ABSTRACT

Title of Document: PARENTAL COMPLIANCE WITH CHILD SALIVARY CORTISOL SAMPLING: IMPACT ON CHILDREN'S CORTISOL DATA

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Studies assessing hypothalamic-pituitary-adrenal axis functioning in young children commonly involve parental collection of salivary cortisol in ambulatory settings. However, no data are available on the compliance of parents in collecting ambulatory measures of children's salivary cortisol. This study examined the effects of parental compliance on the cortisol awakening response (CAR) and diurnal cortisol slopes in a sample of preschoolers. Eighty-one parents were instructed to collect their child's salivary cortisol samples upon their child's waking, 30 and 45 minutes post-waking and before bedtime on two weekdays. Subjective parental compliance was assessed using parent-report, and objective parental compliance was assessed using an electronic monitoring device. Rates of compliance were higher based on parent-report than electronic monitoring. Parental noncompliance as indicated by electronic monitoring was associated with higher waking cortisol and lower CAR. Findings

suggest the need to incorporate electronic monitoring of parental compliance into developmental neuroendocrine research, especially when assessing the CAR.

PARENTAL COMPLIANCE WITH CHILD SALIVARY CORTISOL SAMPLING: IMPACT ON CHILDREN'S CORTISOL DATA.

By

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

In recent decades, research examining hypothalamic-pituitary-adrenal (HPA) axis functioning in children has flourished with the development of salivary cortisol assays (Gunnar & Vazquez, 2006). Salivary cortisol is a valid and reliable measure of plasma levels of cortisol (Hiramatsu, 1981), and its method of collection from children is well established (Jessop & Turner-Cobb, 2008). In contrast to alternative methods requiring urine or blood samples, the collection of salivary cortisol is simple and noninvasive and may be conducted by participants outside of the laboratory. As a result, salivary cortisol assessment has facilitated many naturalistic studies examining HPA axis functioning, which have linked children's cortisol levels to internalizing (Carrion et al., 2002; Goodyer, Park, & Herbert, 2001) and externalizing problems (King, Barkley, Barrett, 1998); day care quality (Gunnar & Donzella, 2002); early maltreatment (Tarullo & Gunnar, 2006); maternal psychopathology (Dougherty, Klein, Olino, Dyson & Rose, 2009; Lupien, King, Meaney, McEwen, 2000) and later child emotional and behavioral problems (Essex, Klein, Cho, & Kalin, 2002).

Although measurement of salivary cortisol has emerged as an increasingly popular method of assessing HPA axis activity in children, its accurate measurement is sensitive to interference and dependent upon numerous factors, including type of assay and interfering substances (e.g., medications, caffeine, dairy, oral stimulants) (Clow, Thorn, Evans, & Hucklebridge, 2004; Jessop & Turner-Cobb, 2008; Kirschbaum & Hellhammer, 1992). While two studies provided investigations and guidelines for limiting interference due to interfering substances (Schwartz, Granger, Susman, Gunnar, & Laird, 1998; Talge et al., 2005), little work has addressed

methodological factors that may influence cortisol measurement in children. One factor that is of critical concern is parental compliance to instructed sampling times. As cortisol levels vary rapidly over time (Clow et al., 2004), deviations from instructed sampling times can compromise the accurate measurement and validity of cortisol data (Kudielka et al., 2003).

Overview of the HPA axis and Cortisol

The HPA axis, one of the body's major stress response systems, plays a critical role in coordinating the body's stress response and maintaining homeostasis (Tsigos & Chrousos, 2002). In response to stress, the hypothalamus triggers the release of corticotrophin releasing hormone (CRH), stimulating the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH then stimulates the adrenal glands to release cortisol (Tsigos & Chrousos, 2002). Cortisol is the major end product of the HPA axis and has widespread effects throughout the body, including effects on metabolism, immune response, cardiovascular function, mood and cognition (Jacobson, 2005; McEwen & Seeman, 1999). As such, although the HPA axis provides an adaptive biological response to acute stress, chronic stress may lead to the dysregulation of cortisol and to adverse effects on health (McEwen, 1998).

Diurnal Cortisol and the Cortisol Awakening Response (CAR). Cortisol levels follow a diurnal rhythm, seen initially in early infancy and stabilizing to an adult-equivalent rhythm during the preschool-age period (Jessop & Turner-Cobb, 2008). This diurnal rhythm is characterized by peak cortisol levels upon waking followed by a gradual decline throughout the day with lowest levels occurring around bedtime

(Fries, Dettenborn, & Kirschbaum, 2009). One distinct aspect superimposing this diurnal rhythm is the cortisol awakening response (CAR), during which cortisol levels rapidly increase about 50-75%, and peak within 30 minutes of waking (Clow, et al., 2004; Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). The CAR has been found to be independent of sleep duration, sleep quality, spontaneous awakening vs. alarm awakening, and disrupted sleep (Clow et al., 2004; Pruessner et al., 1997). In addition, the CAR has shown high intraindividual stability across time and is a reliable index of HPA axis activity (Hellhammer et al., 2007; Wust et al., 2000).

The CAR has gained a great amount of attention in recent years and has been studied extensively as a potential link between physiological functioning and psychosocial factors (Clow et al., 2004). Significant associations have been found between the magnitude of the CAR and physical and psychological health. In a recent meta-analysis, Chida and Steptoe (2009) reported significant associations between the magnitude of the CAR and life stress, fatigue, and post-traumatic stress disorder (PTSD). The CAR has also been hypothesized to serve as a trait-like vulnerability marker for depression (Adam et al., 2010; Bhagwagar, Hafizi, & Cowen, 2003, 2005; Mannie, Harmer, & Cowen, 2007).

Although the CAR holds potential clinical significance, only a few studies have examined the CAR in children (Freitag et al., 2009; Gribbin, Watamura, Cairns, Harsh, & LeBourgeouis, 2011; Hatzinger et al., 2007; Rosmalen et al., 2005). Progress in this line of research may be impeded by the methodological rigor required in assessing the CAR, which includes a time-sensitive collection of multiple morning samples across two or more days, which are commonly collected upon waking and 30

minutes after waking; obtaining the child's compliance to the protocol; and the child refraining from food and drink consumption prior to and during sampling (Clow et al., 2004). These imposed restrictions are necessary to obtain accurate cortisol data, but increase burden on parent and child participants, which may impact compliance with sampling.

Nevertheless, research examining the CAR in children is crucial to our understanding of the development of the HPA axis. Examination of the CAR in the preschool-age period, during which the circadian rhythm of basal HPA axis activity stabilizes into an adult-equivalent rhythm (Jessop & Turner-Cobb, 2008), may provide insight into the origins of HPA axis dysregulation and its potential role in the etiology of physical and psychiatric disorders. To advance research examining the CAR in children, it is critical to address methodological factors affecting the accuracy of cortisol measurement, including compliance with instructed sampling times, which can compromise the accurate measurement of cortisol data and affect subsequent interpretation of findings.

Ambulatory Assessment of Cortisol in Young Children

Ambulatory assessment of children's salivary cortisol activity has emerged as a widely used method as it affords the opportunity to assess the CAR and variations in salivary cortisol levels in the context of children's everyday lives, providing data which is likely more characteristic of children's typical HPA axis functioning than laboratory assessments. However, as accurate measurement of cortisol is highly dependent upon numerous factors (e.g. food, drink, dairy, medications, time of sample collection; see Gunnar & Talge, 2007; Hanrahan et al., 2006), a critical

disadvantage of ambulatory assessments is the lack of experimental control over participant behavior, particularly compliance to sampling times. As studies with ambulatory assessments of cortisol in young children rely upon parents to adhere to the sampling protocol, it is parental compliance that is of foremost concern.

Complicating the issue of parental compliance is that sampling protocols may often be complex and restrictive, to control for the numerous factors that impact the accurate measurement of cortisol. The constraints of the protocol, in conjunction with factors such as the parent's motivation, ability, work and family responsibilities (e.g., getting children to school or daycare), and the compliance of the child (i.e., child refusal to chew on a cotton dental roll), raise significant concerns regarding parental compliance. However, despite these concerns and the widespread use of ambulatory assessments of cortisol in children, little is known about parental compliance, including its impact on children's cortisol data. Moreover, concerns regarding parental compliance are supported by findings in the adult literature highlighting the effects of noncompliance on the integrity of cortisol data.

Compliance with Ambulatory Measures of Cortisol in Studies of Adults

Previous research examining the impact of adult sampling compliance on cortisol data have used electronic monitoring devices, consisting of a bottle in which sampling cotton rolls are placed, and a cap with a microprocessor that records the date and time of each bottle opening, to measure adult participants' objective compliance to instructed sampling times (Broderick, Arnold, Kudiekla, & Kirschbaum, 2004; Jacobs et al., 2005; Kudielka, Broderick, & Kirschbaum, 2003; Kudielka, Hawkley, Adam, & Cacioppo, 2007). Across these studies, overall

objective compliance rates ranged from 61% to 81% among participants uninformed of monitoring (Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003; Kudielka et al., 2007). However, objective compliance rates as high as 90% and 97% have been reported among participants informed of monitoring, suggesting that informing participants of electronic monitoring may enhance compliance (Broderick et al., 2004; Kudielka et al., 2003).

In studies systematically examining adult sampling compliance, cortisol data was consistently found to differ between compliant and noncompliant participants. Noncompliant participants evidenced significantly reduced CAR (Broderick et al., 2004; Kudielka et al., 2003; Kudielka et al., 2007) and flatter diurnal cortisol slope compared to compliant participants (Broderick et al., 2004; Jacobs et. al, 2005; Kudielka et al., 2003). Studies have also reported that the CAR was impacted when participants were discrepant greater than 15 minutes between objective and self-reported wake-times (DeSantis, Adam, Mendelsohn, & Doane, 2010; Dockray, Bhattacharyya, Molloy, & Steptoe, 2008). These findings provide strong evidence that the accurate measurement of cortisol, especially the CAR, is highly dependent upon participant compliance with sampling times. Participant noncompliance can produce spurious results, compromising the interpretation of findings between variables of interest and cortisol. Specifically, without information on objective compliance, it is possible that findings regarding the size of the cortisol estimate (e.g. reduced CAR) are simply an effect of noncompliance rather than a true characteristic of the variable under study. This phenomenon is likely not limited to studies in adults as it may affect studies with ambulatory assessments of cortisol in children.

Compliance with Ambulatory Measures of Cortisol in Studies of Youths

Studies with older youths. No study has systematically examined the effects of older youths' sampling compliance on cortisol data; however, a few studies have relied upon older youths to self-collect samples and electronically monitored their compliance with instructed sampling times (Ellenbogen, Santo, Linnen, Walker, & Hodgins, 2010; Hanson & Chen 2008; Walker & Chen, 2010; Wolf, Nicholls & Chen, 2008). These studies included youths ranging from school-age to late adolescence (i.e., ages 8 to 19) (Ellenbogen et al., 2010; Hanson & Chen 2008; Walker & Chen, 2010; Wolf et al., 2008). In all studies, saliva samples were collected across the day and assessed the CAR (Ellenbogen et al., 2010) and/or diurnal cortisol (Ellenbogen et al., 2010; Hanson & Chen 2008; Walker & Chen, 2010; Wolf et al., 2008). Reported rates of compliance were about 88% across studies (Walker & Chen, 2010; Wolf et al., 2008).

None of these studies provided a comprehensive examination of the effects of youths' compliance on cortisol data. However, a few studies have assessed compliance using electronic monitors to account for its potential effect on data. Ellenbogen and colleagues (2010) assessed compliance in a subset of the sample, providing evidence of high compliance in the sample prior to analyses. Other studies, while reporting the rate of noncompliance, did not report how noncompliance was treated in the data analysis (Hanson & Chen 2008; Walker & Chen, 2010; Wolf et al., 2008). It also should be noted that in the ambulatory assessments of cortisol in youths, without explicit assignment of sampling responsibilities by the researcher, sampling responsibility may in actuality be shared with the parent.

Studies with young children. Similar to the literature in older youths, no study using ambulatory measures of cortisol in young children have systematically examined the effects of parental compliance on the child's cortisol data. However, a few studies have assessed compliance using electronic monitors to account for its potential effect on data. Children in these studies included infants (Dozier et al., 2006), preschool-age children (Dozier et al., 2006; Gunnar et al., 2010), and school-age children (Corbett et al., 2008a, 2008b; Zinke et al, 2010). In most of these studies, children's saliva samples were collected by parents across the day on multiple days to assess diurnal cortisol profiles (Corbett et al., 2008a; Corbett et al., 2008b; Dozier et al, 2006; Gunnar et al., 2010). Only one study examined children's CAR by having parents collect children's saliva samples upon their child's waking and 30 minutes after waking (Zinke et al. 2010). Overall rates of parental compliance in these studies ranged from 86% to 99%.

In these studies, parental noncompliance was addressed by asking parents to resample saliva (Dozier et al., 2006), excluding noncompliant samples from analysis (Gunnar et al., 2010; Zinke et al., 2010), or including noncompliant samples in the analysis after verifying that inclusion had no effect on the results (Corbett 2008a; 2008b). None of these studies provided a comprehensive examination of the impact of parental noncompliance on children's cortisol data.

Limitations of previous research. The few studies that have monitored parental compliance with instructed sampling times are limited for several reasons. First, parental compliance was monitored to account for its effects as a potential confound. As a result, analyses in these studies did not include a systematic

examination of the impact of parental compliance on cortisol data comparable to the studies examining adult sampling compliance. Second, only one study included a sampling protocol to assess the time-sensitive CAR (Zinke et al. 2010), which has also been found to be especially susceptible to the effects of noncompliant sampling in adults (Kudielka et al., 2003; Kudielka et al., 2007). Third, most of the studies monitoring parental compliance only included clinical samples (Corbett et al., 2008a; Corbett et al. 2008b; Dozier et al., 2006; Zinke et al., 2010), which may demonstrate higher sampling compliance in comparison to non-clinical, healthy controls based on the adult literature (Broderick et al., 2004).

Statement of the Problem

Studies assessing HPA axis functioning in young children frequently involve parental collection of salivary cortisol in ambulatory settings. Although ambulatory measures allow for the assessment of children's HPA axis functioning in the context of their everyday lives, a critical disadvantage is the lack of experimental control over parental compliance to sampling times, as accurate measurement of cortisol is dependent on the timing of samplings. Parental compliance is a real and significant concern, as parents must collect the child's samples at specific, instructed times within the constraints and restrictions of the sampling protocol and within the context of the parent's ability, motivation and household responsibilities.

Despite the ramifications of sampling compliance on the integrity of cortisol data and subsequent interpretation, little is known about parental compliance. Research examining parental compliance to sampling times is needed, as it would

provide insight into the accuracy of parent-reports of sampling times, and the impact of parental compliance on children's cortisol data.

Current Study

The present study examined parental compliance with instructed times in a salivary cortisol sampling protocol assessing the CAR and diurnal cortisol in preschool-age children (ages 3-5 years). Preschool-age children and their biological parents were selected from a larger study examining neuroendocrine function and risk for depression. Parents were instructed to collect their child's salivary cortisol samples upon the child's waking, 30 and 45 minutes post-waking, and before bedtime on each of two consecutive weekdays. Parental compliance with the sampling protocol was assessed using parent-report of compliance and an objective measure of compliance using an electronic monitoring device (MEMS Track Cap; AARDEX, Ltd., Zug, Switzerland).

The present study had two aims:

Aim 1: The first aim was to examine concordance between parent-reported compliance and electronic monitoring of parental compliance by comparing a) compliance rates, b) agreement in reported compliance, and c) deviation from instructed sampling times, as reported by the two measures. We hypothesized that parent-reported sampling times would overestimate compliance. We also hypothesized that parent-report and electronic monitor would evidence moderate agreement, and that electronic monitoring would demonstrate greater deviation from instructed sampling times in comparison to parent-report.

Aim 2: The second aim was to examine the effects of parental compliance on young children's cortisol data, as reported by parent-report and electronic monitoring. Similar to findings in the adult literature, we hypothesized that noncompliance would be associated with a reduced CAR and flattened diurnal cortisol slope compared to compliant sampling. We also hypothesized that the effects of noncompliance would be stronger based on electronic monitoring than parent-report.

Chapter 2: Method

Participants

Participants were preschool age children and their biological parents drawn from a larger study examining neuroendocrine function and risk for depression. Participants were identified using a commercial mailing list (27.0%), and print advertisements distributed throughout local schools, daycares, community centers, and health care providers in the greater Washington, DC area (73.0%). A proportion of flyers specifically targeted parents with a history of depression. Families with a child between three and five years of age without any significant medical conditions or developmental disabilities, who were not taking corticosteroids, and who lived with at least one English-speaking biological parent were eligible for the study.

Of the 156 children from the larger study who completed the cortisol assessment, a random subsample of 95 children (50 females; 45 males) were invited to provide objective compliance data, measured by an electronic monitoring device (MEMS Track Cap; AARDEX Ltd., Zug, Switzerland). Of the 95 participants, six participants lost or never returned the electronic monitor. Participants who provided monitor data (n = 89) were compared to those from the larger study who did not provide monitor data (n = 67) on key parent, child, and demographic variables. No differences were found on child age, gender, race/ethnicity, parental marital status, parental education, and parental depression history. Five children were excluded for taking corticosteroid (n = 2), stimulant (n = 1), analgesic (n = 1) medications, and/or because they were sick with a fever (n = 1), as these factors have been shown to impact cortisol levels (Granger, Hibel, Fortunato, & Kapelewski, 2009; Gunnar &

Talge, 2007). Participants were required to provide at least 1 valid cortisol sample, leaving a total of 81 children in the final sample.

Of the children in the final sample, 45 (57.0%) had a family history of depression, based on the non-patient version of the Structured Clinical Interview for DSM-IV (SCID-NP; First, Spitzer, Gibbon, & Williams, 1996). Children were of average cognitive ability as measured by the Peabody Picture Vocabulary Test (*M* = 110.51, *SD* = 14.96, *Range* = 73.00 - 148.00) (PPVT; Dunn & Dunn, 1997). Demographic characteristics of the study sample are presented in Table 1. The study was approved by the human subjects review committee at the University of Maryland, and informed consent was obtained from parents.

Measures

Demographic characteristics. Demographic variables that may potentially affect cortisol levels and participant compliance were assessed using a parent-report questionnaire. Variables assessed included age, gender, race/ethnicity, marital status, household income, and parental education. For the full questionnaire, refer to Appendix B.

Salivary Cortisol. Parents were instructed to obtain salivary cortisol samples from their child immediately upon the child's waking, 30 and 45 minutes post-waking, and 30 minutes before bedtime on two consecutive days, for a total of 8 cortisol samples per child. Of the 638 cortisol samples collected, 28 samples (4.4%) were excluded due to extreme cortisol values (i.e., > 3 standard deviation above the mean; Gunnar & White, 2001), leaving a total of 610 valid cortisol samples. Sampling times were selected to capture the cortisol rise in awakening and nadir

cortisol levels at bedtime. Samples were collected on two days in order to reliably assess the CAR (Hellhammer et al., 2007), and on weekdays only as the type of day has been associated with cortisol levels (Kunz-Ebrecht et al., 2004).

Parents received all sampling materials in a kit and were informed of the use of an electronic monitoring device to monitor sampling times (MEMS ™ Track Cap; Aardex Ltd., Zug, Switzerland). Parents were instructed to open the bottle of the electronic monitoring device only at the child's sampling times, and to remove only one dental cotton roll from the bottle per sampling. Parents were instructed to refrain from sampling if their child was sick or taking antibiotics. In addition, parents were instructed to refrain from the following for the period prior to or during sampling: (1) brushing their child's teeth (2) giving their child food and/or drink, and (3) giving their child caffeine and dairy products, as these factors have been found to influence cortisol levels (Gunnar & Talge, 2008). Parents were given handheld mechanical timers to assist with the timed collection of samples. All verbal and written instructions emphasized the importance of accurate timing and reporting of samples.

To collect cortisol for analysis, parents were instructed to have their child chew on a cotton dental roll dipped in .025 g of Kool-Aid® to stimulate saliva. Previous work shows that the use of Kool-Aid® does not compromise the quality of the assays when used sparingly (Talge et al., 2005). When the cotton roll was saturated, parents were instructed to expel their child's saliva from the cotton roll into a vial using a needleless syringe. Parents were instructed to label and store samples in the refrigerator until their second visit to the laboratory, upon which samples were stored at -20□ C until assayed. Salivary cortisol samples were assayed at the

University of Trier, Germany, in duplicate with a time-resolved immunoassay with fluorometric end point detection (DELFIA). Inter- and intra-assay coefficients of variation ranged between 7.1%-9.0% and 4.0%-6.7%, respectively. For a description of the Salivary Cortisol Sampling protocol see Appendix C.

The following cortisol variables were included in analyses: cortisol values for each time point (waking, 30, 45 minutes post-waking, and bedtime), the CAR, and the diurnal cortisol slope (the rate of decline in cortisol levels from waking to bedtime). The CAR was quantified in two ways: the area under the curve with respect to ground (AUC_g; total cortisol secretion across the morning samples) and with respect to increase (AUC_i; the change in morning cortisol levels over time) for the 0, 30 and 45 minute post-waking samples (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The diurnal slope was calculated by dividing the difference in waking and bedtime cortisol levels by the number of hours between the two samples (Adam & Kumari, 2009). Following Gunnar and Talge (2007), summary variables (i.e., AUC and diurnal slope) were computed using untransformed values.

The distributions of cortisol variables were inspected for normality. Cortisol values for each time point (waking, 30, 45 minutes post-waking, and bedtime) and the diurnal cortisol slope showed positive skew; thus, $\log_{10}$ transformations were applied. As AUC variables were normally distributed, untransformed values were used in all analyses. For ease of interpretation, data presented in all tables and figures reflect untransformed values.

Measurement of Parental Compliance. Two methods were used to measure parental compliance to sampling times: parent-report and electronic monitoring.

Parent-report. Parent-reported compliance was assessed using a diary measure in which parents recorded the child's time of waking, bedtime, and all sampling times. The diary measure also assessed variables known to affect cortisol levels, including recent meal, dairy and caffeine intake, and medication use (see Appendix D).

Electronic monitoring. The MEMS Track Cap (Aardex Ltd., Zug, Switzerland) is an electronic monitoring device, which consists of a bottle in which sampling cotton dental rolls are placed, and a cap with pressure activated microcircuitry that records the dates, times, and duration of each bottle opening. It was used to provide an objective measure of compliance. Data was downloaded from the monitor to the computer using a specialized interface and software program (Aardex Ltd., Zug, Switzerland), and was carefully inspected for times corresponding to unintentional bottle openings (e.g. openings that did not correspond to sampling times, or excessive bottle openings within a limited time period). In such cases, invalid times were removed prior to analyses, and the monitor time that was closest to the sampling assessment time was retained (Broderick et al., 2004).

Compliance was determined for each method at the sample-level and person-level. To define compliance at the sample level, the following time window criteria were applied to samples. Consistent with previous literature (e.g. Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003; Kudielka et al., 2007), a stringent time window of $\pm$ 10 minutes was selected for the samples that compose the CAR (i.e., waking, 30 and 45 minute samples), as cortisol levels change rapidly during the morning (Clow et al., 2004), whereas a more liberal time window of $\pm$ 1 hour was

selected for the bedtime sample, as cortisol levels change more slowly during the evening (Fries et al., 2009). Samples collected within the time window were considered to be collected in compliance with the instructed sampling time.

To define compliance at the person-level, the CAR, diurnal slope, and bedtime cortisol of participants were dummy coded as compliant or non-compliant. For the CAR, participants were coded as compliant if all morning samples (i.e., waking, 30 and 45 minute post-waking samples) were collected within their established time windows; i.e. one or more noncompliant morning samples resulted in the participant being considered as noncompliant. For the diurnal cortisol slope, participants were coded as compliant if both the 'waking' sample and the 'bedtime' sample were collected within their established time windows. For the bedtime sample, participants were coded as compliant if their bedtime sample was collected within the established time window.

Data Analysis Plan. The first aim of the study was to examine concordance between parent-reported compliance and electronic monitoring of parental compliance by comparing a) compliance rates, b) agreement in reported compliance, and c) deviation from instructed sampling times as reported by the two measures. First, we compared compliance rates as reported by parent-report and electronic monitor over the following sampling periods: a) across both sampling days; b) across each sampling day; and c) across specific instructed samples. Compliance rates were expressed as percentages (i.e., the total number of compliant samples divided by the total number of non-missing samples). Paired samples *t*-tests were conducted to compare mean compliance rates between each measure. Next, the agreement between

parent-reported compliance and electronic monitoring of parental compliance was examined using Pearson correlations. Lastly, we conducted paired samples *t*-tests to compare the deviations between instructed sampling times and actual sampling times as reported by each measure.

The second aim of the study was to examine the effects of parental noncompliance on children's cortisol data, as reported by parent-report and electronic monitor. To examine this aim, we conducted repeated-measures analyses using generalized estimating equations (GEE) to account for within-person correlation between repeated-cortisol measurements across both days of sampling. GEE is a statistical approach that accounts for within-person correlations in time-course data (Liang & Zeger, 1986). GEE analyses were conducted separately for parent-report and electronic monitoring. Person-level compliance was entered as an independent variable, and cortisol values corresponding to each time point, $AUC_{g,}$ $AUC_{i,}$, and diurnal slope were entered as dependent variables in separate models.

Chapter 3: Results

Descriptive statistics and preliminary analyses. Table 1 presents descriptive statistics for demographic and cortisol variables. Across all participants, cortisol levels showed the expected diurnal pattern: levels increased to reach a peak 30 minutes post-waking, $t(150) = 6.15$, $p < .001$, declining thereafter to reach lowest levels at bedtime for both sampling days, $t(146) = -27.05$, $p < .001$. Waking values ($M = 7.95$ nmol/l) increased approximately 37% to reach a peak 30 minutes post-waking ($M = 10.88$ nmol/l), declining to reach lower levels at 45 minutes post waking ($M = 8.53$ nmol/l) and lowest levels at bedtime ($M = 2.31$ nmol/l). To assess the stability of cortisol levels across the two sampling days, we conducted Pearson correlations. The correlation between day 1 and day 2 waking, 30 and 45 minute post-waking, and bedtime cortisol were $r = .30$, $r = .31$, $r = .39$, and $r = .55$, respectively (all correlations were significant at $p < .01$). The correlation between day 1 and day 2 AUC_g was $r = .46$, $p < .001$; the correlation between day 1 and day 2 AUC_i was $r = .24$, $p = .01$. The correlation between day 1 and day 2 diurnal cortisol slopes was $r = .33$, $p = .01$. Overall, correlations ranged from $r = .24$ to $r = .55$, indicating moderate stability of cortisol levels across days.

Associations between cortisol and potential covariates were examined. Time of waking was positively associated with AUC_g on day 1 ($r = .27$, $p = .03$). Parental marital status (0 = unmarried, 1 = married) was negatively associated with AUC_i, $t(71) = 3.77$, $p < .001$, and diurnal cortisol slope on day 1, $t(73) = 2.68$, $p = .01$. Parental lifetime depression (0 = no history, 1 = lifetime history) was negatively associated with 30 minute post-waking cortisol on day 2, $F(1, 73) = 5.49$, $p = .02$.

Therefore, time of waking, parental marital status, and parental lifetime depression were included as covariates in all subsequent analyses involving cortisol. Associations between compliance and potential covariates were also examined. Neither parent-reported compliance nor objective compliance was associated with age, gender, ethnicity, parental marital status, parental education, or parental depression status.

Aim 1: To examine concordance between parent-reported compliance with electronic monitoring of parental compliance by comparing a) compliance rates, b) agreement in reported compliance, and c) deviation from instructed sampling times as reported by the two measures.

Parent-reported and objective compliance rates. We compared parent-reported and objective compliance rates. Overall parent-reported and objective compliance for the entire 2-day sampling period was 83.0% and 68.8%, respectively. Comparison of the mean compliance rates revealed that parent-reported compliance was significantly higher than objective compliance, $t(80) = 5.58$, $p < .001$. Examination of compliance rates for each day of sampling revealed that parent-reported compliance dropped from 84.5% to 79.5% from the first to second day of sampling. Objective compliance also declined, from 72.9% to 64.6%. Rates of compliance per instructed sampling time were also examined (Table 2). Parent-reported compliance was higher than objective compliance for each instructed sampling time. Overall, as indicated by both measures, bedtime samples demonstrated the highest rates of compliance, whereas the 45 minute post-waking sample evidenced the lowest rate of compliance.

Agreement between parent-reported and objective compliance. To examine the agreement in compliance as reported by parent-report and electronic monitor, Pearson correlations were computed (Table 2). Overall agreement between compliance as reported by parent-report and electronic monitor for the entire 2-day sampling period was $r = .64$. Agreement between parent-report and electronic monitor for each instructed sampling time was moderate, with correlations ranging from $r = .54$ to .84. Bedtime samples evidenced the highest agreement, whereas the 30 and 45 minute post-waking samples evidenced the least.

Deviation from instructed sampling times. To further compare parent-report and electronic monitor, we examined the deviation between instructed sampling times and sampling times as reported by each measure. The mean deviation between instructed morning sampling times (i.e., waking, 30 and 45 minute post-waking samples) and parent-report was 6.24 ± 18.01 minutes, whereas the mean deviation based on the electronic monitor was 11.99 ± 24.45 minutes. A pairwise *t*-test revealed that across morning samples, timing discrepancies were significantly larger based on the electronic monitor, $t(372) = -11.24$, $p < .001$. The mean deviation between parent-report and the instructed bedtime sampling was 27.00 ± 36.83 minutes, whereas the mean deviation based on the electronic monitor was 32.86 ± 45.57 minutes. A pairwise *t*-test revealed that bedtime sample timing discrepancies were significantly larger based on the electronic monitor, $t(129) = -2.03$, $p = .04$. Table 3 shows the mean discrepancy between instructed sampling times and times as indicated by parent and electronic monitor.

Aim 2: To examine the effects of parental compliance on children's cortisol data, as reported by parent-report and electronic monitoring.

Impact of compliance on children's morning cortisol response. As cortisol levels were nested within individuals, repeated-measures analyses using GEE were conducted to examine the effect of noncompliance on cortisol. Separate models were conducted using person-level compliance as reported by parent-report and electronic monitoring. As shown in Figure 1a, based on parent-report, there were no significant group differences in waking (b = - .02, SE = .06, p = .81), 30 minute (b = .04, SE = .06, p = .50), or 45 minute post-waking cortisol levels (b = .08, SE = .06, p = .17). In contrast, as seen in Figure 1b, based on electronic monitoring, there was a significant group difference in waking cortisol such that children of noncompliant parents evidenced significantly higher waking cortisol levels (M = 9.37, SD = 5.19) compared to children of compliant parents (M =7.53, SD = 5.32; b = - .15, SE = .06, p = .01). No significant group differences were observed for the 30 (b = .02, SE = .05, p = .66) or 45 minute (b = .06, SE = .06, p = .28) post-waking samples.

To assess the effects of parental compliance on children's CAR, we examined whether children of noncompliant and compliant parents showed different total cortisol secretion (AUC_g) and total change in cortisol (AUC_i) after awakening. Based on parent-report, there were no group differences in AUC_g (b = - 5.19, SE = 5.22, p = .32), or AUCi (b = 5.40, SE = 3.61, p = .14). Based on electronic monitoring, there were no group differences in AUC_g (b = - 5.62, SE = 3.54, p = .11). However, there was a significant association between noncompliance and AUC_i (b = 8.93, SE = 2.80, p = .001). Children of noncompliant parents evidenced smaller increases in

AUC_i (*M* = 1.17, *SD* = 19.56) compared to children of compliant parents (*M* = 9.88, *SD* = 16.37) (see Figure 1b). Thus, based on electronic monitoring, parental compliance was associated with blunted CAR or less of a rise in morning cortisol across the waking period.

We next examined the effects of parental noncompliance on children's diurnal cortisol slopes and bedtime cortisol levels. No significant effects of parental noncompliance based on parent-report were observed for the diurnal cortisol slope (*b* = .01, *SE* = .01, *p* = .92) or bedtime cortisol (*b* = -.12, *SE* = .17, *p* = .49). Similarly, no significant effects of parental compliance based on the electronic monitor were observed for the diurnal cortisol slope (*b* = .01, *SE* = .01, *p* = .64) or bedtime cortisol (*b* = -.10, *SE* = .16, *p* = .56).

Chapter 4: Discussion

To our knowledge, this is the first study to examine the effects of parental compliance with a salivary cortisol sampling protocol on young children's cortisol data. As research with ambulatory assessments of HPA-axis activity in children frequently relies upon parents to collect samples, we investigated how closely parents adhere to instructed sampling times, and the impact of their noncompliance on children's cortisol data. We compared rates and effects of compliance as reported by parent-report and electronic monitor. Despite moderate concordance between parent-report and the electronic monitor, we found that parent-reported compliance was consistently higher than objective compliance. We also found that children of noncompliant parents based on the electronic monitor evidenced higher waking cortisol and a lower CAR, compared with children of compliant parents.

This study examined parental compliance by comparing the concordance between parent-report and electronic monitor. We found that parents self-reported higher rates of compliance to sampling than parental compliance rates based on the electronic monitor. Overall parent-reported compliance was 83.0%, whereas objective compliance was significantly lower at 68.8%, suggesting that parents may overestimate their compliance with the sampling protocol. The objective compliance rate we observed is consistent with objective compliance rates ranging from 61% to 81% reported in previous studies examining sampling compliance among adults uninformed of electronic monitoring (Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003, 2007). However, it is noteworthy that the objective compliance rate we observed is lower compared to rates reported for adults informed of

monitoring (90% reported in Broderick et al., 2004; 97% reported in Kudielka et al., 2003). Given that our participants were informed of monitoring, the lower rate of compliance we observed may reflect the difficult nature of assessing cortisol samples in children, for whom parents must collect samples. The objective compliance rate we observed is also lower than rates (86-99%) reported in youth studies (Corbett et al., 2008a, 2008b; Dozier et al., 2006; Ellenbogen et al., 2010; Gunnar et al., 2010; Hanson & Chen, 2008; Walker & Chen, 2010; Wolf et al., 2008; Zinke et al., 2010), likely because our study included an assessment of the CAR, which involves the collection of multiple morning samples within a narrow period of time. In contrast, previous youth studies assessed cortisol throughout the day across larger periods of time, and accordingly, used larger time windows of compliance. Our findings suggest that there may be unique challenges involved in collecting cortisol from young children, particularly when collecting multiple morning samples, and highlight the need for continued research examining methodological issues from a child- and parent-focused perspective.

Although parent-report and electronic monitoring showed moderate agreement in reported compliance, the two measures demonstrated notable differences. First, whereas agreement between the measures was highest for bedtime cortisol, it was lowest for the 30 and 45 minute CAR samples. This indicates that parents are most compliant when collecting evening cortisol and less compliant when collecting CAR samples, suggesting that collecting several cortisol samples across the waking period may be particularly challenging for parents. Second, electronic monitoring indicated that the actual deviation from instructed sampling times was

twice on average what was reported by parents. Similarly, Kudielka et al., (2003) found significant deviations in instructed sampling time between participant report and objective data. The discrepancies between parent-report and electronic monitor of parental compliance may reflect parents' overestimation of their compliance with the protocol or their desire to appear compliant to researchers.

Our study also examined the impact of parental compliance assessed using both parent-report and electronic monitor measures on children's cortisol data. We found that children of parents who were noncompliant based on the electronic monitor evidenced significantly higher waking cortisol and had lower or blunted CAR, as indicated by a lower AUCi. These results provide evidence that parental noncompliance to the waking sample leads to elevated waking values affected by the rapid post-awakening cortisol rise, which in turn, results in a lower or blunted CAR. These findings converge with reports of significantly lower CAR among noncompliant adult participants (Broderick et al., 2004; Kudielka et al., 2003; 2007), and are also similar to emerging evidence from studies using objective measures of waking (e.g. actigraphy) which have shown that delays in collection of the waking sample are associated with reduced CAR (DeSantis, Adam, Mendelsohn, & Doane, 2010; Dockray, Bhattacharyya, Molloy, & Steptoe, 2008; Okun et al., 2010). These findings suggest the need for including an assessment of parental compliance with an electronic monitor, particularly when collecting samples in the morning when rapid changes in cortisol occur, as noncompliance could affect the interpretation of results.

In contrast to results based on the electronic monitor, parental compliance based on parent-reports was not associated with children's cortisol data. The different

findings across the two methods of assessment suggest that researchers should consider assessing compliance using both parent-report and the electronic monitor, particularly when assessing morning cortisol. Nevertheless, parental noncompliance as assessed with parent-report and the electronic monitor did not impact children's diurnal cortisol slopes or bedtime cortisol. Our finding that noncompliance does not affect diurnal cortisol slopes is consistent with Jacobs et al., (2003) who found that noncompliance did not impact the diurnal slope in adults, but is in contrast to other studies (Broderick et al., 2004; Kudielka et al., 2003). These differences in findings may be due to methodological differences in computing the diurnal slope. Similar to Jacobs et al. (2003) and Adam & Kumari (2009), we anchored the slope on the waking sample and excluded the CAR values (i.e., 30 and 45 minutes post-waking samples) from calculation of the slope in order to assess the diurnal slope separately from the CAR. However, previous studies examining adult sampling compliance have included the 30 minute sample in calculation of the slope (Broderick et al., 2004; Kudielka et al., 2003), which may possibly confound the CAR with the diurnal slope.

Overall, the present findings stress that measuring compliance is critical, as parental compliance cannot be assumed. Findings suggest that parent noncompliance may be more of a concern for researchers when assessing morning cortisol samples than samples collected at bedtime. Moreover, comparison of parent-report and electronic monitoring suggests that parents are differentially compliant in collecting waking and evening cortisol. Not only did morning cortisol appear to be more sensitive to the effects of noncompliance, but parents were also found to be less

compliant when collecting morning CAR samples. In contrast, not only did bedtime cortisol appear to be more robust to the effects of noncompliance, but parents also appeared to be reasonably compliant in its collection. Given the discrepancies observed between parent-report and electronic monitoring, the present findings suggest that electronic monitoring devices are necessary when assessing rapid changes in cortisol across the morning. However, we recognize that a significant drawback of using the electronic monitor is the greater experimenter and participant burden, as well as their significant expense. In contrast, in the evening, when cortisol changes more slowly, use of either the parent-report or electronic monitor would be reasonable options for assessing parental compliance.

Strengths and Limitations. Our study was the first to examine systematically parental compliance to child cortisol sampling, which is critical given the widespread reliance on parent-collected child cortisol data in home settings. In addition, this study extended the literature by further examining differences between compliance as assessed by parent-report and electronic monitoring. The study had several methodological strengths, including the collection of multiple cortisol samples, which included assessments of the CAR, across two days to increase reliability of cortisol measurement, and the use of electronic monitors to produce discrete, detailed data that was compared to parent-report.

The study also had several limitations. First, children's wake times were based on parent-report, rather than an objective measure of waking. The use of actigraphy would provide a more objective assessment; nevertheless, evidence suggests that participants are reasonably accurate in reporting wake times (DeSantis, Adam,

Mendelsohn, Doane, 2010; Dockray, Bhattacharyya, Molloy, Steptoe, 2008). Second, although electronic monitoring is a simple, unintrusive method of assessing compliance to sampling times, it is not without limitations. One important limitation is that the electronic monitor is assumed to be the more accurate approach; however, this assumption is not necessarily the case. For example, electronic monitors are not foolproof against participant error. For instance, participants may remove more than one cotton roll at once, which may result in less bottle openings and an underestimation of compliance. Another limitation is that the electronic monitor indicates bottle openings rather than actual sampling behavior. Similar to all studies using electronic monitoring, our study is not exempt from these drawbacks. Fourth, the sample was drawn from a larger study that overselected children with a family history of depression, which may limit the generalizability of results. However, depression history was not associated with significant differences in compliance in our sample.

In closing, consistent with previous research examining cortisol sampling compliance in adults, our findings strongly suggest that compliance is an issue of significant concern in research with ambulatory assessments of cortisol in young children. The present results hold important methodological implications. As meaningful differences were found between data based on parent-report and electronic monitor, findings suggest that future studies cannot merely rely upon parent report of compliance when assessing cortisol in children. Findings speak to the necessity of using the electronic monitor, particularly in studies assessing morning cortisol and the CAR in children. To the extent that parental compliance may

compromise the integrity of cortisol data, it is also imperative for future studies to utilize strategies to maximize parental compliance, including providing parents with mechanical timers, alarms or stop watches; calling or emailing parents the day before sampling; putting parents at ease so they feel that they can fully and candidly report noncompliance; and engaging participants with purpose of the study (see Adam & Kumari, 2009 for a list of suggestions). Such strategies are especially important when assessing children's waking cortisol or the CAR, when parental compliance is a substantial concern.

Tables

Table 1

Subject and cortisol characteristics (N=81)

	% (*N*)	*M (SD)*	Min	Max
Child characteristics				
Gender (male)	46.9 (38)			
Age (months)		49.93 (10.09)	36.00	71.00
Race/ethnicity				
White	49.4 (39)			
Black/African American	36.7 (29)			
Other	13.9 (11)			
Hispanic	17.7 (14)			
Parent characteristics				
Mother age (years)		34.45 (6.15)	21.00	48.00
Father age (years)		36.80 (6.67)	20.00	51.00
Marital status				
Married	67.9 (55)			
Divorced, separated, widowed	8.6 (7)			
Never married	23.5 (19)			
$\geq$ 1 parent college graduate	70.4 (57)			
Parental lifetime depressive disorder	57.0 (45)			
Salivary cortisol indicators				
Time of waking (h)				

Day 1	7:27 (1:08)	4:28	12:45
Day 2	7:28 (1:14)	4:46	11:48
Bedtime (h)			
Day 1	20:42 (2:37)	19:00	00:00
Day 2	20:29 (3:38)	19:00	1:00
Cortisol waking values (nmol/L)			
Day 1	7.38 (4.69)	.12	23.73
Day 2	8.51 (5.67)	1.52	32.36
Cortisol waking + 30 min values (nmol/L)			
Day 1	10.84 (5.70)	1.95	31.02
Day 2	10.92 (4.93)	2.18	25.69
Cortisol waking + 45 min values (nmol/L)			
Day 1	8.92 (5.57)	.15	32.36
Day 2	8.13 (5.11)	.99	32.90
Cortisol evening values (nmol/L)			
Day 1	1.92 (3.77)	.14	19.47
Day 2	2.71 (5.91)	.13	31.04
Diurnal cortisol slope (nmol/L per hour)			

Day 1	-.46 (.35)	-1.73	.36
Day 2	-.46 (.51)	-1.60	1.98
AUC_g (nmol/L)			
Day 1	42.41 (18.01)	8.99	106.40
Day 2	46.76 (24.11)	15.12	150.66
AUC_i (nmol/L)			
Day 1	7.39 (17.65)	-44.53	44.66
Day 2	4.93 (18.73)	-43.76	49.75

Note. Categorical variables are presented as frequency and percentage; continuous variables are presented as mean and standard deviation. Cortisol values reflect raw values for ease of interpretation and are presented in nmol/L. Area under the curve (AUC) was measured with respect to ground (AUC_g) and increase (AUC_i).

Table 2

Percent compliance and correlations between parent-report and electronic monitor for each sampling time

	Percent Compliance		
Instructed Sampling Time	Parent-report	Electronic Monitor	*r*
Waking	85.4	73.1	.58***
Waking + 30	84.6	66.5	.54***
Waking + 45	77.6	55.7	.54***
Bedtime	80.4	76.9	.84***
Overall	83.0	68.8	.64***

***$p < .001$.

Table 3.

Deviance from instructed sampling as indicated by parent-report and electronic monitor and paired samples t-tests between deviance as indicated by parent-report and electronic monitor

	Deviance from instructed sampling time		
	Parent-report	Electronic monitor	
Instructed Sampling Time	Mean (SD)	Mean (SD)	*t* statistic
Overall morning	6.25 (18.00)	11.99 (24.45)	-11.24***
Waking	5.52 (20.62)	9.84 (22.10)	-6.80***
Waking + 30	6.59 (20.86)	11.02 (22.47)	-6.49***
Waking + 45	7.86 (12.20)	16.73 (29.78)	-6.29***
Bedtime	27.00 (36.83)	32.86 (45.57)	-2.03*
Overall (all samples)	11.12 (25.35)	17.38 (32.55)	-6.60***

$^{\dagger}p < .10$; $*p < .05$; $**p$, .01; $***p < .001$. *Note.* Units are in minutes.

Figures

Figure 1. Children's early morning cortisol values as a function of parental compliance status. The graph in (a) compares results for children whose parents were compliant and those whose parents were not compliant to instructed sampling times based on the diary. The graph in (b) compares results for children whose parents were compliant and those whose parents were not compliant to instructed sampling times based on electronic monitoring. Bars reflect standard errors of measurement. $*p < .05$.

Figure 1a.

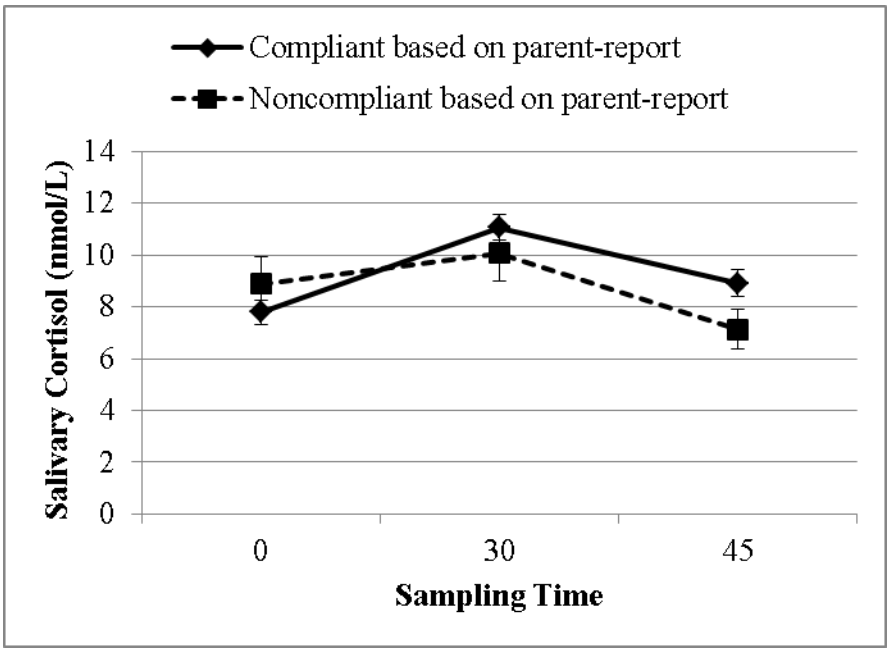
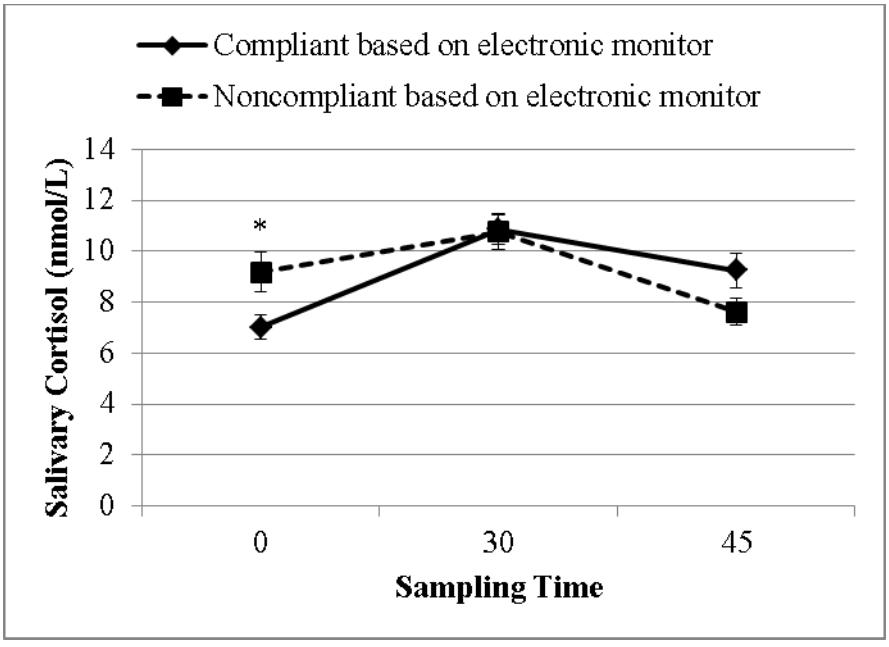


Figure 1b.



Appendices

Appendix A. List and schedule of measures.

Assessment	**Schedule**	**Description**
Demographic Information	Visit 1	Demographic Questionnaire
Salivary Cortisol	Between Visits 1 & 2	Parents will collect cortisol samples from their child on two consecutive weekdays immediately upon the child's waking, 30 and 45 minutes post-waking, and 30 minutes before the child's bedtime.
Electronic Monitoring Device	Between Visits 1 & 2	Medication Event Monitoring System 6 Track Cap (MEMS 6 Track Cap; AARDEX, Ltd., Zug, Switzerland)
Daily Diary	Between Visits 1 & 2	Daily Diary Questionnaire

Appendix B. Demographic Questionnaire

Demographic Data

Child's Age: _____ years

Child's date of birth: _____MM/_____DD/_____YYYY

Your relationship to child: □ Mother □ Father □ Other; Specify_____

Child's Ethnicity: □ White □ African American □ Asian □ Other

Is child of Hispanic descent? □ Yes □ No

With which adults does the child currently live? *(Check all that apply)*

□ Biological mother
□ Biological father
□ Adoptive mother
□ Adoptive father
□ Step-mother or father's companion
□ Step-father or mother's companion
□ Other relative(s)
□ Other non-relative(s)

Marital Status of child's biological parents:

□ Married
□ Living together
□ Mother deceased
□ Father deceased
□ Father remarried
□ Separated
□ Divorced
□ Never married
□ Mother remarried

Please list the child's siblings in order of birth. *(Please indicate first names)*

First Name	Sex	Age	Living at Home
	□ Male □ Female		□ Yes □ No
	□ Male □ Female		□ Yes □ No
	□ Male □ Female		□ Yes □ No
	□ Male □ Female		□ Yes □ No
	□ Male □ Female		□ Yes □ No
	□ Male □ Female		□ Yes □ No

PARENT INFORMATION: *(Please complete for biological parents if known)*

Mother: Age: _____ Mother's date of birth:
_____MM/_____DD/_____YYYY

Mother's present occupation: _____

Father: Age: _____ Father's date of birth:
_____MM/_____DD/_____YYYY

Father's present occupation: _____

Education of Mother:

□ 8th Grade or Less

□ Some High School

□ High School Graduate (or GED)

□ Some College (or 2 Year Degree)

□ 4 Year College Degree

□ Master's Degree

□ Doctoral Degree

Education of Father:

□ 8th Grade or Less

□ Some High School

□ High School Graduate (or GED)

□ Some College (or 2 Year Degree)

□ 4 Year College Degree

□ Master's Degree

□ Doctoral Degree

Yearly Family Income:

□ <$20,000 □ $20,001 - $40,000 □ $40,001 - $70,000 □ $70,001 - $100,000
□ > $100,000

COMPLETE THIS SECTION IF ADULT(S) CARING FOR CHILD IS/ARE NOT BIOLOGICAL PARENTS:

A. Relationship to child: □ Adoptive parent □ Other relative
Age: _____
□ Step parent □ Other non-relative

B. Relationship to child: □ Adoptive parent □ Other Relative
Age: _____
□ Step parent □ Other non-relative

Highest level of education for non-biological caretaker: *(See above)*

Caretaker A (above):
□ 8th Grade or Less
□ Some High School
□ High School Graduate (or GED)
□ Some College (or 2 Year Degree)
□ 4 Year College Degree
□ Master's Degree
□ Doctoral Degree

Caretaker B (above):
□ 8th Grade or Less
□ Some High School
□ High School Graduate (or GED)
□ Some College (or 2 Year Degree)
□ 4 Year College Degree
□ Master's Degree
□ Doctoral Degree

Yearly family income of non-biological caretaker:

□ <$20,000 □ $20,001 - $40,000 □ $40,001 - $70,000 □ $70,001 - $100,000
□ > $100,000

CHILD'S MEDICAL HISTORY:

Does child have any illnesses or disabilities (either physical or mental)? □
Yes □ No

If yes, please describe:
_____

Please mark the circle next to any medical conditions your child has ever had.

□ Epilepsy/seizures/convulsions

□ Seizures with high temperatures

□ Birth abnormalities

□ Heart disease

□ Asthma

□ Food sensitivities

□ Allergies (describe)
_____

□ Chicken pox

□ German measles

□ Whooping cough

□ Problems with vision

□ Problems with hearing

□ Serious accident (describe below)
_____

□ Fever over 104, unknown cause

□ Head injuries or lacerations leading to loss of consciousness

□ Unconscious (other)

□ Anemia

□ Lead poisoning

□ Meningitis

□ Encephalitis

□ Mumps

□ Emergency room visit

□ Poisoning, medicines

□ Poisoning, cleaning agent

□ Poisoning, non-food item

□ Physical handicaps (describe below)
_____

□ Other diseases (describe below)
_____

Is child taking medications for any conditions above? □ Yes □ No

Medication (specify)
_____

Has your child ever been hospitalized for a medical problem? □ Yes □ No
If yes, please specify:
a) Number of times
_____

b) Reason(s)?

_____

CHILDHOOD HISTORY:

How many pregnancies did mother have before the pregnancy with this child?
(Including those not carried to term)

_____ # pregnancies

Check any of the following that occurred during the pregnancy with this child:
(Check all that apply)

□ Severe nausea and vomiting □ Toxemia

□ High blood pressure □ Rubella, Mumps

□ Incompatible Rh factor □ Diabetes

□ Anemia

□ Bleeding 1st 3 months □ Bleeding 2nd 3 months □ Bleeding 3rd 3 months

Medications during pregnancy: □ No □ Yes

Please specify medications (include antidepressants, name of drug, dosage, and duration of use)

(1)
_____
_____

(2)
_____
_____

(3)
_____
_____

(4)
_____
_____

(5)
_____
_____

Check any of the following if they occurred at or following the delivery of the child:
(Check all that apply)

□ Premature delivery
Specify weeks of gestation at birth: _____

□ Infant required oxygen

□ Cesarean section

□ Infant required blood transfusion

□ Breech delivery (feet or buttocks first)

□ Infant was placed in an incubator

□ Infant had cord around neck

□ Infant was blue at birth

□ Other problems (specify)
_____

Child's weight at birth: _____ pounds _____ ounces

Did your child stay in the hospital after mother left? □ Yes □ No

If yes, please specify number of days _____

During the first year of life, did your child have difficulties in any of the following areas? *(Check all that apply)*

□ Sleep problems

□ Excessive crying

□ Feeding problems

□ Difficult to comfort

□ Resisted being held

□ Sluggish, nonresponsive

□ Overly active

□ Fussy much of the time

□ Under active

Was child breast-fed? □ Yes □ No **If yes, for how long?** months

Age child started walking without assistance: _____months

Age child spoke first words: _____months

Age child dressed without supervision: _____months

Did your child have difficulties with the development of speech? *(Check all that apply)*

□ No difficulties
□ Did not use "I" or "me"

□ Delayed speech
□ Often repeated other's words

□ Stammering
□ Talked excessively about one topic

□ Hard to understand
□ Other

Child's primary caregiver(s) are:
(check all that apply)

□ Mother □ Father □ Grandparent □ Live-in nanny/sitter □ Other_____

How many <u>hours per week</u> does your child spend in the following:

_____School _____Daycare _____Other childcare setting

Does mother work outside of the home? □ Yes □ No

If yes, how many hours per week? _____

Does father work outside of the home? □ Yes □ No

If yes, how many hours per week? _____

About how many close friends does your child have? (Do not include brothers and sisters)

□ None □ 1 □ 2 or 3 □ 4 or more

About how many times a week does your child do things with any friends outside of regular school hours?
(Do not include brothers and sisters)

□ Less than 1 □ 1 or 2 □ 3 or more

Compared to others of his/her age, how well does your child:

	Worse	Average	Better	
a) Get along with his/her brothers and sisters? Has no siblings	□	□	□	□
b) Get along with other kids?	□	□	□	
c) Behave with his/her parents?	□	□	□	
d) Play and work alone?	□	□	□	

Does your child receive special education or remedial services or attend a special class or special school?

□ No □ Yes

If yes, please describe the kind of services, class or school
_____

_____
_____

Has your child repeated any grades?

□ No □ Yes

If yes, please describe the grades and reasons

_____

_____

_____

Has your child had any academic or other problems in school?

□ No □ Yes

If yes, please describe._____

_____

_____

_____

Please describe the best things about the child:

_____

_____

_____

_____

Appendix C. Salivary Cortisol Sampling Instructions

INSTRUCTIONS FOR TAKING SALIVA SAMPLES AT HOME (CHILD)

Parent should collect individual samples from their child at the following scheduled times on
TWO consecutive weekdays:

1. **IN THE MORNING UPON CHILD AWAKENING**
2. **30 MINUTES AFTER CHILD AWAKENING**
3. **45 MINUTES AFTER CHILD AWAKENING**
4. **30 MINUTES BEFORE CHILD BEDTIME**

Use the timers to help keep track of the sampling times. Use the Sticker Sheet to help keep track of your progress.

RULES: As you collect saliva, we ask that:

- You select **2 consecutive weekdays** for sampling. Aim for two typical weekdays. AVOID especially troublesome or exciting days or a weekend.
- Do not collect saliva samples if you or your child is sick or taking antibiotics.
- Do not brush your teeth before sampling.
- Drinking a glass of water upon waking is acceptable.
- Do not eat or drink anything prior to sampling, other than water.
- Avoid caffeinated and dairy products prior to sampling.
- Both you and your child MUST use the Kool-Aid crystals to collect saliva samples.
- Complete the Daily Diary for your child at the end of each sampling day.

Step 1: **Set the Timer.** In the morning upon child awakening, set the timer for 30 minutes.

Step 2: **Open Vial.** Pop open the cap from the plastic vial.

Step 3: **Eat Kool-Aid.** Dip the cotton roll in just a few crystals, less than $1/16^{th}$ of a teaspoon. Do not add water to the Kool-Aid.

Step 4: **Chew Cotton.** Chew the cotton in your mouth until it is very moist. Parents should tell the child that she or he is not to swallow the cotton, only to chew it. This usually takes about one minute of chewing. We recommend counting to 60.

Step 5: **Fill Vial.** Separate the plunger from the barrel of the syringe. Put wet cotton roll into barrel. Re-insert plunger, push down, and collect the saliva into vial. Hold the vial firmly, as it can slip. Try to fill at least one-third of the vial. (Discard cotton & syringe.)

Step 6: **Label the Vial.** Record the date and time of day on the correct pre-printed label. Attach this label onto the vial so that it forms a "flag" around the vial. Use a permanent marker/pen to write on the label if you have one. If not, use a pencil.

Step 7: **Refrigerate!** Place the sample into the plastic storage bag. Refrigerate the sample. Refrigeration is a Very Important Step.

Step 8. **When the timer buzzes**, **repeat Steps 1-7** to collect the next sample. Set the timer for 15 minutes as a reminder to collect the saliva for the third time point.

Step 9: **At night, set the timer for 30 minutes before bed.** Repeat Steps 1-7 when the timer buzzes.

Step 10: **Fill out the Daily Diary.** After you have collected all the saliva samples, fill out and your child's Daily Diary for Day 1.

Step 11: **Tomorrow, repeat steps 1-10 for your child.**

Step 12: **Return samples along with the Daily Diaries.** After two days of sampling, your child should have completed a total of 8 samples. Please return the samples, along with the Daily Diaries to our lab on your second lab visit.

If you have ANY questions, please give us a call. No question is strange regarding this process, and we would be happy to answer your questions. Please call the Child Stress and Emotions Lab at 301-405-9880.

Appendix D. Daily Diary

Day 1 – Daily Diary (CHILD Form)
Home Saliva Collection

REMINDER:

Across 2 consecutive weekdays, collect Samples: (1) upon waking, (2) 30 minutes after waking, (3) 45 minutes after waking, and (4) before bed. There should be a total of 4 samples collected from your child on each day. Complete the daily diary after all samples for that day have been collected.

1. Day 1: Date of saliva collection
   _____MM/_____DD/_____YY

2. Day of week (circle one): SUN MON TUES WED THURS FRI SAT

3. Time of child's waking:
   _____AM

4. Was this the child's normal time of waking? NO YES

5. If NO, when does the child normally awaken?
   _____AM

6. Time child went to sleep this evening
   _____PM

7. Time of Sample 1 (to be collected upon waking):
   _____AM

8. Time of Sample 2 (30 minutes after waking):
   _____AM

9. Time of Sample 3 (45 minutes after waking):
   _____AM

10. Time of Sample 4 (30 minutes before bed):
    _____PM

11. Did your child go to school or daycare today? NO YES

12. Does your child have difficulty falling asleep? (Circle one)

    Never Sometimes
    Frequently

13. Circle approximately how long it took your child to fall asleep the night before the
morning sampling. (Circle one)

1.15 min 16-30 min > 30 min

14. How many hours of sleep did your child get on the night prior to the morning sampling? _____hours

15. Circle the best description of your child's health today? (Circle one)

Healthy Sick

If your child was not feeling well today, please comment briefly on his/her symptoms:

16. Does your child use an inhaler for asthma? NO YES

a. If yes, when did your child last use the inhaler?

_____MM/_____DD/_____/YY

b. Did your child use the inhaler the day before or on the day of saliva sampling?
NO YES

c. What is the name of the inhaler? _____

17. Is your child currently using any medications? NO YES

a. If yes, please list medication(s):

18. Please mark which activities your child did on the day of sampling:

____School
____Daycare
____Shopping
____Visiting friends
____Family outing

____Club meeting
____Sport participant
____School event
____Quiet activity at home (homework or TV)
____Playing at home
____Other, please specify: _____

19. Please mark any of the following that apply to your child on the day of the saliva sampling:

_____Argument(s) with parent that lasted more than a few moments
_____Argument(s) with sibling(s) that lasted more than a few moments
_____Argument(s) with friend(s) that lasted more than a few moments
_____Prolonged concerns or things that cause your child to worry
_____Other events causing anxiety or distress for your child
_____None of the above

20. Did your child eat a meal within the hour before any of the samplings?

Before Sample 1? _____NO _____YES If yes, when?_____AM/PM
Before Sample 2? _____NO _____YES If yes, when?_____AM/PM
Before Sample 3? _____NO _____YES If yes, when?_____AM/PM
Before Sample 4? _____NO _____YES If yes, when?_____AM/PM

21. Did your child eat or drink any caffeinated products (e.g., soda, chocolate, iced tea) within two hours prior to any sampling?

Before Sample 1? _____NO _____YES
Before Sample 2? _____NO _____YES
Before Sample 3? _____NO _____YES
Before Sample 4? _____NO _____YES

22. Did your child eat or drink any dairy products within 15 minutes prior to any sampling?

Before Sample 1? _____NO _____YES
Before Sample 2? _____NO _____YES
Before Sample 3? _____NO _____YES
Before Sample 4? _____NO _____YES

23. Has your child had a recent tooth loss? (Circle one) NO YES

a. If yes, when? _____MM/_____DD/_____YY

24. Does your child have any cuts in his/her mouth? Or is there any reason that

there would be blood in your child's mouth? (Circle one) NO YES

a. If yes, what is the reason?

Thanks! One more day to go…

Please label and refrigerate the samples.

Day 2 – Diary (CHILD)
Home Saliva Collection

REMINDER:

Across 2 consecutive weekdays, collect samples: (1) upon waking, (2) 30 minutes after waking, (3) 45 minutes after waking, and (4) before bed. There should be a total of 4 samples collected from your child on each day. Complete the daily diary after all samples for that day have been collected.

1. Day 2: Date of saliva collection
   _____MM/_____DD/_____YY

2. Day of week (circle one): SUN MON TUES WED THURS FRI SAT

3. Time of child's waking:
   _____AM

4. Was this the child's normal time of waking? NO YES

5. If NO, when does the child normally awaken?
   _____AM

6. Time child went to sleep this evening
   _____PM

7. Time of Sample 1 (to be collected upon waking):_____AM

8. Time of Sample 2 (30 minutes after waking):
   _____AM

9. Time of Sample 3 (45 minutes after waking):
   _____AM

10. Time of Sample 4 (30 minutes before bed):
    _____PM

11. Did your child go to school or daycare today? NO YES

12. Does your child have difficulty falling asleep? (Circle one)

    Never Sometimes
    Frequently

13. Circle approximately how long it took your child to fall asleep the night before the morning sampling. (Circle one)

1.16 min 16-30 min > 30 min

14. How many hours of sleep did your child get on the night prior to the morning sampling? _____hours

15. Circle the best description of your child's health today? (Circle one)

Healthy Sick

If your child was not feeling well today, please comment briefly on his/her symptoms:

16. Does your child use an inhaler for asthma? NO YES

a. If yes, when did your child last use the inhaler?

_____MM/_____DD/_____/YY

b. Did your child use the inhaler the day before or on the day of saliva sampling?
NO YES

c. What is the name of the inhaler? _____

17. Is your child currently using any medications? NO YES

a. If yes, please list medication(s):

18. Please mark which activities your child did on the day of sampling:

____School
____Daycare
____Shopping
____Visiting friends
____Family outing
____Club meeting

____Sport participant
____School event
____Quiet activity at home (homework or TV)
____Playing at home
____Other, please specify: _____

19. Please mark any of the following that apply to your child on the day of the saliva sampling:

_____Argument(s) with parent that lasted more than a few moments
_____Argument(s) with sibling(s) that lasted more than a few moments
_____Argument(s) with friend(s) that lasted more than a few moments
_____Prolonged concerns or things that cause your child to worry
_____Other events causing anxiety or distress for your child
_____None of the above

20. Did your child eat a meal within the hour before any of the samplings?

Before Sample 1? _____NO _____YES If yes, when?_____AM/PM
Before Sample 2? _____NO _____YES If yes, when?_____AM/PM
Before Sample 3? _____NO _____YES If yes, when?_____AM/PM
Before Sample 4? _____NO _____YES If yes, when?_____AM/PM

21. Did your child eat or drink any caffeinated products (e.g., soda, chocolate, iced tea) within two hours prior to any sampling?

Before Sample 1? _____NO _____YES
Before Sample 2? _____NO _____YES
Before Sample 3? _____NO _____YES
Before Sample 4? _____NO _____YES

22. Did your child eat or drink any dairy products within 15 minutes prior to any sampling?

Before Sample 1? _____NO _____YES
Before Sample 2? _____NO _____YES
Before Sample 3? _____NO _____YES
Before Sample 4? _____NO _____YES

23. Has your child had a recent tooth loss? (Circle one) NO YES

a. If yes, when? _____MM/_____DD/_____YY

24. Does your child have any cuts in his/her mouth? Or is there any reason that there would be blood in your child's mouth? (Circle one) NO YES

a. If yes, what is the reason?

Thanks again! Please label and refrigerate all samples and bring them with you on your second visit to our lab . If you are not returning to the lab, please mail the envelope and questionnaires to us in your pre-paid envelope.

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