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Noisy Spit: Parental Noncompliance with Child Salivary Cortisol Sampling

ABSTRACT: Studies assessing hypothalamic-pituitary-adrenal (HPA) 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 preschoolage children (ages 3–5). Eighty-one parents were instructed to collect their child's salivary cortisol samples upon their child's waking, 30 and 45 min 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. © 2013 Wiley Periodicals, Inc. Dev Psychobiol 56: 647-656, 2014.

Keywords: compliance; electronic monitoring; salivary cortisol sampling; cortisol; cortisol awakening response; children

INTRODUCTION

The cortisol awakening response (CAR) is characterized by a rapid increase of 50–75% in cortisol levels, which peak approximately 30 min after waking and decline thereafter (Clow, Thorn, Evans, & Hucklebridge, 2004; Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). Research examining the CAR has flourished in recent years as the CAR has been linked to physical and psychological health conditions, including chronic life stress and fatigue, post-traumatic stress disorder, and depression (for a review see Chida & Steptoe, 2009). Moreover, the development of salivary

Contract grant sponsor: UMD Research and Scholars Award Article first published online in Wiley Online Library cortisol sampling procedures has allowed participants to collect early morning cortisol samples in a home setting with relative ease. Although a considerable amount of literature exists on the CAR in adults (see Clow et al., 2004; Fries, Dettenborn, & Kirschbaum, 2009), little is known about the CAR and factors that may influence its accurate measurement in children. Investigating these issues is crucial to our understanding of the development of the hypothalamicpituitary-adrenal (HPA) axis and could also provide insight into its potential role in the etiology of physical and psychiatric disorders.

As the CAR is a dynamic response, a methodological issue of increasing concern is participant compliance to the timing of sample collection. Several studies examining ambulatory sampling compliance in adults have reported that cortisol data may be compromised by noncompliance to sample timing (Broderick, Arnold, Kudiekla, & Kirschbaum, 2004; Jacobs et al., 2005; Kudielka, Broderick, & Kirschbaum, 2003; Kudielka, Hawkley, Adam, & Cacioppo, 2007). Specifically, through the use of electronic monitoring

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devices, the gold standard measure of compliance (Claxton, Cramer, & Pierce, 2001), these studies have revealed significantly lower observed CAR and flatter observed diurnal cortisol slopes among noncompliant adult participants. These findings raise the possibility that noncompliance to sample timing is also an issue of concern in child research, particularly given the many special considerations when collecting salivary cortisol from children (e.g., Jessop & Turner-Cobb, 2008; Schwartz, Granger, Susman, Gunnar, & Laird, 1998). For example, as young children are often resistant to cortisol sampling procedures (Gunnar & Talge, 2007), it may be especially difficult for parents to collect samples in adherence to instructed sampling times. Complicating this issue is that sampling protocols are often complex and restrictive (e.g., collection of multiple time-sensitive samples) which may increase burden on participants. The constraints of the sampling protocol in conjunction with child (e.g., resistance to chewing on a cotton dental roll) and parent factors (e.g., motivation, ability, work, and family responsibilities) raise significant concerns regarding parental compliance.

It remains unclear to what extent parental compliance impacts ambulatory measures of salivary cortisol in research on children. Only two studies assessing the CAR in children have tracked parental compliance using electronic monitoring devices to account for its potential effect on data (Stalder et al., 2013; Zinke, Fries, Kliegel, Kirschbaum, & Dettenborn, 2010). In these studies, parents collected samples from infants (Stalder et al., 2013) or school-age children (Zinke et al., 2010); parental noncompliance was addressed by excluding noncompliant samples from analyses (Stalder et al., 2013; Zinke et al., 2010). A few studies assessing diurnal cortisol levels across the day in children have also tracked parental compliance using electronic monitors (Corbett, Mendoza, Baym, Bunge, 2008a; Corbett, Mendoza, Wegelin, & Levine, Carmean, & Levine, 2008b; Dozier et al., 2006; Gunnar, Kryzer, Van Ryzin, & Phillips, 2010). In these studies, parents collected samples from infants (Dozier et al., 2006), preschool age children (Gunnar et al., 2010), or school age children Corbett et al. (Corbett et al., 2008a, b); parental noncompliance was addressed by asking noncompliant parents to resample saliva (Dozier et al., 2006), excluding noncompliant samples from analyses (Gunnar et al., 2010), or including noncompliant samples in the analyses after verifying that inclusion had no effect on the results (Corbett et al., 2008a, b). Although these previous studies *tracked* parental noncompliance, to date, no study has provided a direct, comprehensive examination of the accuracy of parent-reports of sampling times, and the impact of parental compliance on children's cortisol data.

The purpose of the present study was to examine parental compliance with sample timing in an ambulatory assessment of young children's salivary cortisol. Although effects of noncompliance have been documented in studies of adults, equivalent studies have not been conducted in children. First, given that studies commonly rely on parent-reports of sampling times, we aimed to examine the concordance between parentreported compliance and electronic monitoring of parental compliance. Specifically, we aimed to compare (a) compliance rates, (b) agreement in reported compliance, and (c) deviation from instructed sampling times, as reported by the two methods. In light of previous research examining sampling compliance in adults, we hypothesized that parents would self-report higher rates of compliance to sampling than parental compliance rates based on the electronic monitor. 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 parentreport.

Second, we aimed to examine the effects of parental compliance on young children's cortisol data, as reported by parent-report and electronic monitoring. Specifically, we examined the effects of parental compliance on children's CAR and diurnal cortisol slopes, two integral indices of HPA axis functioning. In light of findings in the adult literature, we hypothesized that parental noncompliance would be associated with children's reduced CAR and steeper diurnal cortisol slopes¹ from waking to bedtime, compared to compliant sampling. We also hypothesized that the effects of parental noncompliance on children's cortisol data would be stronger based on electronic monitoring than parent-report.

METHODS

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

¹In the adult literature, noncompliance was associated with a flatter diurnal slope, computed as the difference between peak and bedtime cortisol. However, given recommendations to separate the CAR from the diurnal slope (Adam & Kumari, 2009), we computed the diurnal slope as the difference between waking and bedtime; thus, we hypothesize that noncompliance will be associated with a steeper slope compared to compliant sampling.

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 3 and 5 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 TrackCap; 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. Three children were excluded for providing samples with extreme cortisol values (n = 3), and five 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). Thus, a total of 81 children were included in the final sample.

Of the 81 children, 45 (57.0%) had a parent with a lifetime history of depression, based on the non-patient version of the Structured Clinical Interview for DSM-IV (SCID-NP; First, Gibbon, Spitzer, & 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

Salivary Cortisol. Parents were instructed to obtain salivary cortisol samples from their child immediately upon the child's waking, 30 and 45 min post-waking, and 30 min before bedtime on two consecutive days, for a total of eight cortisol samples per child. 92.6% of participants provided all eight cortisol samples, and 100% of participants provided at least five cortisol samples. 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 2 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, Kirschbaum, Marmot & Steptoe, 2004).

To monitor sampling times, parents received all sampling materials in a kit and were given an electronic monitoring device (MEMS TrackCap; Aardex Ltd., Zug, Switzerland) containing 1.5 in. Richmond Dental cotton rolls. 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, 2007). Information regarding the occurrence of any such events was recorded by parents in a diary measure, for each sample: 72.7% parents reported compliance to these specific sampling instructions across all samples. Examination of any potential effects of noncompliance to instructions (e.g., recent food or drink intake, consumption of dairy or caffeine) revealed no significant effects on cortisol (all p > .20); thus, samples were retained in all analyses to maintain greater statistical power.

To collect cortisol for analysis, parents were instructed to have their child chew on a cotton dental roll dipped in .025 g of cherry-flavored Kool-Aid to stimulate saliva. A series of experiments conducted by Talge, Donzella, Kryzer, Gierens, and Gunnar (2005) showed that the use of cherry-flavored Kool-Aid does not compromise the quality of cortisol data when used consistently and sparingly. 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, typically occurring within 2 weeks, 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.

In addition to reviewing the sampling protocol with parents, we also implemented a number of methods to improve compliance with the sampling protocol (see Adam & Kumari, 2009). These methods included engaging parents with the purpose of the research, providing parents with information on the circadian rhythm of cortisol and its sensitivity to deviations in timing, emphasizing the importance of accurate timing and reporting of actual sample times, and practicing the sampling protocol with the child and parent. Parents were also informed that their sampling was being monitored (Broderick et al., 2004; Kudielka et al., 2003), and were provided with all sampling materials in an organized and color-coded kit which included handheld mechanical timers to assist with the timed collection of samples.

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Table 1. Subject and Cortisol Characteristics (N = 81)

Developmental Psychobiology

	% (N)	<i>M</i> (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)			
>1 parent college graduate	70.4 (57)			
Parental lifetime depressive disorder	57.0 (45)			
Salivary cortisol indicators				
Time of waking (hr)				
Day 1		7:27 (1:08)	4:28	12:45
Day 2		7:28 (1:14)	4:46	11:48
Bedtime (hr)				
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
Diurnal cortisol slope decline				
Day 1	71 (94.7%)			
Day 2	71 (94.7%)			
AUC_{a} (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
AUC, positive		(
Day 1	49 (67.1%)			
Day 2	46 (63.0%)			

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).

The following cortisol variables were included in analyses: cortisol values for each time point (waking, 30, 45 min 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 min postwaking samples (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). To assess the diurnal cortisol slope separately from the CAR, the diurnal cortisol slope was computed as the difference in waking and bedtime cortisol levels divided by the number of hours between the two samples (Adam & Kumari, 2009). Following Gunnar and Talge (2007), summary variables (i.e., AUCg, AUC_i, 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 min 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-reported compliance was assessed using a diary measure in which parents recorded the child's time of waking, bedtime, and all sampling times. Electronic monitoring of parental compliance was measured with the MEMS TrackCap (Aardex Ltd., Zug, Switzerland), which consists of a bottle in which sampling cotton dental rolls are placed, and a pressureactivated microcircuitry cap that records the date and time of each bottle opening. Data were downloaded from the monitor to the computer using a specialized interface and software program (Aardex Ltd., Zug, Switzerland), and were 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 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 (i.e., Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003, 2007), a stringent time window of ± 10 min was selected for the samples that compose the CAR (i.e., waking, 30 and 45 min samples), as cortisol levels change rapidly during the morning (Clow et al., 2004), whereas a more liberal time window of ± 1 hr 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 noncompliant. For the CAR, participants were coded as compliant if all morning samples (i.e., waking, 30 and 45 min post-waking samples) were collected within their established time windows; that is, one or more noncompliant morning samples resulted in the participant being considered as noncompliant (Kudielka et al., 2007). 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.

Data Analysis Plan. To test our hypothesis that parents would self-report higher rates of compliance to sampling than parental compliance rates based on the electronic monitor, 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, to test our hypothesis that parent-report and electronic monitoring would evidence moderate agreement, Pearson correlations were conducted to examine the agreement between rates of compliance as reported by each measure. Lastly, to test our hypothesis that electronic monitoring would demonstrate greater deviation from instructed sampling times in comparison to parent-report, we computed deviations between instructed sampling times and sampling times as indicated by each measure; deviations were compared using paired samples t-tests.

To examine the effects of parental noncompliance on children's cortisol data, as reported by parent-report and electronic monitor, 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 timecourse data (Liang & Zeger, 1986). Analyses were conducted in SPSS v. 19 (SPSS, Inc., Chicago, IL) with a normal distribution, identity link function, and an unstructured correlation matrix specified. To examine measure-specific effects, GEE analyses were conducted separately for parentreport and electronic monitoring. To test our hypotheses that parental noncompliance would be associated with children's reduced CAR and steeper diurnal cortisol slopes compared to compliant sampling, person-level compliance was entered as an independent variable, and cortisol values corresponding to early morning cortisol time points, AUCg, AUC_i, and diurnal slope were entered as dependent variables in separate models.

RESULTS

Descriptive Statistics and Preliminary Analyses

Table 1 presents descriptive statistics for demographic and cortisol variables. Across all participants for the entire 2-day sampling period, cortisol levels showed the expected diurnal pattern: waking values (M = 7.95nmol/L) increased approximately 37% to reach a peak 30 min post-waking (M = 10.88 nmol/L),t(77) = 5.44, p < .001; declining thereafter to reach lower levels at 45 min post-waking (M =8.53 nmol/L), t(77) = -6.73, p < .001; and lowest levels at bedtime (M = 2.31 nmol/L), t(78) = -20.98, p < .001. 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 min 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 demographic (child age, gender, race/ethnicity, parents' age, marital status, education), health (child's health status, parental lifetime depression), and lifestyle (time of waking) covariates were examined. Cortisol was not significantly associated with these covariates (all p > .17), with а few exceptions: race/ethnicity was significantly associated with AUC_i on Day 1, F(3, 67) = 3.30, p = .03, waking cortisol on Day 2, $F(3, 71) = 3.36, p = .02, and AUC_g$ on Day 2, $F(3, 71) = 3.36, p = .02, and AUC_g$ (66) = 3.77, p = .02. Children of unmarried parents evidenced significantly higher AUC_i, t(71) = 3.77, p < .001, and steeper diurnal cortisol slopes on Day 1, t(73) = 2.68, p = .01. Children of parents with a lifetime history of depression evidenced significantly lower 30 min post-waking cortisol on Day 2, F(1, 73) = 5.49, p = .02. Time of waking was positively associated with AUC_g on Day 1 (r = .27, p = .03). Therefore, race/ethnicity, parental marital status, parental lifetime depression, and time of waking 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 child age, gender, race/ethnicity, parents' age, marital status, income, education, child's health status, or parental depression status.

Concordance Between Parent-Reported Compliance and Electronic Monitoring

Hypothesis: Parents report higher rates of compliance to sampling than parental compliance rates based on the electronic monitor.

As hypothesized, overall parent-reported compliance (83.0%) was significantly higher than objective compliance (68.8%) for the entire 2 day sampling period (see Tab. 2). 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 (see Tab. 2). Parent-reported compliance for each instructed sampling time, with the exception of the bedtime sample, for which no significant differences were observed.

Hypothesis: Parent-report and electronic monitor evidence moderate agreement.

Using Pearson correlations, overall agreement between compliance as reported by parent-report and electronic monitor for the entire 2-day sampling period

Table 2. Comparison of Percent Compliance as Indicated by Parent-Report and Electronic Monitor

Instructed Sampling Time	Percent Compliance		Statistics	
	Parent-Report	Electronic Monitor	Pearson r Statistic	t Statistic
Waking	85.4	73.1	.58***	-3.70***
Waking $+30$	84.6	66.5	.54***	-4.66***
Waking + 45	77.6	55.7	.54***	-3.63***
Bedtime	80.4	76.9	.84***	-1.52
Overall	83.0	68.8	.64***	-5.58^{***}

p < .05; p < .01; p < .01; p < .001.

was r = .64. Agreement between parent-report and electronic monitor for each instructed sampling time was moderate to high, with correlations ranging from r = .54-.84 (Tab. 2).

Hypothesis: Electronic monitoring demonstrates greater deviation from instructed sampling times in comparison to parent-report.

Table 3 shows the mean discrepancy between instructed sampling times and times as indicated by parent-report and electronic monitor. For each sampling time, electronic monitoring indicated that the deviation from instructed sampling times was significantly greater than parent reports of sampling times.

Effects of Parental Compliance on Children's Cortisol Data

Hypothesis: Parental noncompliance is associated with children's reduced CAR.

As cortisol levels were nested within individuals, repeated-measures analyses using GEE were conducted to examine the effect of noncompliance on morning cortisol levels, across the 2-day sampling period. 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 = .71, n = 75), 30 min (b = .03, SE = .06, p = .64, n = 74), or 45 min post-waking cortisol levels (b = .08, SE = .06, p = .16, n = 75). 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

observed waking cortisol levels (M = 9.37, SD = 5.19) compared to children of compliant parents (M = 7.53, SD = 5.32; b = -.12, SE = .06, p = .03, n = 75). No significant group differences were observed for the 30 (b = .02, SE = .04, p = .64, n = 74) or 45 min (b = .07, SE = .05, p = .28, n = 74) post-waking samples.

To assess the effects of parental compliance on summary measures of 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 = -4.27, SE = 5.03, p = .39, n = 72) or AUC_i (b = 3.38, SE = 3.72, p = .36, n = 72). Based on electronic monitoring, there were no group differences in AUC_g (b = -5.11, SE = 3.49, p = .14, n = 71). However, there was a significant association between noncompliance and AUC_i (b = 7.99, SE = 2.77, p = .004, n = 71). Children of noncompliant parents evidenced smaller observed increases in AUC_i (M = 1.17, SD = 19.56) compared to children of compliant parents (M = 9.88, SD = 16.37; see Fig. 1b). Thus, based on electronic monitoring, parental noncompliance was associated with blunted CAR or less of an observed rise in morning cortisol across the waking period.

Hypothesis: Parental noncompliance is associated with children's steeper diurnal cortisol slopes from waking to bedtime.

No significant effects of parental compliance based on parent-report were observed for the diurnal cortisol slope (b = .01, SE = .01, p = .78, n = 75) or bedtime cortisol (b = -.08, SE = .17, p = .66, n = 75). Similarly, no significant effects of parental compliance based on the electronic monitor were observed for the

Table 3. Deviance From Instructed Sampling Times as Indicated by Parent-Report and Electronic Monitor

	Deviance From In	structed 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***

Units are in minutes.

p < .05; p < .01; p < .01; p < .001.



FIGURE 1 Children's 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 parent-report. 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.

diurnal cortisol slope² (b = .01, SE = .01, p = .83, n = 75) or bedtime cortisol³ (b = -.05, SE = .16, p = .75, n = 74).

DISCUSSION

To our knowledge, this is the first study 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. Through comparison of compliance as indicated by parent-report and electronic monitor, we examined how closely parents adhere to instructed sampling and its impact on children's cortisol data. Despite moderate concordance between parent-report and the electronic monitor, we found evidence suggesting that parents overestimate their compliance with the sampling protocol: parents self-reported significantly higher rates of compliance to sampling (83%) than rates based on the electronic monitor (68.8%). Moreover, electronic monitoring indicated that the actual deviation from instructed sampling times was twice on average what was reported by parents. We also found that children of noncompliant parents based on the electronic monitor evidenced higher observed waking cortisol and a lower observed CAR, compared with children of compliant parents.

Although the objective compliance rate we observed (68.8%) is consistent with rates observed among adults uninformed of electronic monitoring (61-81%; Broderick et al., 2004; Jacobs et al., 2005; Kudielka et al., 2003, 2007), it is considerably lower than rates observed among adults *informed* of monitoring (90%) reported in Broderick et al., 2004; 97% reported in Kudielka et al., 2003) and rates reported in child studies (86%-99%; Corbett et al., 2008a, b; Dozier et al., 2006; Gunnar et al., 2010). It is possible that our lower compliance rate is due to our assessment of the CAR, which increases sampling burden as it involves the collection of multiple morning samples within a narrow period of time. Nevertheless, the low parental compliance we observed was surprising, given our considerable efforts to enhance parental compliance, such as informing participants of electronic monitoring (Broderick et al., 2004; Kudielka et al., 2003), practicing the sampling protocol with children and parents, and providing parents with mechanical timers to assist with sample timing. Taken together, our findings underscore the difficult nature of assessing cortisol samples in children, particularly when collecting multiple morning samples.

We found that children of parents who were noncompliant based on the electronic monitor evidenced significantly higher observed waking cortisol and had lower or blunted CAR, as indicated by a lower observed AUC_i. These results provide evidence that parental noncompliance to the waking sample leads to elevated observed waking values affected by the rapid post-awakening cortisol rise, which in turn, results in a lower or blunted observed CAR. These findings converge with reports of significantly lower observed 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

²Given that the best approach to calculating diurnal cortisol slope is debated (Adam & Kumari, 2009), we also calculated diurnal cortisol slopes as the rate of decline from the peak cortisol value (i.e., 30 min post-waking) to bedtime. Results were similar based on this approach: no significant effects of parental noncompliance based on either parent-report (b = .01, SE = .01, p = .84, n = 74) or electronic monitor (b = 01, SE = .01, p = 52, n = 74) were observed for diurnal cortisol slopes.

 $^{^{3}}$ When bedtime compliance was restricted to ± 10 min of bedtime (i.e., the same time window used for morning samples), results were similar.

are associated with reduced CAR (DeSantis, Adam, Mendelsohn, & Doane, 2010; Dockray, Bhattacharyya, Molloy, & Steptoe, 2008; Okun et al., 2010). In contrast to results based on the electronic monitor, parental compliance based on parent-reports was not associated with children's waking cortisol or CAR. The discrepancy across the two methods of assessment suggests that researchers should consider using both parent-report and the electronic monitor to assess parental compliance, particularly when assessing the CAR, as noncompliance could affect the interpretation of results.

We also found that parental compliance was not associated with children's diurnal cortisol slopes. While our findings are in contrast to a few previous studies (Broderick et al., 2004; Kudielka et al., 2003), they are consistent with Jacobs et al. (2005) who found that noncompliance did not impact the diurnal slope in adults. Similar to Jacobs et al. (2005) and Adam and Kumari (2009), we anchored the slope on the waking sample and excluded the CAR values (i.e., 30 and 45 min post-waking samples) from calculation of the slope in order to assess the diurnal slope separately from the CAR. In contrast, previous studies examining adult sampling compliance have based the calculation of the slope on the peak cortisol value of the day (Broderick et al., 2004; Kudielka et al., 2003), which may possibly confound the CAR with the diurnal slope (Adam & Kumari, 2009). Nevertheless, our results remained similar when the effects of parental compliance on diurnal slope were computed based on this latter approach.

Strengths and Limitations

The study had several methodological strengths, including the collection of multiple cortisol samples across 2 days to assess the CAR (Hellhammer et al., 2007), and the use of electronic monitors to produce discrete, detailed data that were 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 parents are reasonably reliable reporters of children's wake times (Tikotzsky & Sadeh, 2001). Second, although electronic monitoring is the gold standard method of assessing compliance, it is not without limitations, such as participant error in its use and the 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 over selected 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 and was included as a covariate in all analyses.

Overall, our findings underscore that measuring parental compliance is critical, as compliance cannot be assumed. Parental noncompliance can meaningfully impact the validity and subsequent interpretation of children's cortisol data, particularly morning cortisol samples. Our findings have significant methodological implications and underscore the importance of using the electronic monitor in conjunction with parentreport and behavioral methods to maximize compliance (see Adam & Kumari, 2009 for a list of suggestions). In closing, our study provides compelling evidence that future studies cannot ignore parental noncompliance, or uncritically rely on parent report, particularly when assessing children's waking cortisol or the CAR.

NOTES

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