (described below) were included in analyses.

Diurnal salivary cortisol. During the third and sixth week of daily diary collection, participants provided saliva samples using timed passive drool four times per day – upon waking, half an hour after waking, before dinner, and before bed -- over four consecutive days: Saturday, Sunday, Monday, and Tuesday. Participants received reminder phone calls prior to each sampling period wherein sampling procedures were reviewed and compliance with sampling procedures was emphasized. Saliva vials were labeled with sampling occasion data and a unique randomly-generated number. Participants were asked to record this number, along with an electronic date/time stamp, on a saliva record sheet each time they sampled saliva. We verified that the tube numbers and date/time data reported matched those assigned to each sampling occasion in order to ensure that the vial labels corresponded with the timing of the

actual sample. We coded samples where there was any question about the accuracy of the sampling occasion data, such as missing tube number or date/time stamp data, with a dummy code (Sample Question, $n = 66$, 5.2% of the final cortisol sample). Participants were instructed to avoid eating, drinking, or brushing their teeth for at least 30 minutes prior to sampling saliva, and to report any food, drinks, caffeine, nicotine, or exercise in the half hour preceding each sample, and any medications taken over the day. Samples in which any of those items were endorsed were identified in a dummy code (Sample Confound, $n = 105$, 8.2%).

Each family member was given a plastic bottle containing straws to assist with saliva collection. Half of the families received bottles with MEMS caps (Aardex, Denver, CO), an electronic monitoring device with a microchip that records bottle openings. The remaining families received bottles with dummy caps resembling the MEMS caps but without microchips. Families were randomly assigned to receive either MEMS or dummy caps; regardless of assignment, all families were told that their caps recorded openings. Participants were instructed to remove a straw and immediately return the cap each time they sampled saliva. They were told to swallow, allow saliva to pool in their mouths, without stimulation, for 60 seconds, and deposit their saliva into the designated vial using the straw. The process was then repeated before participants secured a cap to the vial and stored it in the freezer. Data recorded by the MEMS caps was compared with participant's date/time stamps in order to monitor compliance. Samples in which the MEMS time was more than 30 minutes apart from the date/time stamp information were coded using a dummy code (Mems30, $n = 38$, or 8% of the MEMS samples).

The day after each four-day saliva sampling period, a research assistant picked up saliva samples from the family's home and transported them on ice to the lab where they were frozen and stored at -20° Celsius. Saliva samples were later shipped to the Biological Psychology

Laboratory at the Technical Institute of Dresden (Dresden, Germany), directed by Clemens Kirschbaum, and assayed with commercial kits (IBL, Hamburg, Germany) for free cortisol using chemiluminescence immunoassay (50 µl saliva required; minimum detection limit <.003 µg/dL, mean intra- and interassay coefficients of variance (CV) below 10%; Dressendorfer et al., 1992; Polk et al., 2005).

Measures

Daily Parent-Child Conflict. Children completed three items adapted from the Aversive Behavior with Parent subscale of the Youth Everyday Social Interactions and Mood Scales (YES-I-AM; Repetti, 1996) separately for each parent. The items, which are listed in Appendix A-1, assess children's daily anger with parents, perceptions of parents' anger with children, and punishment received from parents. Items were rated on a 3-point scale (1 = not at all, 2 = some, and 3 = a lot) and daily scores were computed by averaging across the items reported for each parent on each day. The subscale has been shown to have adequate internal consistency with alphas of .77 and .74 for reports of aversive behavior with mothers and fathers, respectively (Repetti, 1996). Children's mean score was 1.20 (Range 1 – 3, SD = .38), for conflict with mothers, and 1.14 (Range, 1 - 3, SD = .32), for conflict with fathers, in the current sample. While children reported low levels of conflict with both mothers and fathers, they reported significantly higher levels of conflict with mothers than with fathers ($t = 2.56, p < .05$). Children completed 93.1% ($n = 350$) of the 376 daily diaries possible from our sample of 47 children over the eight days coinciding with saliva sampling. On average, children completed daily diaries on 7.4 of the eight nights of interest.

Parents completed the 9-item Negative Parent-Child Interactions subscale of the 55-item Parent Home Data Questionnaire (PHDQ; Margolin, 1990). Items, listed in Appendix A-2, assess

the frequency of behavioral and affective indicators of parent-child conflict including yelling, child and parental anger, and punishment. Items were rated on a 3-point scale (1 = not at all, 2 = some, and 3 = a lot). Daily scores were computed by summing and averaging across items for each day. The PDHQ has been validated for use over 6-week periods in families with children between the ages of 8 and 11 (Garcia O'Hearn, Margolin, and John, 1997; Dumas, Margolin, and John, 2003). In the current sample, mothers' mean score was 1.19 (Range 1 – 2.44, SD = .27) and fathers' mean score was 1.11 (Range, 1 – 2.67, SD = .21). While both mothers and fathers reported low levels of daily conflict with children, mothers' reports were significantly higher than fathers' ($t = 4.78, p < .001$). Mothers completed a total of 92.8% ($n = 349, M = 7.4$) and fathers completed 79.5% ($n = 256, M = 6.4$) of the total possible daily diary reports.

Parent-child attachment. In order to assess children's attachment security to parents, children completed the 15-item Security Scale (Kerns et al., 1996; 2000), which is provided in Appendix A-2. The Security Scale measures the degree to which children perceive their parents to be responsive and available, their tendency to depend on parents when stress is encountered, and children's perceived comfort in communicating with parents. Children completed parallel versions of the scale separately for mothers and fathers. All items first require children to decide which of two statements best describes them (for example, "Some kids wish they were closer to their mom." and "Other kids are happy with how close they are to their mom.") and then rate the degree to which that statement describes them as either "really true" or "sort of true". This structure results in a 4-point scale ranging from 1-4, with higher scores indicating more secure attachment. Scores reflecting children's unique attachment security with mothers, and with fathers, were computed by averaging the items on the mother and father versions separately. Cronbach's alphas ranged from .64 to .82 for reports of child's attachment to mothers and from

.82 to .88 for reports of children's attachment to fathers in a study of third-, fifth-, and sixth-graders (Kerns et al., 2000). In the current sample, children's mean scores were 3.32 (Range 2.36 – 3.93, SD = .36) for attachment to mothers, and 3.23 (Range 2.14 – 3.87, SD = .47) for attachment to fathers. The Security Scale questionnaire was completed by all but one child in our sample.

HPA-axis activity. HPA-axis activity was measured through the collection of diurnal salivary cortisol as described in the procedures section above. Two diurnal cortisol outcome variables were created from these data: 1) Predicted daily diurnal cortisol slopes, which were derived from the Ordinary Least Squares (OLS) estimates of the effect of time of day on children's log transformed cortisol values, as described in greater detail in the results section below, and 2) Daily bedtime cortisol levels, which were the log transformed values corresponding with the bedtime sampling occasion on each day. Children completed 88.6% (n = 1,332) of the 1,504 possible diurnal saliva samples. Of those, seven (0.5%) outliers (defined as raw cortisol values over 60 nmol/L) were removed from analyses. Another 49 (3.7%) saliva samples were missing the corresponding time of day data and, since the diurnal rhythm of cortisol is influenced by time of day, these samples were excluded from analyses, leaving 1,276 diurnal cortisol/time pairs. Completion rates were evenly dispersed across sampling occasions with 316 waking, 318 post-wake, 316 dinner, and 326 bedtime samples. The average child completed 3.4 of the possible 4 samples per day.

Data Analysis

Multilevel growth-curve modeling techniques were carried out using HLM (Version 6.06; Raudenbush, Bryk, & Congdon, 2004; SSI Scientific Software International) in order to account for the nested, or non-independent, nature of the data -- repeated measures of diurnal

cortisol nested within individuals – when modeling diurnal cortisol profiles for each child in line with models reported elsewhere (e.g. Adam, Hawkley, Kudielka, & Cacioppo, 2006). Multilevel modeling is uniquely suited to simultaneously detect stable between-person (or between-family) differences, as well as within-person changes over time, and is ideal for analyzing repeated measures over varying lengths of time (Hruschka, Kohrt, & Wortham, 2005). Multilevel models can also accommodate missing data and, because of their use of repeated measures, increase statistical power in small samples, such as ours.

Results

Diurnal Cortisol Patterns, Control Variables, and Predicted Slopes

Cortisol patterns over the day. We observed the expected diurnal rhythm in children's daily cortisol, as shown in Figure 1: Children's cortisol levels were highest in the morning, declined rapidly during the morning hours and then more gradually in the afternoon and evening, with levels near zero at bedtime. In order to model each child's diurnal cortisol rhythm across the eight days of data collection, children's log transformed cortisol values were regressed on the time of day corresponding with each sampling occasion at Level 1 of a multilevel model grouped by participant at Level 2. In line with previous reports (e.g. Saxbe, Repetti, and Nishina, 2008), time variables were centered at 5:00 am so that the intercept would reflect an estimate of morning cortisol levels. We tested both linear and curvilinear models and found that the best fit was achieved with a curvilinear model, with both time and time squared entered as random Level 1 predictors of cortisol values:

$$\text{LNCORT}_{ij} = \beta_{0i} + \beta_{1i}\text{Time}_{ij} + \beta_{2i}\text{Time}_{ij}^2 + \varepsilon_{ij},$$

where i represents the child and j represents the sampling occasion.

As expected, the linear and quadratic time of day variables in this model both significantly predicted diurnal cortisol levels (see Table 1) and accounted for a significant proportion, 79.9%, of the variance in diurnal cortisol. Consistent with the diurnal trend shown in Figure 1, the linear cortisol slope coefficient was negative, with an expected $-.187$ unit decrease in logged cortisol per hour over the day. The quadratic slope coefficient was positive (with an estimated $.003$ unit increase in logged cortisol for each Time^2 unit), reflecting an initial steep decline and subsequent leveling off of cortisol levels over the course of the day. As Figure 1 shows, considerable variation in early morning cortisol were present, while there was less variability in evening cortisol levels.

Cortisol control variables. Next, we added to the Level 1 model each of the three dummy control variables described above under *Procedures* (Sample Question, Sample Confound, and Mems 30) plus a fourth dummy variable (Weekend) to test systematic differences in cortisol levels on weekdays versus weekends. The only control variable observed to significantly predict cortisol levels was the Sample Question dummy variable (Sample Confound, Mems 30, and Weekend dummy $ps = .41, .38, \text{ and } .70$, respectively). Including Sample Question in the model did not reduce residual error or increase model fit but, because it was a significant predictor of diurnal cortisol levels, the Sample Question dummy was retained as a fixed effect in subsequent Level 1 diurnal cortisol models.

Predicted cortisol slope. Next, predicted daily cortisol slopes were calculated for each child on each day with at least three cortisol samples. In order to obtain an estimate of each child's linear diurnal cortisol slope on each of the eight saliva sample days, the Sample Question control variable was added as a fixed effect in the Level 1 model described above with time and time squared entered as random predictors of children's cortisol, and study day and participant

ID were entered as Level 2 and 3 grouping variables, respectively. The person-level ordinary least squares (OLS) estimate of the time of day coefficient was added to the day-level time of day residual for each child on each day, resulting in OLS estimates of daily cortisol slopes to be used as outcomes in subsequent analyses. Since the model used to obtain predicted daily cortisol slopes included time of day and the Sample Question control variable, it was not necessary to include these variables as Level 1 predictors in subsequent analyses with predicted daily cortisol slopes as outcomes.

Parent-Child Conflict and Attachment Predicting Children's Diurnal Cortisol Slopes

Our first two research questions were aimed at testing whether daily parent-child conflict and secure attachment to parents were associated with children's diurnal cortisol slopes at the within- and between-person levels of analysis, respectively. To address the third research question, we tested whether secure attachment to parents moderated associations between daily parent-child conflict and children's diurnal cortisol slopes. Our Level 1 model testing the within-person association between daily parent-child conflict and children's diurnal cortisol slopes was

$$\text{CortSlope}_{ij} = \beta_{0i} + \beta_{1i}\text{DailyConflict}_{ij} + \varepsilon_{ij},$$

where CortSlope_{ij} represents the predicted diurnal slope for child i on day j , and $\beta_{1i}\text{DailyConflict}_{ij}$ represents the within-person effect of day-to-day fluctuations in each of the four parent-child conflict variables (child report of conflict with mothers, child report of conflict with fathers, mother report of conflict with child, or father report of conflict with child) on children's cortisol slopes.

Children's reports of parent-child attachment were entered as Level 2 predictors of the Level 1 intercept (in order to test for effects of attachment on each child's typical cortisol slope) and as predictors of the coefficients representing the effects of daily parent-child conflict on

children's cortisol slopes (in order to test the moderating role of attachment on the relationship between day-to-day fluctuations in parent-child conflict and children's diurnal cortisol slopes). Children's reports of attachment to their mothers were only entered as Level 2 predictors in models with daily mother-child conflict (child or mother report) at Level 1 and, similarly, children's reports of attachment to their fathers were only entered as Level 2 predictors in models with daily father-child conflict (child or father report) at Level 1. Age and sex control variables were also entered as Level 2 predictors of the Level 1 intercept.

Results for diurnal cortisol slope are presented separately for mothers and fathers, respectively, in the top halves of Tables 2 and 3. Daily parent-child conflict was not found to be significantly associated with children's diurnal cortisol slopes in any of the four models tested, suggesting that day-to-day fluctuations in children's diurnal cortisol slopes were not related to day-to-day fluctuations in conflict with mothers nor fathers. Unexpectedly, secure attachment to mothers was found to have a significant positive effect on children's diurnal cortisol slopes in the models with child-reports and mother-reports of conflict. In other words, children who reported higher levels of secure attachment to their mothers had flatter typical diurnal cortisol slopes than children who reported lower levels of attachment to their mothers. Children's reports of secure attachment to their fathers were not associated with children's typical cortisol slopes and neither secure attachment to mother nor father moderated associations between daily parent-child conflict and cortisol slope in any of the four models tested.

Parent-Child Conflict and Attachment Predicting Children's Bedtime Cortisol Levels

Our next set of analyses were aimed at testing whether daily parent-child conflict and secure attachment to parents were associated with children's bedtime cortisol levels at the within- and between-person levels of analysis, respectively, and whether attachment security

moderated associations between daily parent-child conflict and children's bedtime cortisol levels. Our Level 1 model testing within-person associations between daily parent-child conflict and children's bedtime cortisol levels was

$$\text{BedCort}_{ij} = \beta_{0i} + \beta_{1i}\text{Time}_{ij} + \beta_{2i}\text{DailyConflict}_{ij} + \beta_{3i}\text{SampleQuestion}_{ij} + \varepsilon_{ij},$$

where BedCort_{ij} represents the log transformed bedtime cortisol level for child i on day j , $\beta_{1i}\text{Time}_{ij}$ represents the random effect of time of day, $\beta_{2i}\text{DailyConflict}_{ij}$ represents the within-person effect of each of the four parent-child conflict variables on children's bedtime cortisol levels, and $\beta_{3i}\text{SampleQuestion}_{ij}$ represents the fixed effect of the Sample Question dummy variable. Similar to the models predicting cortisol slope above, children's reports of parent-child attachment were entered as Level 2 predictors of the Level 1 intercept and the coefficients representing the effects of daily parent-child conflict on children's bedtime cortisol levels, and child age and sex control variables were included as Level 2 predictors of the Level 1 intercept.

Bedtime cortisol results are presented in the bottom halves of Tables 2 and 3. The coefficient representing the overall effect of daily parent-child conflict across all children in our sample was not significant in any of the four models. However, in one of the two models testing father-child conflict, children's reports of secure attachment to their fathers moderated the association between day-to-day fluctuations in father-child conflict and bedtime cortisol levels. As illustrated in Figure 2, children with lower levels of secure attachment to fathers had higher bedtime cortisol levels on days when fathers reported higher levels of father-child conflict whereas children with higher levels of secure attachment to fathers had lower bedtime cortisol levels on high conflict days.

Children's reports of secure attachment to mothers and fathers did not predict their overall bedtime cortisol levels over the eight days of saliva sampling, and secure attachment to

mothers did not moderate the association between mother-child conflict and bedtime cortisol levels.

Discussion

We examined naturalistic associations between two dimensions of the parent-child relationship (parent-child attachment and daily parent-child conflict) and two indicators of diurnal HPA-axis activity (children's diurnal cortisol slopes and end-of-day cortisol levels) in a small community sample of children aged 8 to 13. At the within-person level of analysis, neither children's nor parents' reports of daily mother- nor father-child conflict were associated with either of the day-level cortisol variables in the overall sample. However, children's reports of secure attachment to their fathers moderated the association between fathers' reports of daily father-child conflict and children's daily bedtime cortisol levels: on higher conflict days, children who reported less secure attachment had higher bedtime cortisol levels compared to children who reported higher levels of attachment to fathers. Surprisingly, at the between-person level of analysis, children's security of attachment to their mothers predicted their diurnal cortisol slopes such that those reporting higher levels of secure attachment had flatter slopes.

Daily Parent-Child Conflict. To our knowledge, no published studies have examined within-family variation in parent-child relationship factors in conjunction with within-person variation in children's diurnal cortisol levels. Therefore, we carried out exploratory analyses to test whether within-person changes in daily parent-child conflict were associated with daily fluctuations in either cortisol metric of interest. Although our analyses were exploratory, given the ways in which individual differences in HPA-axis activity have been linked with parent-child relationship qualities in the limited extant literature, we wondered if we might find that higher

levels of daily parent-child conflict were associated with flatter daily diurnal cortisol slopes or higher daily bedtime cortisol levels.

In the overall sample, daily conflict interactions with mothers and fathers were not associated with children's daily diurnal cortisol slopes or bedtime cortisol levels.

Given our small sample size, our null results may be a consequence of being underpowered to detect effects. Alternatively, our results may be related to the fact that children reported relatively low levels of parent-child conflict: Daily parent-child conflict scores were over 2 (equivalent with "some" conflict in our measures) in only 1.5 and 1.1% of children's reports of conflict with mothers and fathers, respectively, and 1.1 and 0.6% of mothers' and fathers' reports of conflict with children, respectively, across all participants over the eight days of data collection. Thus, children may not have experienced the relatively infrequent, minor parent-child conflict interactions reported in our sample to be stressful, or at least not stressful enough to result in prolonged physiological arousal reflected in their diurnal cortisol slopes and end-of-day cortisol levels. Finally, our null results may be due to individual differences in the association between daily parent-child conflict and children's diurnal cortisol, a possibility we explored further in our next research question.

Secure attachment as a moderator of associations between daily parent-child conflict and daily cortisol. In one of the four models that tested a moderating role of attachment on the association between father-child conflict and children's diurnal cortisol, we found evidence that secure attachment to fathers moderates the association between daily father-child conflict and children's cortisol levels. Specifically, on days when fathers reported higher levels of father-child conflict, children's bedtime cortisol levels were higher among children who reported being less securely attached to their fathers. This pattern may suggest that daily father-child conflict

was more stressful for children who were not as securely attached to their fathers as reflected in poorer end-of-day physiological recovery on higher conflict days compared to more securely attached children. Perhaps less securely attached children experienced conflict with fathers to be more intra- or interpersonally threatening and, therefore, they did not recover as well by bedtime. This interpretation is in line with attachment theory, which holds that children form internalized models about the world and their ability to cope with challenges based on the availability and sensitive responsiveness of attachment figures over time (Bowlby, 1973). Therefore, less securely attached children may interpret daily father-child conflict and their ability to cope with such interpersonal stressors less favorably than more securely attached children, which, in turn, may hinder their ability to physiologically recover from everyday stressors. Similarly, this pattern may be consistent with the notion that stressful family environments exert deleterious influences on health through accumulated wear and tear on regulatory systems, such as the HPA-axis (Repetti et al., 2002; 2011). That is, it is possible that lower security of attachment to fathers may represent an environmental stressor that, over time, reduces one's ability to physiologically recover from interpersonal stressors in daily life. Consistent with this hypothesis, among adults, lower levels of parental warmth during childhood has been shown to predict higher levels of diurnal cortisol output on days characterized by more stress (Hanson & Chen, 2010), suggesting that parent-child relationship factors have long-lasting effects on daily physiological responses to stress. Our results suggest that daily family conflict, in the context of lower security of attachment, may represent a pathway by which childhood family environments exert influences on HPA-axis regulation through daily processes of physiological responses to stress which, over time, may accumulate and lead to wear and tear on regulatory stress systems (Repetti et al., 2002). Notably, our methodological approach of considering between-family predictors of

within-person effects, as well as distinguishing children's relationships and interactions with fathers from mothers enabled our ability to contribute to the small body of research linking parent-child attachment, daily parent-child conflict, and children's diurnal cortisol during developmental periods underrepresented in the literature: middle childhood and adolescence.

Interestingly, our pattern of results suggests that children reporting higher security of attachment to fathers had lower bedtime cortisol levels on days characterized by more conflict with fathers compared to lower conflict days. This pattern is consistent with the "exaggerated" recovery effects Saxbe and colleagues (2008) observed among married women: women more satisfied in their marriages had lower cortisol levels on more stressful work days compared to less stressful days (Saxbe, Repetti, & Nishina, 2008). Similarly, college students who reported receiving higher levels of parental warmth as children had lower levels of daily cortisol output on more stressful compared to less stressful days (Hanson & Chen, 2010). Results like these and our pattern of results suggest that characteristics of the home environment can both enhance and interfere with the process of physiological recovery following a stressful day.

The fact that we only observed security of attachment to moderate associations between daily father-child (and not mother-child) conflict and children's cortisol may be related to the fact that conflict with fathers was less common than conflict with mothers (as noted in the methods section and observed by other researchers (e.g., Fuligni et al., 2009)). In other words, because conflict with fathers, who tend to work more hours outside the home and spend less time with children (Campos, Graesch, Repetti, Bradbury, & Ochs, 2009; B. Reynolds, personal communication, April 7th, 2015), was a more novel experience for children in our sample, it may have been more stressful for these children than more common daily conflict with their mothers. Similarly, mothers tend to be more involved in the day-to-day aspects of childcare than fathers

(Saxbe, Repetti, & Graesch, 2011). As such, children's conflict with mothers may have more often involved relatively benign conflict over things such as homework, household chores, privileges, etc., whereas conflict with fathers, though less common, may have been more likely to involve more serious matters, such as child disobedience, academic problems, or unresolved conflict with mothers that was turned over to fathers. Our measures of daily conflict precluded us from testing this hypothesis, but future research should consider the type and intensity of daily parent-child conflict.

Attachment security did not moderate any of the daily associations between parent-child conflict and children's daily diurnal cortisol slopes. One explanation for this may be that diurnal cortisol slope is derived from samples taken over the course of the day and is correlated with waking cortisol levels which were sampled before the majority of parent-child interactions likely took place. Therefore, the temporal order of parent-child interactions in relation to saliva samples influential in estimating children's diurnal slope may have been a factor.

Secure attachment predicting individual differences in children's cortisol. Based on the limited literature linking nurturant parent-child relationship qualities and diurnal cortisol in school-aged children and adolescents (Booth et al., 2008; Borelli et al., 2010; Pendry & Adam, 2007), we expected that higher levels of secure parent-child attachment would be associated with steeper diurnal cortisol slopes. We sampled multiple cortisol measures over several days, allowing us to model diurnal cortisol slopes and offering increased accuracy in estimating each child's "typical" diurnal cortisol rhythms. Surprisingly, we observed that children's reports of more secure attachment to their mothers were associated with *flatter* diurnal cortisol slopes. Among adults, this diurnal cortisol profile has been associated with chronic stress and adverse health outcomes (e.g. Sapolsky et al., 1986). Similarly, flattened diurnal cortisol slopes have

been associated with indicators of psychosocial distress, such as increased depressive symptoms, among children and adolescents (e.g. Adam 2006).

The counterintuitive association we observed between security of attachment to mothers and children's diurnal cortisol slopes may reflect stress in other areas of children's lives that increase their tendency to rely on their mothers as sources of comfort. That is, while parents' sensitive responsiveness and availability to children during times of stress may be protective (e.g. Bowlby, 1979), children reporting higher than average levels of attachment to their mothers during middle childhood and adolescence may be experiencing inter- and/or intrapersonal distress, such as depressive symptoms or peer problems, that increases their desire for and/or receipt of support from their mothers and may manifest in altered diurnal HPA-axis activity. This explanation is influenced by the notion that adolescence is a period of developmentally normative separation from parents (Csikszentmihalyi and Larsen, 1984). This developmental task may be at odds with some of the constructs tapped by our security of attachment scale, such as children's tendency to rely on their mothers when under stress (e.g., "Some kids go to their mom when they are upset"), engage in self-disclosure (e.g., "[Some kids] like telling their mom what they are thinking or feeling"), and preference for mothers' physical presence (e.g., "Some kids feel better when their mom is around"). Thus, in addition to possibly reflecting parenting characteristics measured by other researchers, such as warmth and supportiveness (e.g. Booth et al., 2008; Pendry & Adam, 2007), higher than average levels of security of attachment during middle childhood and adolescence may reflect characteristics of the child, such as a child's tendency to seek out and/or rely on his/her caregiver more often than other children that, given the age range of our sample, may be driven by distress in other areas of daily life. Testing this hypothesis was beyond the scope of this paper but future research should test for psychosocial

mediators, such as symptoms of depression, anxiety, and/or peer and academic problems, that may mediate the link between parent-child attachment and children's diurnal cortisol rhythms during middle childhood and adolescence.

Importantly, we assessed children's security of attachment to mothers and fathers separately, allowing us to examine the unique effects of attachment to each parent on children's typical cortisol levels. As with the moderation effects discussed above, we found an effect of secure attachment for one parent and not the other, suggesting that the attachment relationships children have with each parent are distinct from one another and may hold unique meaning and significance.

Limitations. The present study was limited by several factors. First, our small sample and missing data limited statistical power. Thus, some of our findings, such as detecting effects for one parent and not the other, or one cortisol outcome variable and not the other, might be artifacts of our limited statistical power. Relatedly, the stressors we measured – daily parent-child conflict – were relatively minor, and the timing of our sampling procedures did not allow us to test short-term, or momentary, cortisol reactivity to their occurrence, which would have required saliva sampling 20 to 40 minutes after parent-child conflict interactions occurred (Dickerson & Kemeny, 2004). We recommend that future studies attempt to identify potentially more stressful events and interactions in the parent-child relationship. Researchers might find the use of event-contingent sampling methods, in which sampling occasions are contingent on the natural occurrence of certain types of events, fruitful in pairing the timing of cortisol collection with potentially more physiologically arousing events. Finally, though ethnically diverse, our sample was comprised of mostly middle-class, two-parent families from the community. More

diverse and/or clinical samples might yield more variability in parent-child relationship factors, with possible consequences for children's naturalistic cortisol output.

Conclusions. Our results highlight the importance of studying children's relationships and interactions with their mothers and fathers, separately. In particular, our results suggest that the attachment relationships children have with each parent are distinct from one another and may hold unique meaning. In terms of attachment to fathers, our results suggest that lower security of attachment may impair children's abilities to physiologically recover from daily stressors, such as interpersonal conflicts with fathers. Regarding attachment to mothers, our results suggest that scores on the secure attachment measure used in this study might mean something different during the pre-adolescence and early adolescence stages represented in our subject pool, than they might for younger children. That is, given the developmental task of individuating from one's parents during adolescence (Csikszentmihalyi and Larson, 1984), above average scores on the security scale in the age range reflected in our sample might be indicative of psychosocial distress that leads children to cling closer to their mothers. Our findings also point to the importance of looking at different indicators of HPA-axis functioning, such as the diurnal cortisol slope and bedtime cortisol level outcome variables used in this study, at both the within- and between-person levels of analysis. Relatedly, our study highlights the importance of considering individual differences in daily processes, or, in other words, moderators of within-person associations. Doing so may uncover otherwise undetected interaction effects and identify conditions under which certain daily effects (such as the effects of daily father-child conflict on children's bedtime cortisol levels) occur. This approach may facilitate more fine-grained analyses and progress toward understanding the ways in which short-term processes may be involved in linking family risk and long-term health outcomes.

Tables

Table 1

Time of day predicting children's log transformed cortisol over eight days

Fixed Effect	Coefficient (SE)	<i>t</i> -ratio	<i>df.</i>
Intercept, β_{00}	3.23 (0.06)	57.02***	46
Time of day, β_{10}	-0.19 (0.02)	-10.13***	46
Time of day squared, β_{20}	0.00 (0.00)	2.873**	46
SampleQuestion, β_{40}	-0.29 (0.09)	-3.23**	1303

** $p < .01$, *** $p < .001$

Table 2

Children's and mothers' daily reports of mother-child conflict and children's reports of secure attachment to mothers predicting children's diurnal cortisol slope and bedtime cortisol levels

Fixed Effect	Child-reported daily conflict			Mother-reported daily conflict		
	Coefficient (SE)	<i>t</i> -ratio	<i>d.f.</i>	Coefficient (SE)	<i>t</i> -ratio	<i>d.f.</i>
<i>Outcome: Diurnal cortisol slope</i>						
Intercept, β_{00}	-0.19 (0.01)	-35.01***	41	-0.18 (0.01)	-33.58***	42
Sex, β_{01}	0.00 (0.01)	0.58	41	0.00 (0.01)	0.36	42
Age, β_{02}	0.01 (0.00)	2.36*	41	0.01 (0.00)	1.84	42
Secure attachment to mother, β_{03}	0.03 (0.01)	3.27**	41	0.04 (0.01)	3.56***	42
Mother-child conflict, β_{10}	0.00 (0.01)	-0.06	43	0.01 (0.01)	0.36	44
Secure attachment to mother, β_{11}	-0.01 (0.02)	-0.56	43	0.01 (0.03)	0.27	44
<i>Outcome: Bedtime cortisol</i>						
Intercept, β_{00}	0.93 (0.06)	14.58***	42	0.93 (0.06)	14.7***	42
Sex, β_{01}	-0.04 (0.06)	-0.60	42	-0.05 (0.06)	-0.79	42
Age, β_{02}	0.05 (0.04)	1.24	42	0.04 (0.04)	1.07	42
Secure attachment to mother, β_{03}	0.15 (0.14)	1.01	42	0.17 (0.14)	1.28	42
Time, β_{10}	0.02 (0.03)	0.56	45	0.01 (0.03)	0.43	45
Mother-child conflict, β_{20}	0.01 (0.08)	0.14	44	-0.10 (0.13)	-0.80	44
Secure attachment to mother, β_{21}	0.02 (0.15)	0.15	44	-0.26 (0.29)	-0.89	44
Sample Question, β_{30}	-0.17 (0.12)	-1.40	229	-0.15 (0.12)	-1.29	229

* $p < .05$, ** $p < .01$, *** $p < .00$

Table 3

Children's and fathers' daily reports of father-child conflict and children's reports of secure attachment to fathers predicting children's diurnal cortisol slope and bedtime cortisol levels

Fixed Effect	Child-reported daily conflict			Father-reported daily conflict		
	Coefficient (SE)	t-ratio	d.f.	Coefficient (SE)	t-ratio	d.f.
<i>Outcome: Diurnal cortisol slope</i>						
Intercept, β_{00}	-0.19 (0.01)	-31.86***	40	-0.19 (0.01)	-28.24***	32
Sex, β_{01}	0.01 (0.01)	1.23	40	0.00 (0.01)	0.23	32
Age, β_{02}	0.01 (0.00)	1.69	40	0.00 (0.00)	0.65	32
Secure attachment to father, β_{03}	0.02 (0.01)	1.99	40	0.02 (0.02)	1.52	32
Father-child conflict, β_{10}	0.01 (0.02)	0.63	42	0.04 (0.03)	1.35	34
Secure attachment to father, β_{11}	-0.04 (0.04)	-1.03	42	-0.10 (0.07)	-1.42	34
<i>Outcome: Bedtime cortisol</i>						
Intercept, β_{00}	0.93 (0.06)	14.91***	41	0.97 (0.07)	13.78***	33
Sex, β_{01}	-0.01 (0.06)	-0.13	41	0.00 (0.06)	-0.06	33
Age, β_{02}	0.04 (0.03)	1.30	41	0.06 (0.03)	1.80	33
Secure attachment to father, β_{03}	0.21 (0.15)	1.42	41	0.13 (0.18)	0.74	33
Time of day, β_{10}	-0.01 (0.03)	-0.22	44	-0.03 (0.03)	-0.92	36
Father-child conflict, β_{20}	-0.02 (0.10)	-0.21	43	0.10 (0.13)	0.74	35
Secure attachment to father, β_{21}	-0.09 (0.25)	-0.35	43	-0.82 (0.30)	-2.72**	35
Sample Question, β_{30}	-0.14 (0.11)	-1.24	232	-0.12 (0.10)	-1.11	256

** $p < .01$, *** $p < .001$

Figures

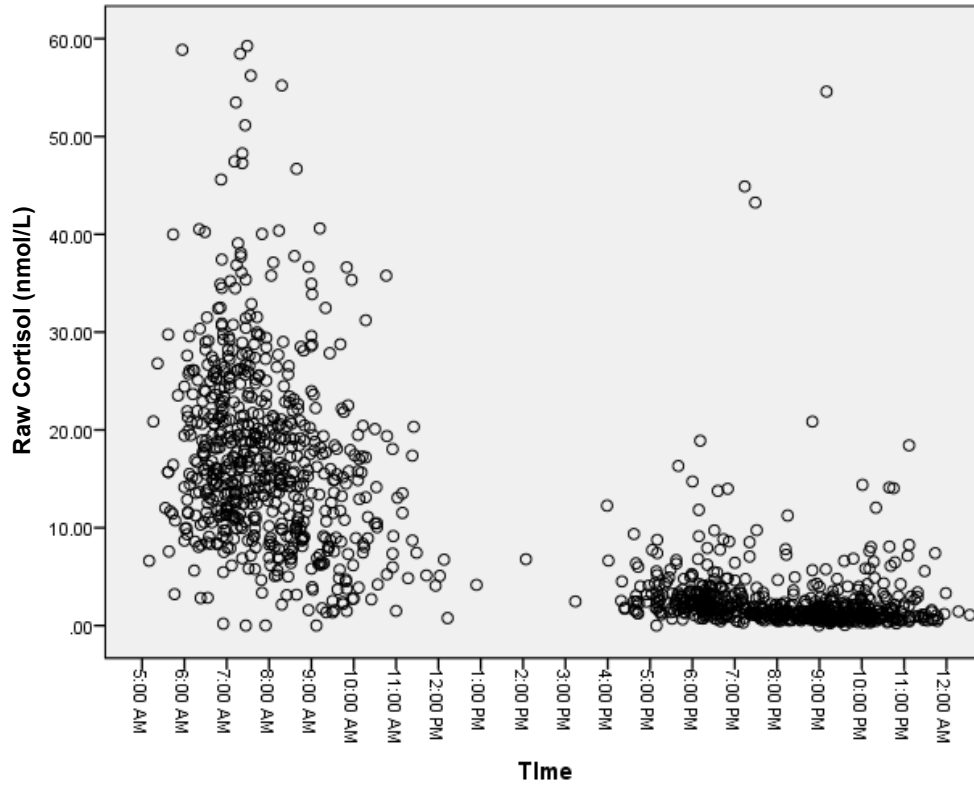


Figure 1. Raw cortisol values by time of day (centered on 5:00 am) for all children over eight days of data collection ($n = 47$ participants).

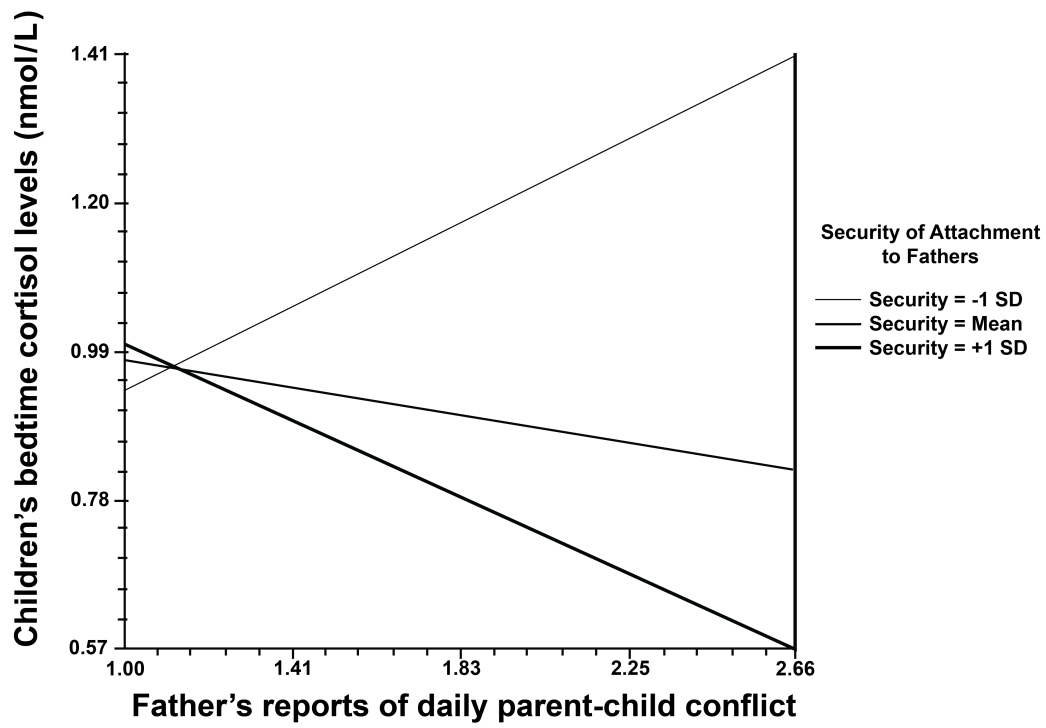


Figure 2. Fathers' daily reports of parent-child conflict and children's reports of secure attachment to fathers predicting children's log transformed bedtime cortisol levels. Average within-person associations between father-child conflict ratings and children's bedtime cortisol levels are depicted for secure attachment to fathers at the sample mean, + 1 standard deviation above the sample mean, and - 1 standard deviation below the sample mean.

Appendix A-1

Daily Parent-Child Conflict Items

Child report items

Please tell us about your day with your MOM/DAD:	<i>Not at all</i>	<i>Some</i>	<i>A lot</i>
1. My mom/dad got mad at me today	1	2	3
2. I was angry at my mom/dad today	1	2	3
3. My mom/dad punished me today	1	2	3

Parent report items

Please complete the following sentences:

Today, I	<i>Not at all</i>	<i>Some</i>	<i>A lot</i>
1. ...punished my child	1	2	3
2. ...nagged my child	1	2	3
3. ...yelled at my child	1	2	3
4. ...was irritated with my child	1	2	3
5. ...was angry with my child	1	2	3
6. ...had to warn my child s/he might be punished	1	2	3
7. ...had to tell my child to stop doing something	1	2	3
8. ...had to ask my child to do something (chore) more than once	1	2	3
9. How angry was your child at you today?	1	2	3

Appendix A-2

Security Scale Items, Mother Version¹

This questionnaire asks about what you are like with your mother – like how you act and feel around her. Before we get to those questions, let’s try a practice question. Each question talks about two kinds of kids, and we want to know which kids are most like you. Decide first whether you are more like the kids on the left side or more like the kids on the right side, then decide whether that is sort of true for you, or really true for you, and circle that phrase. For each question you will only circle one answer.

- | | | | |
|----|--|------------------------|---|
| 1. | Some kids find it easy to trust their mom | BUT | Other kids are not sure if they can trust their mom. |
| | Really true
for me | Sort of
true for me | Sort of
true for me Really true
for me |
| | | | |
| 2. | Some kids feel like their mom butts in a lot when they are trying to do things | BUT | Other kids are feel like their mom lets them do things on their own |
| | Really true
for me | Sort of
true for me | Sort of
true for me Really true
for me |
| | | | |
| 3. | Some kids find it easy to count on their mom for help | BUT | Other kids think it’s hard to count on their mom |
| | Really true
for me | Sort of
true for me | Sort of
true for me Really true
for me |
| | | | |
| 4. | Some kids think their mom spends enough time with them | BUT | Other kids think their mom does not spend enough time with them. |
| | Really true
for me | Sort of
true for me | Sort of
true for me Really true
for me |
| | | | |
| 5. | Some kids do not really like telling their mom what they are thinking or feeling | BUT | Other kids do like telling their mom what they are thinking or feeling. |
| | Really true
for me | Sort of
true for me | Sort of
true for me Really true
for me |

¹ Children completed a parallel version of this scale for fathers with the word “dad” in place of “mom” and all pronouns changed accordingly

- | | | | |
|-----|--|------------------------|---|
| 6. | Some kids do not really need their mom for much | BUT | Other kids need their mom for a lot of things. |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 7. | Some kids wish they were closer to their mom | BUT | Other kids are happy with how close they are to their mom . |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 8. | Some kids worry that their mom does not really love them | BUT | Other kids are <u>really</u> sure that their mom loves them. |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 9. | Some kids feel like their mom really understands them | BUT | Other kids feel like their mom does not really understand them. |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 10. | Some kids are really sure their mom would not leave them | BUT | Other kids sometimes wonder if their mom might leave them |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 11. | Some kids worry that their mom might not be there when they need her | BUT | Other kids are sure their mom will be there when they need her. |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |
| | | | |
| 12. | Some kids think their mom does not listen to them | BUT | Other kids do think their mom listens to them. |
| | Really true
for me | Sort of
true for me | Sort of
true for me |
| | | | Really true
for me |

- | | | | | |
|-----|---|------------------------|---|-----------------------|
| 13. | Some kids go to their mom when they are upset | BUT | Other kids do not go to their mom when they are upset | |
| | Really true
for me | Sort of
true for me | Sort of
true for me | Really true
for me |
| 14. | Some kids wish their mom would help them more with their problems | BUT | Other kids think their mom helps them enough. | |
| | Really true
for me | Sort of
true for me | Sort of
true for me | Really true
for me |
| 15. | Some kids feel better when their mom is around | BUT | Other kids do not feel better when their mom is around. | |
| | Really true
for me | Sort of
true for me | Sort of
true for me | Really true
for me |

References

- Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. *Psychoneuroendocrinology, 31*, 664–679.
- Adam, E. K., Hawkley, L. C., Kudielka, B. M., & Cacioppo, J. T. (2006). Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. *Proceedings of the National Academy of Sciences, 103*(45), 17058-17063.
- Ainsworth, M. D. S., Blehar, M. C., Waters, E., & Wall, S. (1978). *Patterns of attachment*. Hillsdale, NJ: Erlbaum.
- Almeida, D. M., Wethington, E., & McDonald, D. A. (2001). Daily variation in paternal engagement and negative mood: Implications for emotionally supportive and conflictual interactions. *Journal of Marriage and Family, 63*, 417–429. doi:10.1111/j.1741-3737.2001.00417.x
- Ahnert, L., Gunnar, M. R., Lamb, M. E., & Barthel, M. (2004). Transition to child care: Associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75*(3). 639-650.
- Ben-Dat Fisher, D. B., Serbina, L.A., Stacka, D. M. Ruttle, P. L., Ledingham, J. E., & Schwartzman, A.E. (2007). Intergenerational predictors of diurnal cortisol secretion in early childhood. *Infant and Child Development, 16*, 151–170.
- Bernard, K., Butzin-Dozier, Z., Rittenhouse, J., & Dozier, M. (2010). Cortisol production patterns in young children living with birth parents versus children placed in foster care following involvement of child protective services. *Archives of Pediatrics & Adolescent Medicine, 164*(5), 438-443.
- Booth, A., Granger, D. A. & Shirtcliff, E. A. (2008), Gender- and age-related differences in the

- association between social relationship quality and trait levels of salivary cortisol. *Journal of Research on Adolescence*, 18, 239–260. doi:10.1111/j.1532-7795.2008.00559.x
- Borelli, J. L., Crowley, M. J., David, D.H., Sbarra, D. A., Anderson, G. M., & Mayes, L.C. (2010) Attachment and emotion in school-aged children. *Emotion*, 10(4), 475–485
- Bowlby, J. (1973). *Attachment and loss: Vol. 2: Separation. Anxiety and danger*. New York: Basic Books.
- Bowlby, J. (1979). The making and breaking of affectional bonds: Aetiology and psychopathology in the light of attachment theory (An expanded version of the Fiftieth Maudsley Lecture, delivered before the Royal College of Psychiatrists, 10 November 1976). *The British Journal of Psychiatry*, 130, 201-210.
- Bowlby, J. (1988). *A secure base: Parent-child attachment and healthy human development*. New York: Basic Books.
- Campos, B., Graesch, A. P., Repetti, R., Bradbury, T., & Ochs, E. (2009). Opportunity for interaction? A naturalistic observation study of dual-earner families after work and school. *Journal of Family Psychology*, 23, 798–807.
- Chung, G. H., Flook, L., & Fuligni, A. J. (2009). Daily family conflict and emotional distress among adolescents from Latin American, Asian, and European backgrounds. *Developmental Psychology*, 45, 1406–1415. doi:10.1037/a0014163.
- Cicchetti, D., & Rogosch, F.A. (2001). Diverse patterns of neuroendocrine activity in maltreated children. *Development and Psychopathology*, 13, 677-693
- Csikszentmihalyi, M. and Larson, R. (1984). *Being adolescent: Conflict and growth in the teenage years*. New York: Basic Books.

- Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355-391.
- Dickerson, S. S., & Zoccola, P. M. (2009). Toward a biology of social support. In C. R. Snyder & S. J. Lopez (Eds.), *Oxford Handbook of Positive Psychology* (2nd ed., pp. 519-526). New York: Oxford University Press.
- Doumas, D. M., Margolin, G., & John, R. S. (2003). The relationship between daily marital interaction, work, and health-promoting behaviors in dual-earner couples: An extension of the work-family spillover model. *Journal of Family Issues*, *24*, 3-20.
- Dressendorfer, R. A. et al. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *The Journal of Steroid Biochemistry and Molecular Biology*, *43*(7), 683-92.
- Edwards, V. J., Holden, G. W., Felitti, V.J., & Anda, R. F. (2003). Relationship between multiple forms of childhood maltreatment and adult mental health in community respondents: Results from the adverse childhood experiences study. *American Journal of Psychiatry*, *160*, 1453-1460.
- Fuligni, A. J., & Masten, C. L. (2010). Daily family interactions among young adults in the United States from Latin American, Filipino, East Asian, and European backgrounds. *International Journal of Behavioral Development*, *34*, 491-499.
doi:10.1177/0165025409360303
- Fuligni, A. J., Telzer, E. H., Bower, J., Cole, S. W., Kiang, L. & Irwin, M. R. (2009). A preliminary study of daily interpersonal stress and C-reactive protein levels among adolescents from Latin American and European backgrounds. *Psychosomatic Medicine*, *71*(3), 329-333.

- Garcia O'Hearn, H., Margolin, G., & John, R. S. (1997). Mothers' and fathers' reports of children's reactions to naturalistic marital conflict. *Journal of the American Academy of Child & Adolescent Psychiatry*, 36, 1366-1373.
- Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology. Special Issue: Stress and Drug Abuse*, 27(1-2), 199.
- Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development & Psychopathology* 13(3), 515-538.
- Hanson, M. D., & Chen, E. (2010). Daily stress, cortisol, and sleep: The moderating role of child- hood psychosocial environments. *Health Psychology*, 29, 394–402.
doi:10.1037/a0019879
- Hoffman, L. (2007). Multilevel models for examining individual differences in within-person variation and covariation over time. *Psychology, Department of Faculty Publications, Department of Psychology, University of Nebraska – Lincoln*.
- Hruschka, D. J., Kohrt, B. A., & Worthman, C. M. (2005). Estimating between-and within-individual variation in cortisol levels using multilevel models. *Psychoneuroendocrinology*, 30(7), 698–714.
- Kaufman, J., & Charney, D. (2001). Effects of early stress on brain structure and function: Implications for understanding the relationship between child maltreatment and depression. *Development and Psychopathology. Special Issue: Stress and Development: Biological and Psychological Consequences*, 13(3), 451.

- Kerns, K. A. (2008). Attachment in middle childhood. In Cassidy, J. & Shaver, P. R. (Eds.), *Handbook of attachment: Theory, research, and clinical applications, 2nd edition*, 366–382. New York: Guilford Press.
- Kerns, K. A., Lepak, L., & Cole, A. K. (1996). Peer relationships and preadolescents' perceptions of security in the child-mother relationship. *Developmental Psychology*, *32*, 457-466.
- Kerns, K. A., Tomich, P. L., Aspelmeier, J. E., & Contreras, J. M. (2000). Attachment-based assessments of parent-child relationships in middle childhood. *Developmental Psychology*, *36*, 614-626.
- Kiang, L., & Buchanan, C. M. (2014). Daily stress and emotional well-being among Asian American adolescents: Same-day, lagged, and chronic associations. *Developmental Psychology*, *50*, 611–621. doi:10.1037/a0033645
- Kirschbaum, C. & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology* *22*(3), 150–169.
- Luecken, L. J. (1998). Childhood attachment and loss experiences affect adult cardiovascular and cortisol function. *Psychosomatic Medicine*, *60*, 765–772.
- Lupien, S. J., King, S., Meaney, M. J., & McEwen, B. S. (2001). Can poverty get under your skin?: Basal cortisol levels and cognitive function in children from low and high socioeconomic status. *Developmental Psychopathology*, *13*, 653-676.
- Margolin, G. (1990). *Parent Home Data Questionnaire*. Los Angeles: University of Southern California.
- McEwen, B. S. (1998). Stress, adaptation and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*, 33–44.

- McEwen, B. S. (2007) Physiology and neurobiology of stress and adaptation: central role of the brain, *Physiological Reviews*, 87, 873–904.
- McEwen, B. S., & Seeman, T. E. (1999). Protective and damaging effects of mediators of Stress: Elaborating and testing the concepts of allostasis and allostatic load, *Annals of the New York Acadademy of Sciences*, 896, 30–47.
- Miller, G. E., Chen, E., & Parker, K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. *Psychological Bulletin*, 137(6):959-97. doi: 10.1037/a0024768
- Pendry, P., & Adam, E. K. (2007). Associations between parents' marital functioning, maternal parenting quality, maternal emotion and child cortisol levels. *International Journal of Behavioral Development*, 31(3), 218-218.
- Polk, D. E. et al. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology*, 30(3), 261-272.
- Raudenbush, S. W., Bryk, A. S., Cheong, Y., & Congdon, R. T. (2004). HLM 6: Hierarchical linear and nonlinear modeling. Lincolnwood, IL: Scientific Software International.
- Repetti, R. L. (1996). The effects of perceived daily social and academic failure experiences on school-age children's subsequent interactions with parents. *Child Development*, 67, 1467-1482.
- Repetti, R. L., Reynolds, B. M., & Sears, M. S. (2015). Families under the microscope: Repeated sampling and recordings of perceptions, experiences, biology and behavior. *Journal of Marriage and Family*, 77(1), 126-146. DOI: 10.1111/jomf.12143
- Repetti, R. L., Robles, T. F., & Reynolds, B. M. (2011). Allostatic processes in the family. *Development and Psychopathology*, 23, 921–938.

- Repetti, R. L., Taylor, S. E., & Seeman, T.E. (2002). Risky families: Family social environments and the mental and physical health of offspring. *Psychological Bulletin*, *128*, 330–366.
- Sapolsky, R. M., Krey, L .C, & McEwen, B.S. (1986). The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocrine Reviews* *7*, 284-301.
- Saxbe, D. E., Repetti, R. L., & Graesch, A. P. (2011). Time spent in housework and leisure: Links with parents' physiological recovery from work. *Journal of Family Psychology*, *25*, 271–281.
- Saxbe, D.E., Repetti, R. L., & Nishina, A. (2008). Marital satisfaction, recovery from work, and diurnal cortisol among men and women. *Health Psychology*, *27*(1), 15-25.
- Seeman, T. E. (1996). Social ties and health: The benefits of social integration. *Annals of Epidemiology*, *6*(5), 442-451
- Slatcher, R. B., & Robles, T. F. (2012). Preschoolers' everyday conflict at home and diurnal cortisol patterns. *Health Psychology*, *31*, 834–838. doi:10.1037/a0026774
- Smyth, J. M., Ockenfels, M.C., Porter, L. S., Kirschbaum, C., Hellhammer, D. H., Stone, A. A. (1998). Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology*, *23*, 353-370.
- Stawski, R. S., Cichy, K. E., Piazza, J. R., & Almeida, D. M. (2013). Associations among daily stressors and salivary cortisol: Findings from the National Study of Daily Experiences. *Psychoneuroendocrinology*, *38*, 2654–2665. doi:10.1016/j.psyneuen.2013.06.023
- Taylor, S. E., Lerner, J. S., Sage, R. M., Lehman, B. J., & Seeman, T. E. (2004). Early environment, emotions, responses to stress, and health. Special issue on personality and health. *Journal of Personality*, *72*, 1365-1393.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, 53, 865-871.

Uchino, B. N., Uno, D., & Holt-Lunstad, J. (1999). Social support, physiological processes, and health. *Current Directions in Psychological Science*, 8, 145-148.

Vining, R. F., McGinley, R.A., Maksvytis, J. J., & Ho, K. Y. (1983). Salivary cortisol: A better measure of adrenal cortical function than serum cortisol. *Annals of Clinical Biochemistry*, 20(6), 329-335.

STUDY 2

Stability in Children's Diurnal Cortisol and Connections with Cortisol

Reactivity to and Recovery from a Laboratory Stressor

Abstract

Despite a rich and growing body of evidence linking early stress, HPA-axis functioning, and mental and physical health outcomes, there is surprisingly little known about the temporal stability of children's diurnal cortisol and how children's reactivity to acute stressors is concurrently related to their diurnal cortisol profiles. To address these gaps in the literature, we calculated multilevel-model derived ICCs to assess short-term temporal stability in four metrics of diurnal cortisol - waking cortisol levels, cortisol awakening response (CAR), diurnal slope, and bedtime cortisol levels - over a range of 2 to 8 sample days spanning a three week study period in a community sample of eight- to 13-year-olds. We also explored associations between each diurnal cortisol metric and children's reactivity to and recovery from the Trier Social Stress Task for Children (TSST-C). We found that, overall, children's diurnal cortisol metrics were moderately stable, with the highest stability levels observed in bedtime cortisol levels and lowest stability estimates observed in the CAR. Surprisingly, on the whole, increasing the number of sample days did not result in improvements in stability. Bedtime cortisol levels were more stable on weekdays while the CAR was more stable on weekends and there was more within-person variability in girls' waking cortisol levels compared to boys. Better cortisol recovery from the laboratory stressor task was significantly correlated with higher waking cortisol levels and correlated with higher CARs at the marginal significance level, while cortisol reactivity to the laboratory tasks was not associated with any of the diurnal cortisol variables.

The Hypothalamus Pituitary Adrenal axis (HPA-axis) is a critical component of the stress response system affecting emotional, cognitive, and physiological responses to stress. Its end product, the hormone cortisol, is released in response to a range of psychological and physical stressors with widespread influences including those on neural processes essential to cognition, emotion, memory, and metabolism (McEwen, 2007; Sapolsky, Romero, & Munck, 2000). In addition to the vital role that stress-induced increases in cortisol play in facilitating adaptation to stress, basal levels of this hormone, released in a diurnal rhythm over the course of the day, are also essential in supporting basic physiological functioning (Tsigos & Chrousos, 2002). Although short-term increases in cortisol in response to acute threats (reactivity) facilitate adaptive responses to environmental challenges, frequent and or prolonged activation of the HPA-axis is believed to result in cumulative “wear and tear”, or *Allostatic load* (McEwen, 1998). Chronic stressors, such as adverse early rearing conditions, have been linked with dysregulation of both the diurnal and stress-responsive components of the HPA-axis (Gunnar & Donzella, 2002; Repetti, Robles, & Reynolds, 2011; Repetti, Taylor, & Seeman, 2002). Furthermore, dysregulation of both diurnal cortisol activity and stress-induced reactivity has been associated with poor long-term mental and physical health outcomes, suggesting a mechanistic role in the link between stress and disease (McEwen & Seeman, 1999; Repetti et al., 2002; 2011).

As the developmental period directly preceding increased onset of various psychological disorders (Oskis et al., 2009), middle childhood, along with early adolescence, represent important periods from which to understand typical HPA-axis functioning and may provide a foundation from which to further explore emerging neuroendocrine-health associations. However, despite a robust literature connecting chronic psychosocial stress, particularly in the context of early rearing conditions, changes in HPA axis activity and reactivity, and long-term

mental and physical health outcomes, important questions still remain: 1) Although the Allostatic load literature has used children's diurnal cortisol as an individual difference variable, little is known about the temporal stability of diurnal cortisol activity during childhood, such as whether and which diurnal cortisol metrics may reflect stable between-person differences. 2) To our knowledge, no published studies have investigated links between children's diurnal cortisol activity and acute stress reactivity and recovery, despite the posited mechanistic link between chronic stress and health by way of wear and tear on the HPA-axis. We aim to address these gaps in the literature by assessing the magnitude of short-term stability in various metrics of children's diurnal cortisol, as well as differences in magnitude related to the number of days of data collection; we also explore age and sex differences in intra-individual variability of children's diurnal cortisol activity and effects of weekend versus weekday on stability of children's diurnal cortisol. Finally, we explore correlations between multiple parameters of children's diurnal cortisol activity and their cortisol reactivity to and recovery from an acute laboratory stressor.

Stability in Diurnal HPA-axis Activity

The scientific community has treated indicators of children's diurnal cortisol as stable, individual differences. The Allostatic load literature, for example, and models drawing on it, such as the Risky Families model (Repetti et al., 2002), posit that we can begin to see differences in HPA-axis functioning due to cumulative wear and tear associated with early stress exposure during childhood. This notion is reflected in numerous studies testing linkages between various metrics of children's diurnal cortisol and a multitude of stable, individual difference, or trait, variables such as family environment, temperament, and mental and physical health. Embedded in these designs is the assumption that children's diurnal cortisol profiles represent the emergence of stable individual differences in children's HPA-axis functioning. However,

stability in children's diurnal cortisol measures has not been adequately investigated, and limitations of the studies that have explored this concept make it difficult to draw conclusions. For example, while experts in the field recommend collecting diurnal saliva samples over multiple days to improve reliability (Adam & Kumari, 2009; Stewart & Seeman, 2000), empirical evidence on incremental improvements in stability associated with increased sample days and the relative stability of different parameters of children's diurnal cortisol is lacking.

Stability in Adults' Diurnal Cortisol. Assumptions regarding stability in children's diurnal cortisol parameters have largely been influenced by evidence from the adult diurnal cortisol literature. Among adults, there is evidence of moderate¹ to high within-person stability in the cortisol awakening response (CAR) and the diurnal slope over the day (Edwards, Clow, Evans, & Hucklebridge, 2001; Wuest, Hellhammer, Federenko, Schommer, & Kirschbaum, 2000), signifying that they are reliable indices of trait characteristics in adults. Over two consecutive or closely spaced days, correlations between the CAR measures (as indexed by Area Under the Curve, or AUC) ranged from 0.52 – 0.63 (Edwards et al., 2001, Wuest et al., 2000) and across day correlations for diurnal slope ranged from $r = .45 - .66$ (Edwards et al., 2001; Kraemer et al., 2006) in samples of healthy adults. Over the past decade, researchers have extended these findings by utilizing Intraclass Correlation Coefficients (ICC; Shrout & Fleiss, 1979) to parse out sources of variance due to differences within- and between-individuals in estimations of diurnal cortisol stability; ICCs provide an estimate of the proportion of variance that is attributed to between-person differences relative to total variance (within-person plus between-person). In line with previous bivariate correlational findings, ICCs for waking cortisol profiles over two consecutive or closely-spaced days ranged from 0.62 – 0.69 among samples of

¹ Our use of qualitative terms, such as modest, moderate, and high, to describe estimates of stability in the extant literature is based on interpretations provided by the study authors in the cited peer-reviewed articles.

healthy women (Ranjit, Young, Raghunathan, & Kaplan. 2005) and healthy men and women (Hellhammer et al., 2007). These findings indicate that the majority of variance -- approximately two thirds -- in adults' awakening responses over relatively short timeframes can be attributed to between-person differences.

Researchers have noted, however, that the CAR and diurnal slope are influenced by situational, or state, factors and, for some metrics of cortisol, such as AUC with respect to increase, more sample days (five) were necessary to achieve stability results comparable to those observed in other metrics, such as AUC with respect to ground, over only two consecutive days (Hellhammer et al., 2007; Stewart & Seeman, 2000; Thorn, Hucklebridge, Evans, & Clow, 2009). Moreover, effects of weekend versus weekday sampling status have been shown to influence cortisol responses to awakening in adults (Karlamanla, Friedman, Seeman, Stawski, & Almeida, 2013; Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). And, when time between samples is increased to two to three months, ICCs have been shown to decrease (total daily output: ICC = .30, diurnal slope: ICC = .23, CAR: ICC = .10; Ross, Murphy, Adam, Chen, & Miller, 2014) relative to measurements taken days and weeks apart. Thus, while substantial between-person variability exists in these indices of adult diurnal cortisol secretion, particularly over short-term time frames, they also seem to be influenced by time-varying within-person factors. To our knowledge, there are no published studies reporting stability in single sample measures of diurnal cortisol, such as waking or bedtime levels, in adult samples.

Stability in Children's Diurnal Cortisol. Research on the stability of diurnal cortisol in child and adolescent populations is sparse, and the majority of existing studies represent sampling methodologies that limit generalizability of findings and/or prohibit investigation of the full diurnal cortisol profile. However, the limited number of studies in the extant literature

suggest moderate to high stability in various aspects of children's and adolescents' diurnal cortisol profiles. For example, moderate to high stability has been observed in the CAR ($r = .39 - .67$; Oskis et al., 2009; Pruessner et al., 1997; ter Wolbeek et al., 2007) and diurnal slope ($r = .73$; Oskis et al., 2009) over two consecutive days. Similarly, single sample measures have demonstrated moderate to high stability over five consecutive days at 9 am ($\alpha = .73$) and 4 pm ($\alpha = .68$; Cicchetti & Rogosch, 2001) and over 3 consecutive days at awakening, 30 minutes post-wake, in the afternoon, and at bedtime ($\alpha = .49, .77, .58, \text{ and } .75$, respectively; O'Connor et al., 2005). Most recently, multilevel model-derived ICCs indicated moderate to high stability in a variety of metrics of children's and adolescents' diurnal cortisol profiles across two to three days sampled over a two week timeframe: ICCs averaged 0.54 for aggregate measures (e.g. total cortisol output over the day), 0.22 for dynamic measures (e.g. diurnal slope, AUC increase), and 0.28 for single sample (e.g. wake, bedtime) metrics (Rotenberg, McGrath, Roy-Gagnon, & Thanh Tu, 2012). As with adults, however, stability estimates were lower when samples were spaced months to a year apart, rather than days and weeks: Collected annually over the course of two to three consecutive years, stability of the CAR in adolescents was modest, with stability estimates ranging from .09 to .17 (Ross et al., 2014; Platje et al., 2013). Similarly, samples taken at 6 month to two year intervals over the course of two to six years yielded modest stability estimates, with 12 to 13% of variability in diurnal slopes and total cortisol output attributed to stable, inter-individual differences (Ross et al., 2014; Shirtcliff et al., 2012).

Taken together, these limited findings suggest moderate to high short-term stability in various metrics of the diurnal cortisol profiles of children and adolescents. However, the generalizability and robustness of these findings has been limited by methodologies that prohibit tests of within-person stability over the full diurnal profile due to the timing of cortisol samples

(e.g. Cicchetti and Rogosch, 2001; Pruessner et al., 1997), or are constrained by sample characteristics, such as samples that include only girls (e.g. ter Wolbeek et al., 2007; Oskis et al., 2009). Moreover, while researchers have recommended collecting diurnal cortisol over multiple days to improve accuracy in estimating person-level diurnal cortisol parameters (e.g. Adam & Kumari, 2009; Stewart & Seeman, 2000), there is a lack of empirical evidence guiding research on the optimal number and spacing of days, and relative stability in one metric compared to another. Using estimates of sources of variance derived from multilevel models, researchers have calculated the projected number of days necessary to obtain optimal levels of reliability (.80; Hruschka et al., 2005; Rotenberg et al., 2012). According to those calculations, when multiple samples are collected over the day, as few as one to four days of sampling might suffice for reliable estimates of total cortisol output (AUCg) and mean levels, whereas daily samples would be needed for one week to obtain reliable awakening estimates and for two weeks to obtain reliable estimates of bedtime levels and slope (Hruschka et al., 2005; Rotenberg et al., 2012). Similarly, guidelines put forth by the MacArthur Foundation Research Network recommend 4 days of data to achieve reliable AUCg estimates and 8 days for stable estimates of slope when cortisol is sampled repeatedly over each day (Stewart & Seeman, 2000). To our knowledge, however, no studies have empirically tested whether gains in children's diurnal cortisol ICC levels are obtained with incremental increases in the number of sample days. Furthermore, to our knowledge, predictors of variability such as weekend sample status, age, and sex have not been investigated in the child diurnal cortisol literature.

These important remaining questions call for a thorough investigation of stability in children's diurnal cortisol. We seek to add to and extend the literature on children's stability by answering the following questions: a) What is the proportion of variance in children's diurnal

cortisol attributed to between-subject factors when measured over relatively short-term timeframes (e.g. days and weeks)?, b) Are some indices of children’s diurnal cortisol more stable than others?, c) Does increasing the number of sample days have a dose-response effect on stability estimates?, and d) Do stability estimates vary depending on weekend/weekday sampling, or the child’s age or gender? The answers to these questions will help to identify the metrics of cortisol that are optimal to use when addressing different research goals. For example, if a researcher were interested in exploring individual differences in diurnal cortisol production, cortisol metrics with adequate between-person variability would be appropriate metrics for this type of investigation, whereas metrics with proportionately more within-subject variability would be appropriate for questions involving “state” constructs that also vary within individuals, such as day-to-day changes in mood. The results can also inform study design, such as the number of cortisol sampling days, and consideration of other factors that might affect stability.

Associations Between Children’s Diurnal Cortisol and Stress-Induced Cortisol Responses

As noted above, the HPA-axis releases cortisol both in a diurnal rhythm over the course of the day and in short-term increases in response to stress. While reactivity to stress is an essential function of the HPA-axis that helps organisms mount an adaptive response to challenges encountered, wear and tear can occur when the HPA-axis is activated frequently or for prolonged periods of time due to exposure to chronic or frequent stress (McEwen, 1998; 2000). Resulting dysregulation of the underlying diurnal cortisol profile has been posited as a mechanism linking early exposure to stress, such as family environments ranging from non-nurturant or conflict-ridden to abusive or neglectful, with long-term mental and physical health outcomes such as depression, cardiovascular disease, and a host of other conditions (Repetti et al., 2002; 2011). However, despite the theoretical and empirical links between frequent or

sustained stress-induced HPA-axis reactivity and suspected alterations in basal HPA-axis activity in the pathogenesis of disease, there is a dearth of research testing concurrent associations between diurnal HPA-axis functioning and neuroendocrine reactivity to and recovery from acute stressors. Similarly, while an abundance of research shows that acute psychosocial stressors such as the Trier Social Stress Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) and TSST for Children (TSST-C; Buske-Kirschbaum, Jobst, Wustmans, & Kirschbaum, 1997) are reliably experienced as stressful and trigger short-term increases in cortisol (e.g. Dickerson & Kemeny, 2004), demonstrating high internal validity, there is a lack of evidence supporting the external validity of these tasks. For instance, while laboratory stressor tasks like the TSST-C reliably increase cortisol levels, it is not known if an individual's pattern of cortisol reactivity and recovery in this context inform us about their naturalistic diurnal cortisol activity. Addressing these gaps in the literature has the potential to advance our understanding of the developmental trajectory in dysregulation of HPA-axis functioning implicated in links between early stress exposure and later adverse mental and physical health outcomes.

To our knowledge, no published studies have assessed associations between children's diurnal cortisol profiles with their reactivity to and recovery from laboratory stressor tasks, and only a handful of studies have carried out this line of investigation in adult samples. Of the four studies that have investigated associations between adult participants' CAR and cortisol reactivity to laboratory stress tasks, three found no associations (Kidd, Carvalho, & Steptoe, 2014; Schmidt-Reinwald et al., 1999; Wolfram, Bellingrath, Feuerhahn, & Kudielka, 2013), while one study reported a negative correlation: individuals with greater cortisol reactivity in the laboratory had smaller CARs (Quirin, Pruessner, & Kuhl, 2008). To our knowledge, adult cortisol reactivity to laboratory tasks has been tested as a correlate of diurnal cortisol slope and

mean cortisol levels over the day in one study each, with no associations found for either diurnal slope (Kidd et al., 2014) or mean cortisol levels over the day (van Eck, Nicolson, Berkhof, & Sulon, 1996). However, Kidd and colleagues (2014) found that laboratory cortisol reactivity was positively associated with total daily cortisol output (AUC) among a large sample of older men and women. With these limited and inconsistent findings, it is difficult to draw conclusions about links between stress-induced laboratory cortisol and diurnal cortisol activity over the day among adult populations. As noted, analogous studies on children are absent from the literature. It should also be noted that, to the best of our knowledge, existing studies have only explored links between diurnal cortisol activity and laboratory *reactivity* to, but not *recovery* from, stressor tasks (indicated by a return to baseline levels). This is an important distinction because an individual's ability to down regulate the neuroendocrine stress response and quickly return to baseline levels after a stressor has passed is considered adaptive, whereas slower returns to baseline levels prolong exposure to stress hormones, increase risk of deleterious health outcomes, and may signify HPA-axis dysregulation (McEwen, 1998; Seeman & Robbins, 1994; Sapolsky et al., 2000). Therefore, the second major aim of the present study is to assess correlations between various metrics of children's diurnal cortisol activity and laboratory stress-responsive reactivity and recovery.

The Present Study

The present study aims to investigate the magnitude of temporal stability in four metrics of diurnal cortisol believed to characterize children's HPA-axis activity during middle childhood and early adolescence: Waking cortisol levels, cortisol awakening response, diurnal slope, and bedtime cortisol levels. As evidenced by the literature review above, various analytic approaches have been used to test stability in child and adult diurnal cortisol such as bivariate correlations

(e.g. Preussner et al, 1997) and Cronbach's alpha (e.g. Cicchetti & Rogosch, 2001). We use multi-level modeling (MLM) techniques, which allow for the calculation of ICCs by simultaneously modeling within- and between-person effects (and variance components), make adjustments for nested data (e.g. repeated observations nested within persons), are robust to missing data, and are appropriate for the size of our study sample. ICCs will be calculated over an increasing number of days for each cortisol metric (beginning with the first 2 sample days through 8 days sampled over a three week timeframe) in order to 1) determine the relative stability of each cortisol metric of interest and 2) empirically evaluate whether increases in stability are gleaned by incrementally increasing the number of days of data included in analyses. Based on the relatively small extant literature, we expect to find moderate levels of stability across our indices of diurnal cortisol and we expect ICCs levels to increase with corresponding increases in sample days included in analyses. Comparisons between weekend and weekday stability, and tests of age and sex differences in intra-individual variability will also be explored in all four parameters of children's diurnal cortisol; specific predictions are withheld for these exploratory analyses.

The second major aim of this paper is to explore whether any of the metrics of diurnal cortisol of interest are associated with children's reactivity to and recovery from an acute psychosocial stressor. To our knowledge, there are no published studies addressing this research question in children, and the scant findings in the adult stress and health literature do not provide a strong foundation upon which to form hypotheses. However, given that allostatic load presumes cumulative wear and tear on the HPA-axis through chronic and/or frequent activation of the stress response system, we suspect that cortisol responses to laboratory stressors will be related to one or more diurnal cortisol parameter. For example, flatter diurnal slopes might be

associated with hyper- or hypo-reactivity, or sluggish recovery, reflecting dysregulation in both stress-responsive and basal components of the HPA-axis. Importantly, we specifically test associations between daytime cortisol metrics and both reactivity to and recovery from a laboratory stressor.

Methods

As part of a larger study examining family life and health, a variety of data were collected from children and their families using multiple methods such as interviews, questionnaires, an eight-week daily diary period, repeated naturalistic saliva sampling, the collection of other biological samples, and a laboratory stressor task. Since our study is concerned with children's HPA-axis activity, we focus on the methods relevant to estimating children's diurnal cortisol, and cortisol reactivity to and recovery from a stressor task.

Participants

47 children (28 girls) ranging in age from eight to 13 years ($M = 11.8$, $SD = 1.5$) and grades third through ninth (8.5% third, 10.6% fourth, 14.9% fifth, 14.9% sixth, 25.5% seventh, 23.4% eighth, and 2.1% ninth grades) participated. Representative of the ethnically diverse population from which our sample was drawn (the greater Los Angeles area), children were identified as Caucasian (38%), African-American (15%), Hispanic (15%), Asian (11%), and other or mixed ethnicity (21%).

Procedures

Recruitment, inclusionary criteria, and compensation. Our community sample was recruited using a variety of methods such as advertisements in school newsletters, flyers at local schools, clinics, and libraries, and direct mailings to middle class families in the Los Angeles area. In order to participate, children had to 1) be between the ages of 8 and 13 (target child), b)

be able to read and speak English at a 3rd grade level or better, and c) be in normal health, with the exception of a history of frequent upper respiratory infections (URIs), which was recruited for in half of all children as part of the larger study's research aims. Children were excluded if they had medical conditions or engaged in behaviors that can affect neuroendocrine function. Since the occurrence of URIs was an outcome assessed in the larger study, participation coincided with cold and flu season. Children and their participating family members were compensated with cash and gift cards for their participation, with children receiving a base honorarium plus weekly bonus honoraria awarded for thorough completion of measures.

Diurnal salivary cortisol sampling. During the third and sixth weeks of the larger eight-week daily diary study, children provided timed passive drool saliva samples four times per day (at waking, 30 minutes after waking, before dinner, and before bed) over four consecutive days (Saturday through Tuesday). At each sampling occasion, children were instructed to allow saliva to pool in their mouths for 60 seconds before depositing it into a designated vial, repeating the process once more before storing the vial in the freezer. We took several steps to monitor sampling compliance. Along with sample occasion identifiers, saliva vials were labeled with a unique random code which participants recorded alongside an electronic date/time stamp when sampling saliva. These data were later reviewed and any sampling occasions that could not be verified (e.g. data were missing or inconsistent) were identified in a dummy variable (Sample Question, n = 66, 5.2% of the final cortisol sample). Additionally, we flagged participant reports of engaging in potentially confounding activities that they had been instructed to avoid during the half hour before each sampling occasion, such as eating, drinking, or brushing teeth, in another control variable (Sample Confound, n = 105, 8.2%). Finally, half of all participants were randomly assigned MEMS caps (Aardex, Denver, CO) -- an electronic device that records bottle

openings -- on bottles holding straws used for saliva collection, and were told to remove one straw immediately before each sampling occasion. The MEMS recordings allowed us to objectively verify the accuracy of participants' self-reported sampling times and flag any samples in which the two sources of data were more than 30 minutes apart (Mems 30, n = 38, 8% of MEMS samples). Research assistants transported saliva samples to our laboratory where they were stored at -20° Celsius until they were shipped to the Biological Psychology Laboratory at the Technical Institute of Dresden (Dresden, Germany) and assayed with commercial kits (IBL, Hamburg, Germany) for free cortisol using chemiluminescence immunoassay (50 µl saliva required; minimum detection limit <.003 µg/dL, mean intra- and inter-assay coefficients of variance (CV) below 10%; Dressendorfer et al., 1992; Polk et al., 2005).

Laboratory stressor task. Following the eight-week daily diary phase of the larger study, children came to the laboratory and were asked to complete five-minute speech and mental arithmetic tasks before an evaluative audience using an established acute laboratory stressor protocol: the Trier Social Stress Task for children (TSST-C; Buske-Kirschbaum et al., 1997). One child elected not to participate in the TSST-C, and another three children became distressed during the stressor tasks and, therefore, the protocol was aborted in those instances. Thus, a total of 43 of the 47 children participating in the larger study completed the TSST-C.

All laboratory sessions took place in the afternoon and early evening. Salivary cortisol was collected using the passive drool collection method described above at the end of a 35-minute baseline period, and four times over a 30-minute recovery period at 1, 10, 20, and 30 minutes after completing the stressor tasks. After the baseline saliva sample was collected, two confederates posing as evaluators delivered instructions for the speech task. Children received the beginning of a story (see Buske-Kirschbaum et al., 1997) and were given five minutes to

prepare to finish telling the story in a five-minute speech before the evaluators. During the speech task, confederates gave the appearance that they were evaluating children's performance without providing any feedback. After the speech task, children were asked to serially subtract the number 7 from 758 as quickly and accurately as possible for five minutes and prompted to start over when they made a mistake. After completing the stressor tasks, children were debriefed and the remaining four saliva samples were collected over the 30-minute recovery period.

Measures

Due to the positive skew observed in diurnal cortisol and cortisol responses to the TSST-C, all cortisol values were natural log transformed to normalize their distribution.

Diurnal HPA-axis activity. Diurnal HPA-axis activity was measured through the collection of naturalistic salivary cortisol as described in the procedures section above. Children completed 88.6% ($n = 1,332$) of the 1,504 possible diurnal saliva samples. Of those, seven (0.5%) outliers (defined as raw cortisol values over 60 nmol/L) were excluded from analyses. Another 49 (3.7%) saliva samples were missing the corresponding time of day and, since the diurnal rhythm of cortisol is influenced by time of day, these samples were excluded from analyses, leaving 1,276 diurnal cortisol/time pairs. Completion rates were evenly dispersed across sampling occasions with 316 waking, 318 post-wake, 316 dinner, and 326 bedtime samples. The average child completed 3.4 of the possible 4 samples per day.

Four diurnal cortisol metrics were created from these data at both the day and person level of analysis: 1) Waking cortisol levels, which, at the day level, were the log transformed values corresponding with the waking sampling occasion and, at the person level, were estimated in multilevel models described below 2) The cortisol awakening response (CAR), which was estimated at the day level by subtracting the log transformed waking sample from the log

transformed 30-minute post-wake sample (CAR difference score), and was estimated at the person level by including a Level 1 dummy variable indicating the 30-minute post-wake sample in a multi-level model with all cortisol samples as outcomes and participant ID as the Level 2 grouping variable, as described in more detail in the results section below 3) Diurnal slope, which was estimated at the day level by subtracting the log transformed waking sample from the log transformed bedtime sample (Slope difference score), and was estimated at the person level by regressing time of sampling occasion at Level 1 on all cortisol samples available for each person in a multilevel model with participant ID as the Level 2 grouping variable, as described in more detail in the results section below, and 4) Bedtime cortisol levels, which, at the day level, were the log transformed values corresponding with the bedtime sampling occasion on each day, and, at the person level, were estimated in multilevel models described below.

HPA-axis reactivity and recovery.

Children's HPA-axis reactivity to and recovery from a laboratory stressor task was measured through the collection of children's salivary cortisol before and after they completed the speech and math task portions of the TSST-C, as described in the procedures section above. Of the 43 children who completed the TSST-C, there was either missing or insufficient saliva to estimate cortisol levels in two children's baseline cortisol levels and another two children's 30-minute post-task saliva sample. Therefore, we were able to compute reactivity scores for 41 children and recovery scores for 39 children as follows: 1) Reactivity to the laboratory stressor task was computed by subtracting children's log transformed baseline cortisol levels from their log transformed peak post-task cortisol levels ($M = .55$, $SD = .76$, $Range = -.42$ to 2.47); 2) Recovery from the laboratory stressor task with respect to baseline cortisol levels was computed by subtracting children's log transformed 30-minute post-task cortisol levels from their log

transformed baseline cortisol levels ($M = -.23$, $SD = .73$, Range = -2.14 to 1.35); 3) Recovery from the laboratory stressor task with respect to peak post-task cortisol levels, was computed by subtracting children's log transformed 30-minute post-task cortisol levels from their log-transformed peak post-task cortisol levels ($M = .27$; $SD = .20$, Range = .00 - .92).

Results

Cortisol Patterns

Diurnal cortisol patterns and control variables. As reported in paper 1, using multilevel modeling techniques (HLM Version 6.06; Raudenbush, Bryk, & Congdon, 2004; SSI Scientific Software International) we observed the expected diurnal trend in children's diurnal cortisol. Testing linear and curvilinear models revealed that the best fit was achieved with a curvilinear model with time and time squared entered as random Level 1 predictors. As noted in paper 1, the only control variable found to significantly predict children's diurnal cortisol levels was the Sample Question dummy variable, which was retained as a fixed effect in subsequent Level 1 diurnal cortisol models.

Cortisol reactivity and recovery. As shown in Figure 1, we observed the expected increase in children's cortisol levels following the completion of the TSST-C stressor tasks, with mean levels peaking approximately 10 minutes after completion of the stressor tasks and trending back toward baseline levels 30 minutes after the stressor tasks were completed; this pattern and magnitude of response is comparable to previous reports for healthy children (e.g. Buske-Kirschbaum et al., 1997). Children's log transformed mean cortisol levels were 1.48 (Range 0.18 – 3.09; $SD = 0.54$) at the pre-task baseline sample, 2.02 (Range 0.88 – 3.73; $SD = 0.77$) at the peak post-task sample, and 1.72 (Range 0.65 – 3.57; $SD = 0.70$) at the 30-minute post-task sample.

Stability in Children's Diurnal Cortisol

Our first research question was aimed at exploring the temporal stability of four aspects of children's diurnal cortisol rhythms: waking cortisol levels, the CAR, diurnal slope, and bedtime cortisol levels. With 8 days of diurnal cortisol data, we were able to assess the relative stability across metrics of diurnal cortisol as well as whether incrementally increasing the number of sample days results in improved stability estimates. To address this question, we used multilevel modeling techniques to compute intraclass correlation coefficients (ICCs; Shrout and Fleiss, 1979) -- an estimate of the proportion of variance in each metric that is attributed to differences between children -- for each of the four daily cortisol metrics described in the Methods section. To compute ICCs, we regressed each day-level cortisol metric on a random intercept, random group-centered sample time (waking sample time was used in models predicting waking cortisol levels, the CAR, and diurnal slope, whereas bed sample time was used in models predicting bedtime cortisol levels) and fixed dummy control variable (Sample Question) at Level 1, and we grouped data by participant ID at Level 2 to obtain estimates of the variance within and between individuals, controlling for the effects of time and potential sampling errors. ICCs were then computed for each metric by dividing the corresponding model's between-person variance component by the total variance (within plus between) to estimate the relative proportion of variance in children's diurnal cortisol that can be attributed to differences between-persons. By estimating the degree to which cortisol values are influenced by stable, underlying characteristics of an individual, the ICC provides an estimate of test-retest reliability, in this case, the extent to which we can expect values within a given metric of diurnal cortisol to correlate with one another within the same person across days (Hruschka et al., 2005).

In order to explore whether incremental increases in the number of days of cortisol data included in analyses resulted in corresponding increases in ICCs, we used an iterative process

whereby we computed ICCs for subsets of sample days beginning with two days and adding one day at a time until reaching the full 8 saliva sample days. In order to also explore whether there were differences in the stability of children's cortisol profiles related to weekend versus weekday sampling, we also computed ICCs for the first two weekday sample days (Monday and Tuesday of the first week of saliva collection) to compare with the ICCs computed for the first two days of saliva sampling, which fell on a consecutive Saturday and Sunday.

Results are presented in Figures 2 and 3. As Figure 2 shows, ICCs ranged from 0.14 to 0.69 across all four diurnal cortisol metrics and across 2 to 8 sample days included in analyses. Thus, we found that anywhere from 14% to 69% of the variance in children's diurnal cortisol was attributed to differences between children, depending on the cortisol metric and number of sample days under consideration. Put another way, we can expect the within-individual correlations in children's diurnal cortisol to vary from .14 to .69 depending on which cortisol metric and timeframe we're interested in. In general, ICCs and, thus, stability or reliability, did not increase as we increased the number of days included in analyses. Overall, children's bedtime cortisol levels were found to be the most stable diurnal cortisol metric, with the highest ICC values regardless of days included, and, conversely, the CAR was found to be the least stable diurnal cortisol metric with the lowest ICCs value across each combination of sample days included in analyses. As shown in Figure 3, shifting from two weekend to two weekday sample days had the biggest impact on ICCs for the CAR, with a 39.1% decrease in weekday ICC compared to weekend, for this metric. Conversely, stability in bedtime cortisol levels increased by 25% when shifting from two weekend days to two weekend days. ICCs for children's waking cortisol levels and diurnal slope difference scores were virtually unchanged regardless of whether the corresponding saliva samples were collected on two weekdays or two weekends.

Age and sex differences in the stability of children's diurnal cortisol. A related aim of our exploration of stability in children's diurnal cortisol profiles was to explore whether stability, or within-person variability, in children's diurnal cortisol levels varies with children's age or sex. Modeling our analytical approach after one previously used to test individual differences in intra-individual variability in mood (Penner, Shiffman, Paty, & Fritzsche, 1994), we computed the standard deviation in each daily cortisol metric for each child across all eight days of data collection, and then, to control for potential intercorrelation between stability and mean scores, we divided each standard deviation score by the corresponding child's mean score on the respective cortisol metric, resulting in the coefficient of variation (CV). This produced four new variables: Child-level CV scores for each of our four metrics of diurnal cortisol. Since within-person variability in each of our cortisol metrics may be related to within-person variability in sampling time, we computed CV scores for each child for the time associated with waking and with bedtime saliva samples to serve as control variables in analyses.

Bivariate partial correlations were computed between the CV corresponding with each metric of cortisol and children's age and sex, controlling for variability in sampling time (the CV for waking sampling time was entered in analyses involving waking cortisol, CAR, and diurnal slope; the CV for bed sampling time was entered in analyses involving bedtime cortisol) to determine if either age or sex were associated with intra-individual variability in each of the cortisol measures of interest. As can be seen in Table 1, sex was significantly positively correlated with within-person variability in waking cortisol levels, controlling for within-person variability in waking sample time. That is, there was significantly more variability (less stability) in girls' waking cortisol levels over the eight days of naturalistic saliva collection that was not attributable to fluctuations in the timing of waking saliva samples than there was in boys' waking

cortisol levels. Sex was not correlated with any of the other metrics of diurnal cortisol and age was not correlated with any diurnal cortisol metrics.

Age and Sex Differences in Children's Cortisol

We also tested age and sex differences in children's diurnal cortisol and cortisol reactivity to and recovery from a laboratory stressor. Our analytic approach and overview of results are described in Appendix B-1, and tables are presented in Appendix B-2 and B-3.

Correlations Between Children's Diurnal Cortisol and Cortisol Reactivity and Recovery

Our last set of analyses were aimed at testing whether any of our metrics of children's diurnal cortisol were correlated with any of our metrics of cortisol reactivity and recovery. In order to obtain person-level estimates of each diurnal cortisol metric of interest for each child, Empirical Bayes estimates were computed, as follows: Both of the Level 1 models presented in the tests of age and sex differences in diurnal cortisol (see Appendix B-1) were entered separately into HLM, and grouped by participant at Level 2; the residuals from each model were saved and the person-level Empirical Bayes estimates of the coefficients corresponding with the intercept, time, and CAR dummy variable, in the first model, and with the intercept in the second model served as estimates of each child's waking cortisol levels, diurnal slope, CAR, and bedtime cortisol levels, respectively. Bivariate correlations between the Empirical Bayes estimates of our four diurnal cortisol metrics and the TSST-C reactivity and recovery scores were computed.

As can be seen in Table 2, children's waking diurnal cortisol levels were significantly positively correlated with their cortisol recovery from the TSST-C with respect to baseline; that is, higher waking cortisol levels were associated with greater recovery from the TSST-C relative to baseline cortisol levels. Similarly, children's CAR estimates were marginally significantly

positively associated with the TSST-C recovery with respect to baseline scores; in other words, greater increases in cortisol sampled approximately 30 minutes after waking, were marginally associated with better recovery from the TSST-C acute stressor tasks, relative to baseline cortisol levels. Neither cortisol reactivity to the TSST nor cortisol recovery relative to peak cortisol levels were correlated with any of the diurnal cortisol metrics.

Discussion

Impaired HPA-axis regulation -- believed to result from wear and tear due to repeated and/or prolonged reactivity to environmental stressors -- has been implicated as a mechanism linking stressful rearing conditions with later adverse health outcomes (e.g. Repetti et al., 2002). However, despite a rich and growing body of evidence linking early stress, HPA-axis functioning, and mental and physical health outcomes, there is surprisingly little known about the temporal stability of children's diurnal cortisol and how children's reactivity to acute stressors is concurrently related to their diurnal cortisol profiles. To address these gaps in the literature, we calculated multilevel model-derived ICCs to assess short-term temporal stability in four metrics of diurnal cortisol - waking cortisol levels, the cortisol awakening response (CAR), diurnal slope, and bedtime cortisol levels - over a range of 2 to 8 sample days spanning a three week study period in a community sample of 8- to 13-year-olds. We also explored associations between each diurnal cortisol metric and children's reactivity to and recovery from the Trier Social Stress Test for Children (TSST-C).

Overall, children's diurnal cortisol metrics were moderately¹ stable, with bedtime cortisol emerging as the most stable metric assessed and the CAR as the least. Interestingly, overall, increasing the number of sample days did not result in improvements in stability. Compared to

¹ Our qualitative interpretations of stability estimates reflect conventions used in the published articles also using multi-level model derived ICCs to estimate stability in children's and adults' diurnal cortisol.

weekdays, stability was higher for the CAR and lower for bedtime cortisol levels over two consecutive weekend days. Age was not correlated with intra-individual variability in any of our diurnal cortisol metrics, and sex was only correlated with waking cortisol levels, with more within-person variability in girls' waking cortisol levels. Better recovery from the TSST-C, with respect to baseline cortisol levels, was significantly correlated with higher waking cortisol levels and correlated at the marginal level of significance with higher CARs. In contrast, cortisol recovery with respect to peak post-task cortisol levels, and cortisol reactivity to the laboratory tasks was not associated with any of the diurnal cortisol variables.

Stability in Children's Diurnal HPA-axis Activity

Magnitude and comparisons across metrics. As expected based on the limited number of relevant studies, overall, we found moderate stability in children's diurnal cortisol with the most stability in bedtime levels (highly stable), and least stability in the CAR (modestly to moderately stable). Contributing to a small, but growing, literature, our estimates were generally consistent with previous reports in the child and adolescent cortisol literature over similarly short timeframes for waking cortisol levels (O'Connor et al., 2005; Rotenberg et al., 2012), bedtime cortisol levels (O'Connor et al., 2005), and the CAR (Rotenberg et al., 2012; ter Wolbeek et al., 2007), with our diurnal slope estimates falling between corresponding reports in the extant cortisol literature (Oskis et al., 2009; Rotenberg et al., 2012). Notably, however, our bedtime stability estimates were substantially higher than those reported by Rotenberg et al. (2012) and some have observed higher stability in child and adolescent CAR relative to our estimates (Oskis et al., 2009; Pruessner et al., 1997).

In our data, between-subject differences accounted for approximately one fourth of the variability in children's bedtime cortisol levels, one third of the variability in children's waking

cortisol levels and diurnal slopes, and one half to two thirds of the variability in children's bedtime cortisol levels when sampled across 2 to 8 days over a three week timeframe. These findings suggest that, while a considerable portion of the variability can be explained by individual differences across metrics, in our sample, the CAR was influenced more by within-person changes, or day-level factors (which might include mood, school workload, interpersonal stressors, etc.), while bedtime cortisol levels may be more reflective of a child's underlying diurnal cortisol profile. Our analytic approach of using multilevel models to partition variance into sources attributed to differences within- and between-individuals to calculate ICCs was based on the notion that diurnal cortisol is influenced by both within- and between-person factors. Accordingly, all metrics demonstrated non-negligible proportions of each source of variability, with the relative proportion of each varying across metrics. Importantly, our results show that some metrics might be more fruitful for researchers wishing to explore day-to-day processes and fluctuations in children's cortisol, while others might be more promising for tests of individual differences. Thus, this paper makes important methodological contributions that could be useful in informing future study designs.

We suspect that sampling compliance and dynamics of the diurnal rhythm may have influenced the higher stability observed in children's bedtime levels and lower stability in their CARs. The morning is often a busy time for families; competing demands and limited time resources likely impair compliance with fairly demanding morning sampling procedures sensitive to deviations in time since waking. While we took several measures to monitor sampling compliance (as described in the Methods section), we were limited to self-reports of participants' wake times and, therefore, we weren't able to objectively measure compliance with the timing of morning samples relative to wake times. Among adult women, Ranjit et al. (2005)

reported that waking and wake plus 30 minute samples were collected, on average, 14 and 90 minutes after awakening, respectively, even though participants were instructed to sample saliva as soon as they woke up and 30 minutes later, respectively. Kudielka et al. (2003) has also noted challenges with morning sampling compliance and possible consequences for reliability.

Consistent with our results, poor morning sampling compliance is likely to effect CAR stability estimates more than single sample morning estimates or diurnal slope estimates due to the limited window of time to capture the morning rise, with cortisol secretion peaking within 30 minutes after awakening (Pruessner et al., 1997), and the fact that the CAR is a composite measure dependent on at least two morning samples, compounding the opportunity for error due to compliance issues. When considering our pattern of results, it is noteworthy that bedtime cortisol was the only metric assessed that didn't involve at least one morning cortisol sample. In contrast to the morning rush, children's bedtimes are likely more slow-paced and, by bedtime, diurnal decline has leveled off, rendering variations in sample times less influential (Tsigos and Chrousos, 2002). Future studies using morning sampling procedures may be aided by electronic accelerometer devices that sense and record body accelerations such as ActiGraph (Pensacola, FL) activity monitors to verify participant wake time in relation to morning sample time.

Stability over an increasing number of sample days. To our knowledge, no other study has empirically investigated whether increasing the number of sample days results in a corresponding increase in stability levels in children's diurnal cortisol parameters. Based on mathematically-derived estimates (Hruschka et al., 2005; Rotenberg et al., 2012) and limited evidence that stability in adult diurnal cortisol can be improved by including more sample days (Hellhammer et al., 2007), we expected ICCs for our metrics of children's diurnal cortisol to increase with corresponding increases in days included in analyses. Surprisingly, through an

iterative process of calculating ICCs over an increasing number of days (from 2 to 8 days over three weeks), we found that, with only one exception¹, increasing the number of sample days did not result in meaningful changes in stability estimates. Notably, as shown in Figure 2, stability estimates remained consistent across the subsets of sample days despite differences in the spacing between days included in analyses, which included consecutive days within the same week versus days spread over two sample weeks three weeks apart.

To our knowledge, these are the first empirical findings on the effects of increased sample days on the stability of children's diurnal cortisol. These findings do not support the hypothesis that increasing the number of sample days over which various metrics of diurnal cortisol are measured, beyond two to three days, results in related increases in stability as previously suggested and mathematically projected (e.g. Hruschka et al, 2005; Nicolson, 2008; Rotenberg et al., 2012). It should be noted that our ICCs did not approach the optimal stability level (ICC = .80) targeted in previous mathematical projections drawing on Classical Measurement Theory (bedtime cortisol levels came the closest, with the highest ICC estimate in this metric equaling .69). However, our data suggest that the association between number of days and stability in children's diurnal cortisol may not be linear, and thus, stability estimates in these metrics may never reach .80 even with more and more sample days. Even so, we observed meaningful levels of between-person variability across all metrics of diurnal cortisol, suggesting opportunities to model individual differences in diurnal cortisol (and, just as importantly, significant proportions of within-person variability within each measure suggest that tests of within-person effects of day-to-day fluctuations in things such as mood, workload, and family conflict, may also be particularly productive pursuits in children's diurnal cortisol research).

¹ Going from two to three sample days resulted in a nearly 33% increase in bedtime ICCs. Bedtime stability estimates returned to their initial level when additional sample days were added (specifically when data from the second week of saliva collection were added to the first) and remained consistent for the remainder of iterations.

In sum, despite theoretical evidence that stability would increase with corresponding increases in sample days, our data show that, overall, after the first two to three days, stability estimates remained consistent regardless of the number of days included in analyses. Therefore, we did not observe a payoff, in terms of short-term stability, with increased sample days. These results have important implications for future study designs, as researchers often make tradeoffs between sample size and intensity of sampling. Our results suggest that, at least where individual differences in children's diurnal cortisol are concerned, researchers may find it more effective to opt for larger samples with fewer sampling days per person.

Predictors of stability and within-person variability. We explored whether stability in children's cortisol profiles differed depending on weekend versus weekday sampling by comparing ICCs for the first two weekend sample days to the first two weekday sample days (collectively sampled over four consecutive days). The bedtime ICC score was 25% higher while, conversely, the CAR ICC score was nearly 40% lower on weekdays compared to weekends. Type of day (weekend versus weekday) was not associated with meaningful changes in ICC scores for waking cortisol levels or diurnal slopes over the day. To our knowledge, the effect of weekend versus weekday sample day status has not previously been investigated in children, and has only minimally been assessed in adults (Karlman et al., 2013; Hellhammer et al., 2007). Among adults, weekend versus weekday status has been associated with changes in stability levels in the CAR (Hellhammer et al., 2007), and differences in peak cortisol levels, and AUC (Karlman et al., 2013). Notably, however, among adults, The CAR was found to be more stable on weekdays than weekends, which is the inverse of our findings.

Variations in stability may be explained by varying degrees of consistency in routines and activities on weekdays versus weekends (Hellhammer et al., 2007), above and beyond the

effect of variability in sample time, for which we statistically controlled. For example, children may have more consistency in their end-of-day activities and bedtime routines on weekdays or “school nights”, whereas weekend evenings may be more loosely structured and characterized by greater variability in pre-bedtime activities. While weekday mornings might also be characterized by more consistent morning routines, compared to weekend mornings, it is also possible that anticipatory stress may be more variable on weekdays than weekends due to variability in academic demands (for example, school exams); although the function of the CAR is not completely understood, some researchers have suggested that it is an anticipatory response to the day ahead (Fries, Dettenbord, and Kirschbaum, 2009). It is also likely that children had greater difficulty with morning sampling compliance on weekday mornings, when children and families were preparing to leave the house for school and work, whereas weekend mornings may have been more slow-paced, thus facilitating better compliance with sampling procedures.

It should be noted, however, that post-hoc analyses revealed that approximately two thirds of weekend CAR estimates fell at or below zero, indicating non-response to awakening, compared to approximately one third of weekday samples (see appendix B-4 for histograms and descriptive statistics of weekend and weekday CAR estimates). While this is likely influenced by the fact that weekend wake times were significantly later than weekday wake times ($\beta = 1.59, p < .001$), weekend sample status remained a significant predictor of lower CAR estimates after controlling for the effects of differences in wake time ($\beta = -0.17, p = .04$). Thus, in our sample, weekend sampling status predicted significantly lower CARs compared to weekdays, and this effect was above and beyond the effects of differences in waking sample time. As noted above, it is possible that lower levels of anticipatory stress on weekends, compared to weekdays, may have influenced this pattern of results.

We also assessed whether within-person variability co-varied with participants' age and sex and found that girls displayed more within-person variability in their waking cortisol levels than did boys. In other words, girls' waking cortisol levels were found to be significantly more variable within the same individual from day-to-day over the 8 days of saliva collection compared to boys' waking cortisol levels. Age was not correlated with intra-individual variability in any of the diurnal cortisol metrics we investigated, and sex was not associated with any of the other metrics; our small sample size and limited variability in participant age, however, may have limited our ability to test group differences. To our knowledge, age and sex correlates of within-person variability in diurnal cortisol have not previously been reported in any published studies concerning children or adults. Consideration of age and sex differences in intra-individual variability in children's cortisol may be especially important given that several mental health disorders onset during adolescence and may be mediated by alterations in HPA-axis functioning (e.g. Oskis et al., 2009; Shirtcliff & Essex, 2008). Even in the absence of differences in mean levels, within-person variability in diurnal cortisol has been associated with mental health (Goodyer, Tamplin, Herbert, & Altham, 2000; Posener et al., 2004; Yehuda, Teicher, Trestman, Levengood, & Siever, 1996). Our findings that girls' waking cortisol levels were more variable than boys' may be relevant for researchers who have observed sex differences in the prevalence of mental and physical health conditions that might be mediated by HPA-axis activity (e.g. Kudielka & Kirschbaum, 2005).

Associations Between Children's Diurnal Cortisol and Stress-Induced Cortisol Responses

We explored whether any of the metrics of diurnal cortisol of interest were associated with children's reactivity to and recovery from an acute psychosocial stressor (the TSST-C). We are not aware of any published findings on associations between children's diurnal cortisol and

acute laboratory stress responses, and this question has only been minimally investigated in adults. Therefore, exploratory analyses were carried out without specific predictions about results. We found that recovery from, but not reactivity to, the laboratory stress tasks was associated with naturalistic morning cortisol secretion such that poorer recovery from the TSST-C, with respect to baseline cortisol levels, was associated with significantly lower waking cortisol levels and marginally significantly smaller CARs. Thus, poorer recovery from acute stress, relative to baseline cortisol levels, was found to be associated with patterns of diurnal cortisol profiles -- lower waking values and an attenuated post-wake rise in cortisol levels -- that have been associated with chronic stress and negative health outcomes in adults, and may indicate biological pathways linking stress and health (Miller, Chen, & Zhou, 2007; Preussner et al, 1999; Sapolsky, Krey & McEwen, 1986). These findings provide evidence supporting the ecological validity of laboratory stressor tasks, such as the TSST-C, suggesting that individual differences in how children recover from certain stressors in the laboratory signify individual differences in children's naturalistic diurnal cortisol activity. Importantly, connecting laboratory and real-life measures of children's HPA-axis functioning may help advance our understanding of biopsychosocial pathways linking stress and health through dysregulation of the HPA-axis.

Notably, associations were observed with one of our two metrics of stress recovery but not with our metric of stress reactivity, suggesting that dysregulation of and wear and tear to the diurnal profile may be more closely tied to sustained HPA-axis activity, or difficulty down regulating cortisol secretion after a threat has passed, than it is to the magnitude of reactivity itself. Although cortisol recovery has been identified as an important, yet understudied, indicator of HPA-axis functioning (Kudielka & Kirschbaum, 2005; Sapolsky, 2000; Seeman & Robbins, 1994), to our knowledge, we are the first to explore associations between diurnal cortisol and

laboratory recovery in either child or adult samples. “Wear and tear” to the HPA-axis may be both reflected in and predicted by impaired recovery from stress, with dysregulation in both stress-related and diurnal cortisol secretion related to long-term health outcomes. Longitudinal studies are needed to better understand the process of HPA-axis dysregulation and potential biological pathways linking stress and health. Moreover, tests of associations between children’s diurnal cortisol and their neuroendocrine responses to naturalistic stressors is an important next step in understanding links between HPA-axis activity and reactivity.

Limitations and Future Directions

While our study had many strengths, such as eight days of salivary cortisol data sampled multiple times per day, multi-level modeling statistical techniques, and both diurnal and laboratory cortisol samples, we were also limited by a number of factors. First, particularly for tests of individual or group (e.g. age, sex) differences, our study was limited by sample size, which affects statistical power to detect between-person effects. It is recommended that future studies employ larger samples to test predictors of within-person variability in children’s diurnal cortisol and associations between children’s diurnal profiles and reactivity to and recovery from acute laboratory stress tasks. Additionally, our study measured temporal stability in diurnal cortisol over a relatively short timeframe (days and weeks) and over a relatively restricted age range. Longitudinal designs (e.g. Shirtcliff et al., 2012), in contrast, would allow researchers to assess stability over longer timeframes as well as explore potential developmental changes related to stability in children’s and adolescents’ diurnal cortisol. Finally, while our tests of correlations between diurnal cortisol and laboratory responses to an acute psychosocial stressor offered an important contribution toward our understanding of connections between these two components of the HPA-axis, a recommended future direction is for researchers to extend this

design to connections between children's diurnal cortisol and their responses to naturalistic, everyday stressors.

Summary and Conclusions

This study makes several important contributions to the scientific literature concerning children's diurnal and stress-responsive cortisol. Our results indicate that children's diurnal cortisol profiles are moderately stable, with high stability in bedtime cortisol levels, moderate stability in waking cortisol levels and diurnal slope, and more modest stability in the CAR. Thus, the present study provides evidence in support of the use of children's diurnal cortisol metrics as individual differences in explorations of HPA-axis mediated links between early and/or chronic stress and later health outcomes. Given the relative patterns of stability we observed between metrics of children's diurnal cortisol, our results suggest that children's bedtime cortisol metrics might be particularly fruitful in tests of individual differences, while the CAR, with the highest proportion of within-person variability observed across metrics, might be more relevant for research on daily processes. Additionally, we observed that increasing the number of sample days beyond two to three days did not improve children's diurnal cortisol stability estimates. Thus, given the tradeoff that researchers often make between sample size, sampling intensity, and participant burden, our results suggest that inquiries regarding individual differences in children's diurnal cortisol may be most efficient when sampling days are limited in favor of larger sample sizes. Our results also suggest that researchers might obtain more stable estimates of children's bedtime cortisol on weekdays compared to weekends (with no differences in stability observed for waking cortisol levels or diurnal slope on weekends compared to weekdays, and with our observation of higher weekend CAR stability complicated by higher rates of non-responses to awakening on weekends compared to weekdays). Thus, the results of

the present study make important contributions that may inform methodological considerations and decisions of future studies involving child and adolescent diurnal cortisol. Moreover, our results support the treatment of children's diurnal cortisol as a fairly stable individual difference variable implicit in the allostatic load literature.

Finally, the present study also provides the first published findings, to our knowledge, assessing links between children's diurnal cortisol and responses to a laboratory stress task. Our findings provide ecological validity in support of the TSST-C; that is, not only does the TSST-C produce reliable increases in children's cortisol levels which have been connected with health (Buske-Kirschbaum et al., 1997), but our results indicate that children's recovery from the TSST-C is also related to children's everyday diurnal cortisol functioning. Notably, we only observed correlations with recovery from, but not reactivity to, the TSST-C, highlighting the importance of considering both the magnitude of increase in cortisol levels in response to a stressor and the process of down-regulating the HPA-axis following a stressor, especially since sustained exposure to circulating stress hormones is thought to be relevant in stress-mediated health outcomes (e.g. McEwen, 1998; Sapolsky et al., 2000; Repetti et al., 2002). Taken together, the results of the present study provide evidence that we can observe alterations in both diurnal cortisol profiles and children's stress-responsive cortisol in middle childhood.

Tables

Table 1

Partial Correlations Between Intra-individual Variability in Each of Four Diurnal Cortisol Metrics (as Indexed by Coefficients of Variation for Each Child on Each Metric) and Children's Age and Sex, Controlling for Intra-individual Variability in Cortisol Sampling Time (df = 44)

Variable	Age	Sex (boys 0, girls 1)
<i>Diurnal Cortisol Coefficient of Variation</i>		
Wake	-0.11	0.29*
CAR	-0.24	-0.04
Diurnal Slope	0.07	0.12
Bed	-0.04	-0.04

* $p < .05$

Note: Since variability in children's diurnal cortisol might be related to variability in sampling time, the Coefficient of Variation for each child's waking sampling time was entered as a control variable in analyses involving variability in children's wake cortisol, CAR, and Diurnal Slope, and the Coefficient of Variation for each child's bed sampling time was entered as a control variable in analyses involving variability in children's bedtime cortisol levels.

Table 2

Correlations Between Children's Cortisol Reactivity to and Recovery from the Trier Social Stress Task for Children (TSST-C) and Empirical Bayes Estimates of Diurnal Cortisol Metrics for Each Child Over 8 Days of Naturalistic Saliva Sampling

Variable	Empirical Bayes Estimates of Children's Diurnal Cortisol			
	Wake	Slope	CAR	Bed
Children's Cortisol Reactivity to the TSST-C ($n = 41$)	-0.25	-0.01	-0.21	-0.01
Children's Cortisol Recovery from the TSST-C with Respect to Baseline ($n = 39$)	.44**	0.01	0.29+	0.13
Children's Cortisol Recovery from the TSST-C with Respect to Peak ($n = 39$)	.03	-.07	-.07	-.03

+ $p < 0.10$, ** $p < 0.01$

Note: For variables representing reactivity to and recovery from the TSST-C, higher values indicate greater reactivity and better recovery, respectively

Figures

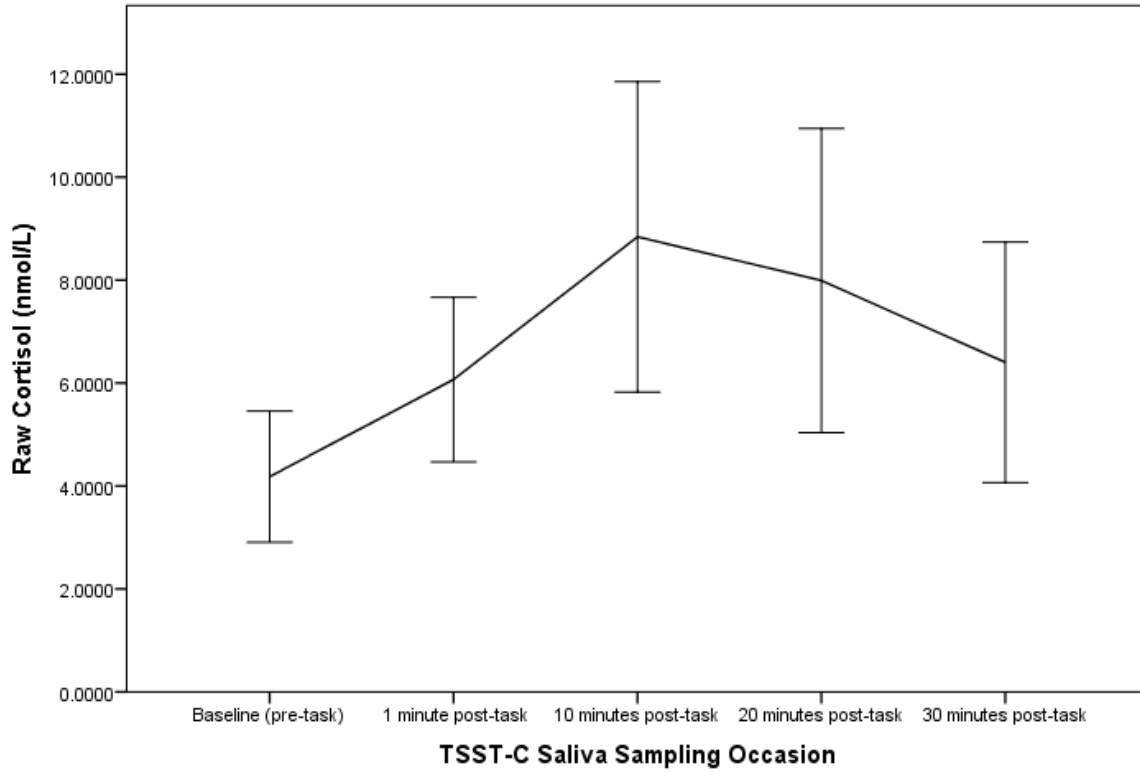


Figure 1. Children's mean raw salivary cortisol levels at each sampling occasion before and after completion of the TSST-C stressor tasks ($n = 41$).

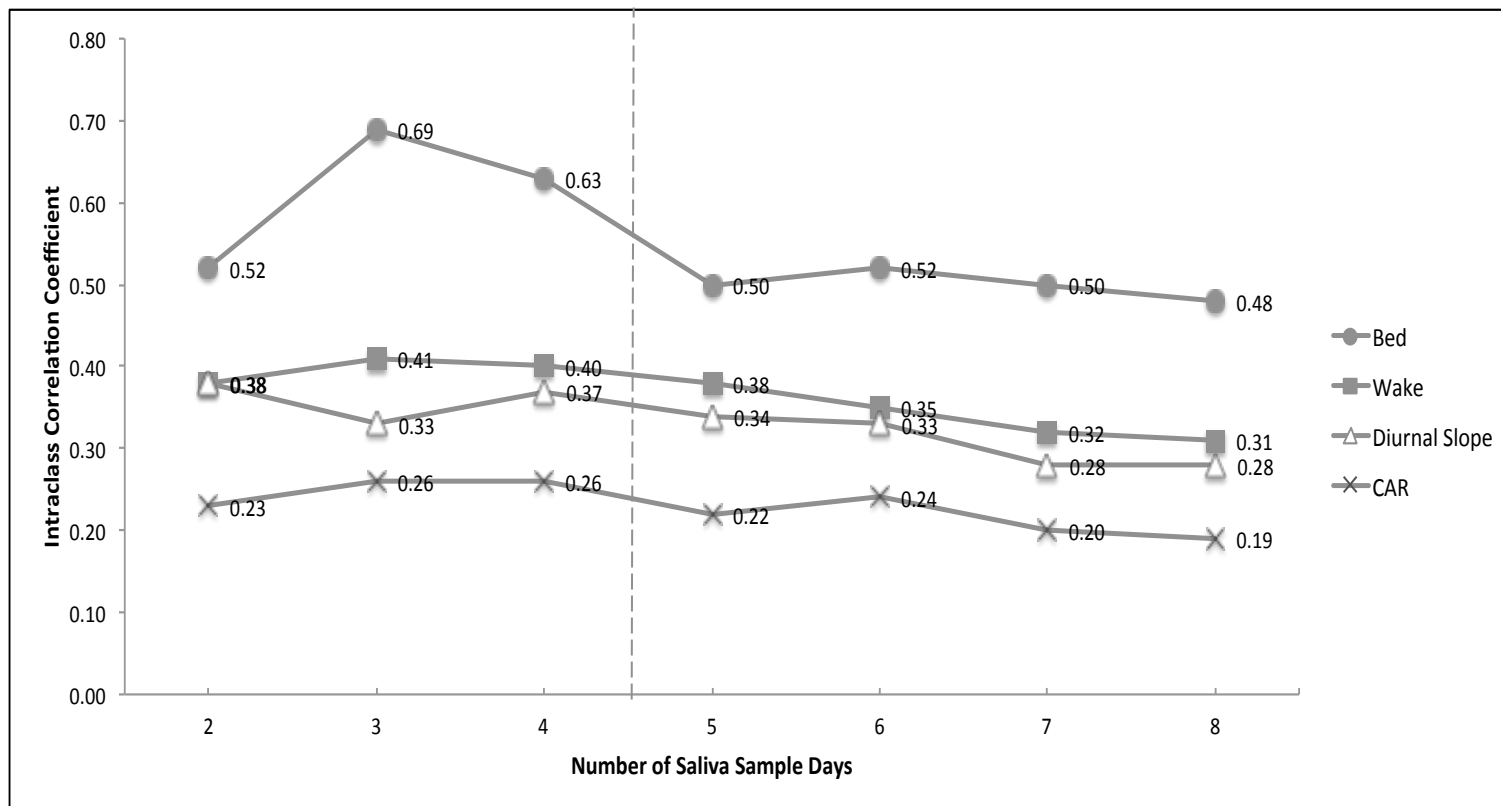


Figure 2. Intraclass correlation coefficients corresponding with four metrics of children’s diurnal cortisol over an increasing number of sample days ranging from two to eight days (n = 47). Elapsed time between data collected during weeks 3 and 6 of the larger study is represented by a vertical dashed line.

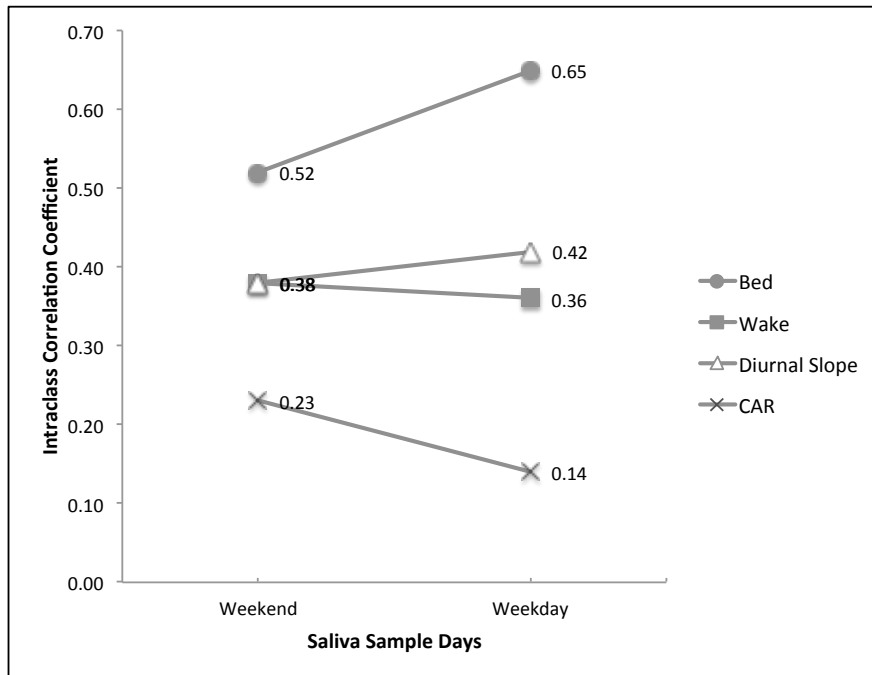


Figure 3. Intraclass correlation coefficients corresponding with four metrics of children’s diurnal cortisol over two consecutive weekend days and two consecutive weekdays (n = 47).

Appendix B-1

Tests of Age and Sex Differences in Children's Cortisol

Diurnal cortisol. As noted in the Results section, we tested the effects of age and sex on each of our four cortisol metrics of interest using two multilevel models. In our first model, we added a Level 1 dummy variable indicating the 30-minute post-wake sample to our basic model (described in the diurnal cortisol patterns section above) with time, time squared, and the Sample Question control variable at Level-1 predicting children's log transformed diurnal cortisol (across all sampling occasions). The resulting Level-1 model provided estimates of children's waking cortisol levels (intercept), linear and quadratic diurnal slope (time and time squared, respectively), and the CAR (30-minute post wake dummy code). At Level-2, we added children's age, sex, and their cross product as predictors of the Level-1 effects of intercept, time, time-squared, and the CAR dummy variable to test for age, sex, and age by sex effects in children's waking cortisol levels, linear and quadratic slope, and CAR, respectively.

Our second Level 1 model regressed children's daily log transformed bedtime cortisol levels on a random intercept, the time of day corresponding with each bedtime sample, and our Sample Question control variable in order to obtain an estimate of each child's typical bedtime cortisol levels (intercept), controlling for time of day and potential sampling errors. At Level 2, we added children's age, sex, and their cross product as predictors of the coefficient representing the random intercept to test for age and sex differences in children's typical bedtime cortisol levels.

As can be seen in the table in Appendix B-2, there was a significant negative effect of age and positive age by sex interaction effect on the coefficient predicting children's bedtime cortisol levels (the intercept in the bedtime cortisol model), controlling for the effects of sample time and

potential sample errors. That is, we found that girls had significantly lower bedtime cortisol levels than boys, and this effect was moderated by age such that younger girls had lower cortisol levels than older girls. We did not find significant age, sex, nor age by sex effects on any of the other metrics of diurnal cortisol.

Cortisol reactivity and recovery. In order to test for age and sex effects in children's HPA-axis response to an acute stressor, children's cortisol reactivity to and recovery from the TSST-C (defined in the Methods section) were entered in bivariate correlations with children's age and sex. As shown in the table in Appendix B-3, we found that sex was significantly correlated with both the reactivity and recovery scores, while age was significantly correlated with the recovery score and was marginally significantly correlated with reactivity. Specifically, we found that girls displayed significantly higher cortisol reactivity to the stressor task (as indexed by increases in post-task cortisol levels over baseline levels), while boys had significantly better recovery from the tasks (as indexed by number of units of cortisol below baseline levels at 30 minutes post-task). With regard to age, younger children displayed marginally significantly more reactivity to the TSST-C and significantly poorer HPA-axis recovery 30 minutes after the task, compared with older children.

Appendix B-2

Children's Age, Sex (boys 0, girls 1), and Their Cross Product Predicting Children's Waking Cortisol Levels, CAR, Slope, and Bedtime Cortisol Levels

Fixed Effect	Coefficient (SE)	<i>t</i> -ratio	d.f.
<i>Outcome: Diurnal cortisol</i>			
Intercept, β_{00}	3.13 (0.51)	6.17***	43
Sex, β_{01}	0.17 (0.83)	0.21	43
Age, β_{02}	0.02 (0.05)	0.50	43
Age x Sex, β_{03}	0.00 (0.07)	-0.04	43
Time, β_{10}	0.05 (0.15)	0.36	43
Sex, β_{11}	-0.40 (0.26)	-1.53	43
Age, β_{12}	0.00 (0.01)	0.28	43
Age x Sex, β_{13}	0.03 (0.02)	1.27	43
Time Squared, β_{20}	0.00 (0.01)	-0.80	43
Sex, β_{21}	0.02 (0.02)	1.09	43
Age, β_{22}	0.00 (0.00)	-0.59	43
Age X Sex, β_{23}	0.00 (0.00)	-0.88	43
CAR, β_{30}	0.49 (0.33)	1.38	43
Sex, β_{31}	-0.58 (0.53)	-1.09	43
Age, β_{32}	-0.01 (0.03)	-0.34	43
Age x Sex, β_{33}	0.06 (0.04)	1.38	43
SampleQuestion, β_{40}	-0.28 (0.10)	-2.91**	1303
<i>Outcome: Bedtime cortisol</i>			
Intercept, β_{00}	2.35 (0.42)	5.59	43
Sex, β_{01}	-2.38 (0.65)	-3.64***	43
Age, β_{02}	-0.07 (0.04)	-1.76	43
Age x Sex, β_{03}	0.20 (0.06)	3.44**	43
Time, β_{10}	0.02 (0.02)	0.95	46
SampleQuestion, β_{20}	-0.19 (0.10)	-1.82	281

** $p < .01$, *** $p < .001$

Appendix B-3

Correlations Between Children's Cortisol Reactivity to and Recovery from the Trier Social Stress Task for Children (TSST-C) with their Age and Sex

Variable	Children's Demographics	
	Age	Sex (boys 0, girls 1)
Children's Cortisol Reactivity to the TSST-C (<i>n</i> = 41)	-0.30+	0.43**
Children's Cortisol Recovery from the TSST-C with Respect to Baseline (<i>n</i> = 39)	0.44**	-0.45**
Children's Cortisol Recovery from the TSST-C with Respect to Peak (<i>n</i> = 39)	- 0.54	- 0.50

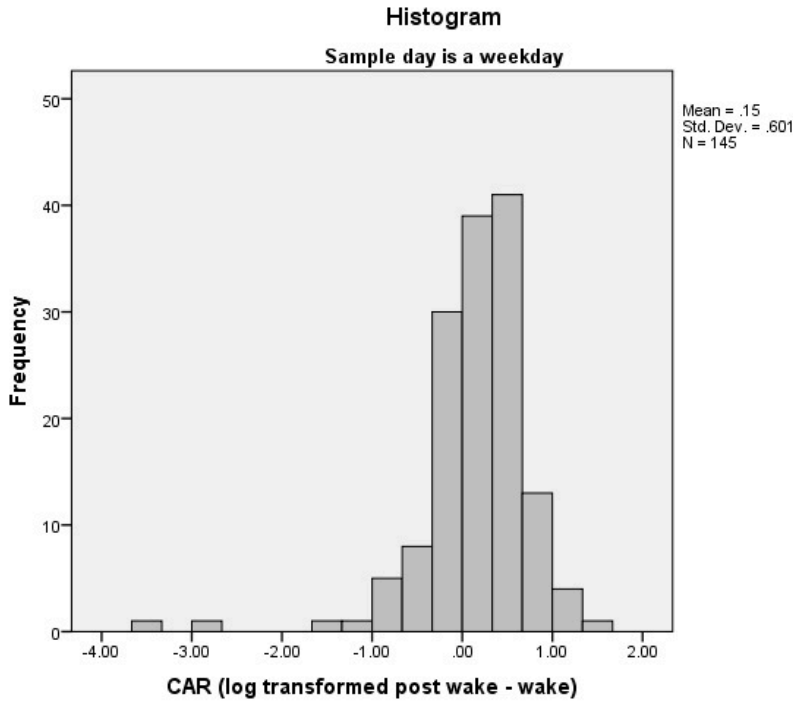
+ $p < 0.10$, ** $p < 0.01$

Note: For variables representing reactivity to and recovery from the TSST-C, higher values indicate greater reactivity and better recovery, respectively

Appendix B-4

Histograms and Descriptive Statistics of Children's Cortisol Awakening Response Difference

Scores Reported Separately for Weekdays and Weekend Days

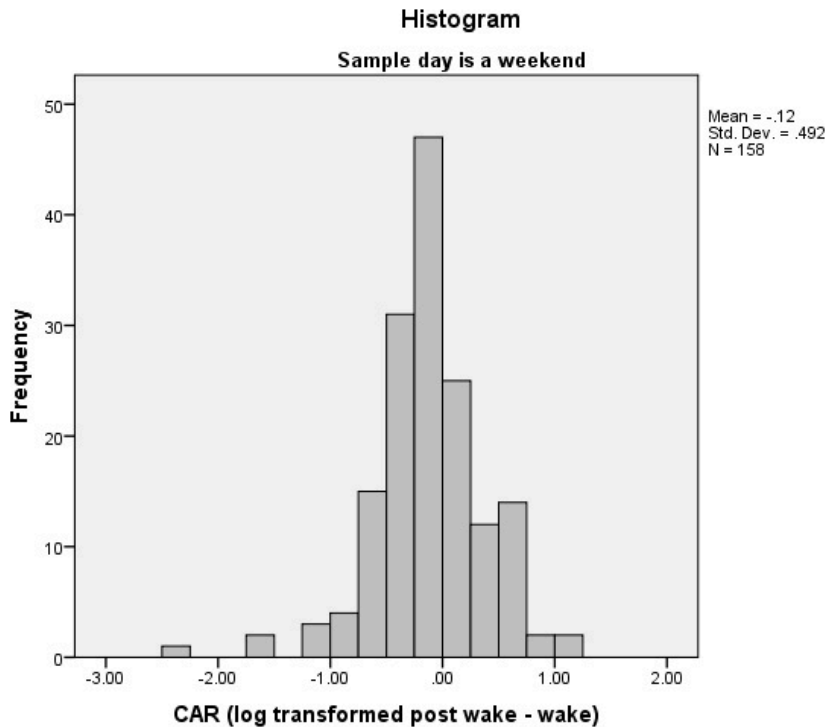


CAR Weekday

(LN post wake – LN wake)

N	Valid	145
	Missing	43
Mean		.1519
Median		.1957
Std. Deviation		.60147
Range		5.01

a. Sample day is a weekday



CAR Weekend

(LN post wake – LN wake)

N	Valid	158
	Missing	30
Mean		-.1179
Median		-.1373
Std. Deviation		.49161
Range		3.48

a. Sample day is a weekend

References

- Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*, 1423-1436.
doi:10.1016/j.psyneuen.2009.06.011
- Buske-Kirschbaum, A., Jobst, S., Wustmans, A., & Kirschbaum, C. (1997). Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosomatic Medicine*, *59*(4), 419-419.
- Cicchetti, D., & Rogosch, F. A. (2001). Diverse patterns of neuroendocrine activity in maltreated children. *Development and Psychopathology*, *13*, 677-693.
- Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355-391.
- Dressendorfer, R. A. et al. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *The Journal of Steroid Biochemistry and Molecular Biology*, *43*(7), 683-92.
- Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sciences*, *68*(18), 2093–2103.
- Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology*, *72*, 67-73.
- Goodyer, I. M., Tamplin, A., Herbert, J., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents, *The British Journal of Psychiatry*, *177*, 499–504.
- Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human

- development. *Psychoneuroendocrinology*, 27, 199–220.
- Hellhammer, J., Fries, E., Schweisthal, O. W., Scholtz, W., Stone, A. A., & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state-and-trait components. *Psychoneuroendocrinology* 32, 80-86.
- Hruschka, D. J., Kohrt, B. A., & Worthman, C. M. (2005). Estimating between-and within-individual variation in cortisol levels using multilevel models. *Psychoneuroendocrinology*, 30(7), 698-714.
- Karlamangla, A. S., Friedman, E. M., Seeman, T. E., Stawski, R. S., & Almeida, D. M. (2013). Daytime trajectories of cortisol: Demographic and socioeconomic differences—Findings from the National Study of Daily Experiences. *Psychoneuroendocrinology*, 38(11), 2585–2597.
- Kidd, T., Carvalho, L. A., & Steptoe, A. (2014). The relationship between cortisol responses to laboratory stress and cortisol profiles in daily life. *Biological Psychology*, 99(100), 34–40. doi:10.1016/j.biopsycho.2014.02.010
- Kirschbaum, C., Pirke K. M., & Hellhammer, D. H. (1993). The ‘Trier Social Stress Test’ – A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, 28, 76–81.
- Kraemer, H. C., Giese-Davis, J., Yutsis, M., O'Hara, R., Neri, E., Gallagher-Thompson, D., & Taylor, C. B. (2006). Spiegel D. Design decisions to optimize reliability of daytime cortisol slopes in an older population. *The American Journal of Geriatric Psychiatry*, 14, 325–333.
- Kudielka, B. M., Broderick, J. E., & Kirschbaum, C. (2003). Compliance with saliva sampling

- protocols: Electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine*, 65(2), 313–319.
- Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology*, 69(1), 113–132.
- Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M., Steptoe, A. (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, 29, 516–528.
- McEwen, B. S. (1998). Stress, adaptation and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, 840, 33–44.
- McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain, *Physiological Reviews*, 87, 873–904.
- McEwen, B. S., & Seeman, T. E. (1999). Protective and damaging effects of mediators of Stress: Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences*, 896, 30–47.
- Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, 133(1), 25–45.
- Nicolson, N. A. (2008). Measurement of cortisol. In L. J. Luecken & L. C. Gallo (Eds.), *Handbook of physiological research methods in health psychology* (pp. 37–74). Thousand Oaks, CA: Sage.
- O'Connor, T. G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., & Glover, V. (2005). Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biological Psychiatry*, 58(3), 211–217.

- Oskis, A., Loveday, C., Hucklebridge, F., Thorn, L., & Clow, A. (2009). Diurnal patterns of salivary cortisol across the adolescent period in healthy females. *Psychoneuroendocrinology, 34*, 307–316.
- Penner, L. A., Shiffman, S., Paty, J. A., & Fritzsche, B. A. (1994). Individual differences in intraperson variability mood. *Journal of Personality and Social Psychology, 66*(4), 712-721.
- Platje, E., Vermeiren, R. R., Branje, S. J., Doreleijers, T. A., Meeus, W. H., Koot, H. M., Frijns, T., van Lier, P. A., & Jansen, L. M. (2013). Long-term stability of the cortisol awakening response over adolescence. *Psychoneuroendocrinology, 38*, 271–280.
- Polk, D. E. et al. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology, 30*(3), 261-272.
- Posener, J. A., DeBattista, C., Veldhuis, J. D., Province, M. A., Williams, G. H., & Schatzberg, A.F. (2004). Process irregularity of cortisol and adrenocorticotropin secretion in men with major depressive disorder. *Psychoneuroendocrinology, 29*(9), 1129-1137.
- Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., & Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences, 61*, 2539—2549.
- Pruessner, J. C., Hellhammer, D. H. & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61*, 197–204.
- Pruessner, M., Hellhammer, D. H., Pruessner, J. C., & Lupien, S. (2003). Self-reported

- depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. *Psychosomatic Medicine*, *65*, 92–99.
- Quirin, M., Pruessner, J. C., & Kuhl, J. (2008). HPA system regulation and adult attachment anxiety: Individual differences in reactive and awakening cortisol. *Psychoneuroendocrinology*, *33*, 581–590.
- Ranjit, N., Young, E. A., Raghunathan, T. E., & Kaplan, G. A. (2005). Modeling cortisol rhythms in a population-based study. *Psychoneuroendocrinology*, *30*, 615–624.
- Raudenbush, S. W., Bryk, A. S., Cheong, Y., & Congdon, R. T. (2004). *HLM 6: Hierarchical linear and nonlinear modeling*. Lincolnwood, IL: Scientific Software International.
- Repetti, R. L., Robles, T. F., & Reynolds, B. M. (2011). Allostatic processes in the family. *Development and Psychopathology*, *23*, 921–938.
- Repetti, R. L., Taylor, S. E., & Seeman, T. E. (2002). Risky families: Family social environments and the mental and physical health of offspring. *Psychological Bulletin*, *128*, 330–366.
- Ross, K. M., Murphy, M. L., Adam, E. K., Chen, E., & Miller, G. E. (2014). How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. *Psychoneuroendocrinology*, *39*, 184–93. doi:10.1016/j.psyneuen.2013.09.016.
- Rotenberg, S., McGrath, J. J., Roy-Gagnon, M., & Thanh Tu, M. (2012). Stability of the diurnal cortisol profile in children and adolescents. *Psychoneuroendocrinology*, *37*, 1981–1989.
- Sapolsky, R. M., Romero, M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*, 55–89.
- Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and

- aging: The glucocorticoid cascade hypothesis. *Endocrine Reviews*, 7, 284-301.
- Schmidt-Reinwald A., Pruessner J. C., Hellhammer D. H., Federenko I., Rohleder N., & Schurmeyer T. H. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences*, 64, 1653–1660.
- Seeman, T. E., Robbins, R. J., (1994). Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrine Reviews*, 15, 233–260.
- Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychological Bulletin*, 36, 420–428.
- Shirtcliff, E. A., Allison, A. L., Armstrong, J. M., Slattery, M. J., Kalin, N. H., & Essex, M. J. (2012). Longitudinal stability and developmental properties of salivary cortisol levels and circadian rhythms from childhood to adolescence. *Developmental Psychobiology*, 54, 493–502.
- Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology*, 50, 690–703.
- Stewart, J., & Seeman, T. (2000). Salivary cortisol measurement. Summary of a meeting of the John D. and Catherine T. MacArthur Foundation Research Network on Socioeconomic Status and Health. Retrieved January 22, 2015, from <http://www.macses.ucsf.edu/research/allostatic/salivarycort.php>
- ter Wolbeek, M., van Doornen, L. J. P., Coffeng, L. E., Kavelaars, A., & Heijnen, C. J. (2007). Cortisol and severe fatigue: a longitudinal study in adolescent girls. *Psychoneuroendocrinology* 32, 171 - 182.
- Thorn, L., Hucklebridge, F., Evans, P., & Clow, A. (2009). The cortisol awakening response,

- seasonality, stress and arousal: A study of trait and state influences.
Psychoneuroendocrinology, 34(3), 299-306.
- Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, 53, 865-871.
- van Eck, M. M., Nicolson, N. A., Berkhof, H., & Sulon, J. (1996). Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. *Biological Psychology*, 43, 69–84.
- Wolfram, M., Bellingrath, S., Feuerhahn, N., & Kudielka, B. M. (2013). Cortisol responses to Naturalistic and laboratory stress in student teachers: comparison with a non-stress control day. *Stress and Health*, 29(2), 143-9. doi:10.1002/smi.2439
- Wuest, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response—normal values and confounds. *Noise Health*, 2, 79-88.
- Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A. and Siever, L. J., (1996). Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biological Psychiatry*, 40, 79–88.