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# Adherence to Diurnal Cortisol Sampling among Mother-Child Dyads from Maltreating and Nonmaltreating Families

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## Abstract

There has been increasing interest in evaluating whether interventions for child maltreatment can improve and/or prevent child physiological dysregulation via measurement of diurnal cortisol. The assessment of diurnal cortisol typically involves the home-based collection of saliva multiple times per day, bringing forth important methodological considerations regarding adherence to collection instructions. To date, there has been no data regarding adherence to home collection of diurnal cortisol among maltreating families. The current study provides data on adherence to in-home sampling of salivary cortisol among 166 maltreating and demographically similar nonmaltreating mother-child dyads using electronic monitoring devices (Medication Event Monitoring System (MEMS) caps). Mothers collected saliva samples on themselves and their children three times per day (waking, midday, evening) for two consecutive days. Analyses reveal that although maltreating families were more likely to be nonadherent to the collection protocol on their initial attempt, with additional support and resampling, maltreating and nonmaltreating families were comparable on most measures of adherence. Suggestions for best- practices, including the use of electronic monitoring devices, for diurnal cortisol collection with maltreating families are provided.

Among the most exciting and important advancements in child maltreatment research in the last decade has been the discovery that relational interventions for maltreated children and their caregivers can improve and/or prevent the development of physiological dysregulation among maltreated children (i.e., Cicchetti, Rogosch, Toth, & Sturge-Apple, 2011; Dozier, Peloso, Lindhiem, … & Levine, 2006; Fisher, Stoolmiller, Gunnar, & Burraston, 2007). Given extensive data regarding links between early adversity and later risk for physical and mental health (i.e., Anda, Feletti, Bremner… & Giles, 2006), in part due to physiological dysregulation (Hertzman & Boyce, 2010), interventions that are able to improve outcomes at both behavioral (i.e., socio-emotional) and biological levels are appealing. For child maltreatment in particular, there has been a focus on evaluating the extent to which interventions may affect children's physiological regulation via diurnal cortisol in the context of randomized clinical trials (RCTs). The assessment of diurnal cortisol typically

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involves the home-based collection of saliva multiple times per day, bringing forth important methodological considerations in relation to participant adherence to collection instructions. To date, little data regarding adherence to home collection of diurnal cortisol among special populations are available, and none regarding maltreating families specifically. The current study seeks to fill this gap by providing data on adherence to in-home sampling of salivary cortisol among maltreating and demographically similar nonmaltreating mothers and their preschool-aged children using electronic monitoring devices (Medication Event Monitoring System (MEMS) caps).

The hypothalamic-pituitary-adrenal (HPA) axis, with the end product cortisol, is responsible for being both the body's stress response system, as well as the primary synchronizer of multiple physiological systems around the 24-hour dark/light cycle (i.e., a diurnal rhythm; Smyth, Hucklebridge, Thorn, Evans, & Clow, 2013). Deficiencies in adequate caregiving behavior are related to dysregulations in stress physiology including the (dis)organization of cortisol rhythms across the day. In particular, maltreated children tend to exhibit dysregulation characterized by a flattening of diurnal cortisol activity, including lower early morning cortisol, and less cortisol decline across the day (for review see Tarullo & Gunnar, 2006), although elevations in cortisol levels have also been noted among maltreated children (i.e, Cicchetti & Rogosh, 2001).

The stress-response system is highly sensitive to the immediate context, and interventions enhancing caregiver behavior have been show to improve children's cortisol regulation (e.g., Cicchetti, et al., 2011, Dozier et al., 2006; Fisher et al., 2007). Thus despite early adversity, relational interventions that support the caregiver-child relationship have the capacity to improve maltreated children's stress response systems. Such work has also provoked important questions regarding whether or not physiologically remediating interventions are more effective at preventing long-term adverse mental and physical health outcomes than interventions that do not (Valentino, 2017). With this increased interest, there is a need for information regarding the feasibility and reliability of diurnal cortisol collection among maltreating families, including data on adherence to collection procedures, to inform best practices with this population.

Salivary cortisol sampling procedures are minimally invasive and thus appealing for use in the home environment to track diurnal release. However, despite the relative ease of salivary sampling over blood draws, salivary sampling procedures require participants to closely comply with numerous behavioral restrictions while adhering to an intensive sampling design at predetermined times. For example, participants are given specific instructions dictating how much sample to provide, what information to record about the sample, procedures for storing the sample and restrictions on their eating, drinking, and smoking behavior. Further, participants must follow a stringent timeline of saliva sampling because of cortisol's defined diurnal rhythm (i.e., high morning and low evening cortisol levels). Studies assessing this rhythm therefore generally require at least three samples across the day (waking, midday, bedtime or 12 hours post waking) to estimate diurnal cortisol levels and slopes (Smyth et al., 2013). Lastly, the HPA axis has evolved to help individuals respond to immediate environmental demands (Sapolsky, Romero, & Munck, 2000). This means that cortisol varies moment to moment, requiring multiple days of sampling to allow for the

accurate measurement of an individual's trait cortisol rhythm, as opposed to state variations (Hellhammer et al., 2007). For example, reports suggest samples must be collected on at least two consecutive days to assess the diurnal rhythm (Thorn et al., 2011). Together, this means that participants must be able to understand the strict behavioral restrictions and collection design, organize their day and potentially change their behavior (i.e., restrain from smoking prior to sample collection) to accommodate for these directions, and stay motivated to participate over multiple days.

To facilitate these collection requirements, researchers typically provide participants with labor intensive, and costly, researcher man-hours. Specifically, researchers must provide an in- person description and instruction of saliva collection and explanation of the collection design. Researchers must explain saliva collection kits, which include instructions, collection supplies (labeled vials and straws for passive drool, and/or cotton swabs and tubes), and a log book for recording the time of collection. Further, multiple supports such as phone call reminders, color-coding of supplies, and electronic monitoring devices have been used to increase and monitor participant adherence to this protocol (Laudenslager et al., 2013).

Despite these supports, many participants still struggle with compliance (Kudielka, Broderick, & Kirschbaum, 2003), prompting studies to utilize electronic monitoring devices to verify participants' self-reported collection times. Electronic monitoring devices, such as MEMS caps, can be used on bottles where participants are instructed to store their samples or collection materials, and the caps will record the exact date and time of each opening. Electronic monitoring devices are thought to be the gold standard measure of compliance (see Claxton, Cramer & Pierce, 2001, for review) as these objective measures of time can be compared to participant self-reported collection times.

Very little is known about compliance to diurnal sampling procedures with children or other special populations. One study of parental compliance to the collection of their preschool-aged children's diurnal cortisol revealed that parent self-reported rates of compliance were higher than rates verified by electronic monitoring devices (Smith & Dougherty, 2013). Specifically, electronic monitoring devices indicated that participants were compliant with the timing of collection protocol 68.8% of the time, and noncompliance was related to elevated waking cortisol, but not the diurnal slopes or cortisol levels at bedtime. In a separate study with low income school-aged children, only 54% of the self-reported sample timing was considered accurate (within 10 minutes for the first 2 samples and within 30 minutes for the bedtime sample); however, adding the results of noncompliance into their models did not significantly alter the results (Willner, Morris, McCoy & Adam, 2014). Alternately, research with adults has demonstrated that cortisol data may be compromised by noncompliance to sample timing (e.g., Kudielka et al., 2007). Specifically, because cortisol levels vary by time of day, collection times must be accurate in order to interpret the individual's regulatory functioning. Cortisol levels one standard deviation above an individual's own mean in the morning would be expected, but might be indicative of pathology if found in the evening. Thus, having accurate checks of participant adherence is critical in determining who is at risk for physiological dysregulation across the day, and which interventions are effective at ameliorating these physiological patterns.

Yet the extent to which maltreating families are able to adhere to diurnal sampling procedures remains unknown. Several previous studies of diurnal cortisol with maltreated children have circumvented the need for home-collection procedures by either collecting multiple cortisol samples in the lab during a summer day-camp program (e.g., Cicchetti & Rogosch, 2001), or by focusing on morning cortisol levels and collecting samples during lab visits all scheduled in the morning (Cicchetti et al., 2011; Trickett ,Noll, Susman, Shenk, & Putnam, 2010). In those cases, the timing of the cortisol collection could be verified by the experimenters in lab. Few studies assessing maltreated children's diurnal cortisol levels have included home-based collection and to our knowledge, only one has also tracked compliance to sample timing using electronic monitors (Dozier et al., 2006). In Dozier's study, saliva was collected from maltreated children by foster parents, and noncompliance was defined as a discrepancy of greater than one hour between the electronically-recorded and self-reported time. Parental noncompliance was rare, and cortisol was resampled in those cases. To date, there have been no studies to examine adherence to diurnal cortisol collection procedures among maltreating mothers with regard to collection of their own cortisol samples or of their children's. Maltreating families are generally characterized by higher levels of home chaos, with increased unpredictability and lower levels of social support (Cicchetti & Valentino, 2006); as such, maltreating mothers may be at risk for poor adherence to cortisol collection procedures. Surveillance technology, such as electronic monitoring devices may be critical for identifying invalid data and signaling the need for additional support for in-home salivary collection.

The purpose of the present study is to examine parental adherence to in-home sampling of salivary cortisol, collected three times a day (waking, midday, evening) for two consecutive weekend days, among maltreating and nonmaltreating mother-child dyads. We examined adherence to protocol instructions on all participants' first diurnal cortisol collection. Based on initial adherence, some families were asked to re-sample cortisol. We then examined adherence from participants' best collection and compared protocol adherence by maltreatment status. Specifically, we compared maltreating and nonmaltreating dyads on 1) the number of samples the dyads returned; 2) the number of adherent samples returned based on a large adherence window (i.e., objective morning collections within 30 minutes and objective afternoon and evening collections within an hour of self-reported times); 3) the number of adherent samples returned based on a small adherence window (i.e., objective morning collections within 15 minutes and objective afternoon and evening collections within 30 minutes of self-reported times); and 4) the number of samples that were sufficient for assay. We anticipated that maltreating families would be less adherent than nonmaltreating families on their first collection attempt, but would have similar rates of adherence on their best collection.

## Method

### Participants

The participants included 166 mothers and their children, aged 3 to 6 years from a medium-sized Midwestern city. The mother was named as a perpetrator in 102 families with substantiated cases of child maltreatment. Families (n = 64) with no child welfare system

history were recruited to be demographically similar to the maltreating families. The nonmaltreatment group was matched on child age, gender, and family income. In all families, eligibility criteria specified that children must be living with their biological mothers. Participants were screened for endocrine disorders (e.g., Cushing's Syndrome, Addison's disease) or continual corticosteroid use (Granger, Hibel, Fortunato, & Kapelewski, 2009), which affect cortisol levels; however no families were excluded for these reasons. Maltreated and nonmaltreated dyads did not differ on a number of important demographic characteristics, with the exception of child race (see Table 1). Additionally, maltreating and nonmaltreating mothers did not differ in language abilities on a standardized assessment of receptive language (PPVT-4; Dunn & Dunn, 2007).

Maltreating families were recruited through the Department of Child Services (DCS). DCS Family Case Workers provided eligible participants with an informational flyer and asked whether they would be interested in sharing their contact information with project staff. Project staff contacted interested families to discuss enrollment. Nonmaltreating families were recruited from the local community in locations such as the WIC office, the housing authority, and Head Start, which typically serve a similar demographic population to the maltreating families. All participating families provided informed consent and signed release forms granting access to their DCS records. The presence or absence of maltreatment was subsequently verified through extensive examinations of each family's case history and through maternal interview. Only families who have never received child protective services through DCS and indicated no maltreatment on the maternal interview were included in the nonmaltreating comparison sample.

## Maltreatment Classifications

DCS records were coded using the Maltreatment Classification System (MCS; Barnett, Manly, & Cicchetti, 1993). The MCS utilizes operational criteria for determining the occurrence of subtypes of maltreatment which includes sexual abuse, physical abuse, physical neglect, and emotional maltreatment. Sexual abuse is coded when any sexual contact or attempted sexual conduct occurred between the child and an adult. Physical abuse is determined by injuries that had been inflicted upon a child by nonaccidental means. Physical neglect is coded for failure of the primary caregiver to meet a child's needs for food, clothing, shelter, health care, education, hygiene, or safety. Emotional maltreatment is coded for chronic or extreme neglect or disregard of children's emotional needs and includes witnessing domestic violence (see Barnett et al.,1993). Additionally, the severity, chronicity, perpetrator, and the developmental timing of each maltreatment incident were assessed. MCS ratings were supplemented by information obtained during the Maternal Maltreatment Classification Interview (MMCI; Cicchetti, Toth, & Manly, 2002), a structured interview based on the MCS. Approximately 30% of the maltreated sample was double coded ($n = 32$) by two coders, and reliability was established ($\kappa = .84\text{-}1.0$).

Within the maltreatment group, 4.6% of the children experienced sexual abuse, 12.5% experienced physical abuse, 53.9% experienced emotional maltreatment and 70.3% experienced physical neglect. Subtype comorbidity was high, with 60.9% of the sample experiencing more than one subtype of maltreatment; this includes 37.5% who experienced

2 subtypes, 19.5% who experienced 3 subtypes, and 3.9% who experienced 4 subtypes of maltreatment. The average length of time since the last maltreatment incident was just under 1 year (356 days), with a range of 20 to 1704 days.

### Procedure

Data for the current study were drawn from the baseline assessment of an ongoing longitudinal RCT of an intervention for maltreating mothers and their preschool-aged children. Families in all conditions completed an assessment consisting of one session in the home followed by one in the laboratory. Research staff conducting baseline assessments was naive to families' maltreatment status. The current study involves data from the baseline home assessment only. At the time of writing, participants were still being enrolled in this study; as such, this report provides data for only a subsample of the full sample. As part of the home assessment, mothers engaged in free-play with their children and were then trained to collect three saliva samples (waking, midday, and evening) on themselves and their children for two consecutive weekend days. To control for daily fluctuations in cortisol associated with sleep, stress and affect, mothers also completed sleep logs to report bed and wake times, and questionnaires including measures of perceived daily stress (Almeida, Wethington, & Kessler, 2002), parenting stress (Fisher & Stoolmiller, 2008), and positive and negative affect (Watson & Clark, 1994); they also reported medication use. Participants were compensated for the entire home-visit and lab-visit with $90, which included $20 specifically for completion of the cortisol collection and a $10 gas card to assist with transportation costs to the lab. The study protocol was approved by the Institutional Review Board of the University of Notre Dame.

## Measures

### Cortisol.

For diurnal cortisol collection, saliva collection kits were brought to participants' homes. Collection kits included pre-labeled sample vials, straws (for the mothers), saliva collection sponges (for the children; SalivaBio, LLC), and two collection bottles with MEMS 6 TrackCap Monitors (Medication Event Monitoring System; WestRock Switzerland, Ltd.). One collection bottle was for mothers' samples and one for children's samples. Mothers were all informed that the MEMS caps would track the date and time of each cap opening, thus assessing their collection times. They were asked to keep the bottles in the freezer with the caps on, to add each sample to the appropriate bottle as they were collected across the two days, and to close the bottles with the MEMS cap after each opening. Mothers were trained to collect saliva via passive drool (Granger et al, 2007) for themselves and via sponge for their children. Research assistants observed mothers collect one practice sample from herself and her child during training, including placing the samples into the MEMS cap sealed bottles, and provided corrective feedback to ensure comprehension of the collection procedures.

Participants provided salivary samples: immediately upon waking, before lunch, and before bed (Adam & Kumari, 2009) on two consecutive weekend days when mother and child were home together. Participants were asked to sample on weekend days to ensure that mothers

and children would be home together. In addition to placing their samples in the MEMS cap sealed bottles, participants recorded the times at which they provided their salivary samples on a written log. Participants were instructed to drink water 10 minutes before collection (except at waking), and not to eat, smoke, or drink alcohol or caffeine within 20 minutes of providing salivary samples. To enhance adherence to the protocols, mothers were also given a cell phone to facilitate cortisol collection. Mothers were asked estimate their waking, lunch and bedtimes for the following day; based on this schedule we sent text-based reminders to the cell phone we provided for the family 20 minutes before each collection time and mothers were asked to respond. If they did not respond within 30 minutes, mothers received a phone call.

Respondents were instructed to keep the saliva samples in the freezer and to bring samples to the laboratory on ice in provided portable coolers when they attended their lab assessment. Families without freezers were provided a cooler in which samples could be kept; these samples were picked up by staff and transported to the lab each morning. Samples were stored in an ultralow freezer (−80 C) with back-up generator until analysis (Granger et al., 2007).

When participants attended their lab session, the MEMS cap times were downloaded by placing the caps on a MEMS reader-device. Dates and times of cap openings were compared with the self-reported log sheets. Initial adherence data was based on all participants' "first attempt" at diurnal cortisol collection. In cases where there was not at least one adherent day (defined as a waking, midday, and bedtime sample all from the same day for each mom and child with MEMS times that corresponded to self-report log times within one hour), mother-child dyads were asked to redo the cortisol collection, generally over the next weekend. For these families, research assistants repeated all cortisol instructions and training as described above. Additional attention was paid to addressing the specific type of nonadherence that was problematic during participants' first attempt, and research assistants problem-solved around how to further support the mother to adhere to the cortisol protocol.

## Coding

**First collection.—**Basic adherence data were coded for participants' first attempt at home-based diurnal cortisol collection. Specifically, we coded whether families were fully adherent or not to the collection protocol (1 = returned all 12 cortisol samples with electronically recorded times that corresponded within 1 hour to the self-reported log times, from two consecutive days of collection, 0 = less than 12 samples with corresponding electronically recorded and log times). Among the nonadherent families, we coded whether the adherence problem was identified by the electronic cap data (1 = yes, 0 = no). Nonadherence identified by the caps included large discrepancies of greater than one hour between the electronically-recorded time and the self-reported log time for one or more sample, samples taken on nonconsecutive days or on more than two days, or missing cap openings without corresponding missing cortisol samples. Other adherence problems that could be identified without the electronic monitoring primarily included missing samples. Lastly, we coded for the presence of missing cap openings (1 = missing electronically recorded time, 0 = no missing times), for cases in which there were more samples returned

than corresponding electronically recorded times, suggesting that more than one sample could have been done at the same time.

**Best collection.—**Second, we coded adherence for all participants 'best-attempt" at diurnal cortisol collection. The cortisol samples provided during participants' best attempt were those retained for assay. Coding for the best attempt adherence included determining the number of samples returned from the dyad (0-12). Next, we coded the number of samples collected within predetermined 'large' and 'small' adherence windows. Adherence windows were adapted from Moeller et al., 2014, Kudeilka et al., 2003, and Dozier et al., 2006. In the first 5 to 10 minutes post waking, cortisol levels are fairly stable; though in the subsequent hour levels rapidly increase and then decrease. Reliable reports of collection times in the morning are therefore especially critical for estimating a participant's waking cortisol, and diurnal decline (Stalder et al., 2016). For the 'large' adherence window, adherent morning samples had electronically recorded times within 30 minutes. Because cortisol fluctuations in the afternoon and evening are relatively minimal, afternoon and evening samples with electronically recorded times within one hour of self-reported times were considered adherent. For the 'small' adherence window, adherent morning samples had electronically recorded times within 15 mins of participant self-reported times and afternoon and evening adherent samples had electronically recorded times within 30 minutes of the self-reported time. Dyads could have between 0 and 12 adherent samples.

Finally, we coded the number of samples that were sufficient for assay (0-12). Assayable samples were defined as those samples from which cortisol could be extracted at assay. Reasons samples were insufficient for assay could be due to sample quantity (e.g., insufficient sample for assay) or quality (e.g., high coefficient of variation between duplicates, biologically implausible cortisol levels > 3.0 μg/dl), and are thus likely to be indicative of participant nonadherence to the protocol (e.g., not collecting for a sufficient amount of time, collecting immediately after oral consumption).

**Analytic strategy.—**For participants' first collection data, we examined overall sample descriptives and then compared our dichotomous indices of adherence by maltreatment group with Chi Square analyses. For participants' best collection data, we examined differential adherence by maltreatment group and we determined if these adherence measures differed by sampling day (1 or 2) or collection time point (waking, midday, or bedtime). These analyses used t- tests for group comparisons and ANOVA for examination of maltreatment group, sampling day/time, and their interaction on adherence outcomes of: total number of samples returned, number of samples within the large adherence window, number of samples within the small adherence window, and number of samples sufficient for assay.

## Results

### First Collection

Across the whole sample, 62.7% of dyads were adherent to the protocol on their first attempt at diurnal cortisol collection. Analyses by maltreatment group indicated that maltreating dyads were significantly more likely to be nonadherent (45.5%) than were nonmaltreating

dyads (19.7%; $\chi^2(1) = 11.08$, $p<.001$). Among those families who were nonadherent, the data provided by the MEMS cap identified the adherence problem 77.6% of the time. A particularly problematic form of nonadherence was missing MEMS cap openings relative to the number of samples provided, which suggests that more than one sample may have been provided at the same time of day. Maltreating dyads were more likely to have had missing MEMS cap openings (22.0%) compared to nonmaltreating dyads (6.6%), $\chi^2(1) = 7.20$, $p=.007$. Based on participants first attempt at cortisol collection, 12.4% of families (n = 20) were asked to re-do their cortisol collection. These were families where there was not at least one adherent day (defined as a waking, midday, and bedtime sample all from the same day for each mom and child with MEMS times that corresponded to self-report log times within one hour). There was a trend such that maltreating families were more likely to have been asked to redo the cortisol collection (16%) compared to the nonmaltreating families (6.6%; $\chi^2(1) = 3.11$, $p=.078$).

## Best Collection

**Descriptive statistics.—**On average mothers' self-reported collection times were 0:15 ($SD$ = 0:52), 0:20 ($SD$ = 1:04), and 0:25 ($SD$ = 1:13) minutes different than the objective measures of sample collection time. Likewise, children's objective-subjective time differences were 0:17 ($SD$ = 0:52), 0:26 ($SD$ = 1:13) and 0:16 ($SD$ = 0:47) minutes. Mother and child objective-subjective time differences were not significantly different than each other at any of the three time points ($t(149) = -0.163$, $p = .871$; $t(149) = -0.805$, $p = .422$; $t(143) = 1.579$, $p = .117$). Because of this finding, and the fact that mothers collected on themselves and their children, in all subsequent analyses we average mother and child time differences and report overall adherence for the dyad.

**Number of samples returned and maltreatment status.—**There was no difference in the number of saliva samples returned by the maltreating compared to the non-maltreating dyads, $t(164) = 1.630$, $p = .105$ (Table 2). Likewise, the maltreating and non-maltreating dyads did not differ in the number of samples returned on day one compared to day two, $F(1, 164) = 1.096$, $p = .297$ (Table 3). Nor did the number of samples returned based on collection time, $F(2, 328) = 0.532$, $p = .588$ (Table 4), differ by maltreatment group.

**Large adherence window and maltreatment status.—**Based on an adherence window of 30 mins at sample 1 (morning), and one hour at samples 2 and 3 (midday and evening), there was no difference in the number of saliva samples returned $t(164) = 1.238$, $p = .217$ (Table 2) when maltreating dyads were compared to non-maltreating dyads. Likewise, the maltreating and non-maltreating dyads did not differ in the adherence across the collection times, based on the large adherence window $F(2, 328) = 0.155$, $p = .857$ (Table 4). However, a day X maltreatment group interaction emerged, $F(1,164) = 8.225$, $p = .010$). Specifically, although the non-maltreating dyads were similarly adherent on day one ($M$ = 5.0, $SD$ = 1.9) as day two ($M$ = 5.1, $SD$ = 1.8), the maltreating dyads showed reduced adherence on day two ($M$ = 4.4, $SD$ = 2.0) compared to day one ($M$ = 5.0, $SD$ = 1.7; Table 2).

**Small adherence window and maltreatment status.—**Restricting the adherence to 15 minutes at sample 1, and 30 minutes at samples 2 and 3 still did not produce differences in the number of saliva samples returned $t(164) = 1.318$, $p = .189$ (Table 2) or adherence across the collection times $F(2, 328) = 0.360$, $p = .698$ (Table 4) when maltreating dyads were compared to non-maltreating dyads. Further, like the large adherence window, a day X maltreatment group interaction emerged using the small adherence window $F(1,164) = 8.408$, $p = .004$. Though the non-maltreating dyads were similarly adherent on day one ($M = 4.8$, $SD = 1.9$) as day two ($M = 4.9$, $SD = 1.8$), the maltreating dyads showed reduced adherence on day two ($M = 4.2$, $SD = 2.0$) compared to day one ($M = 4.8$, $SD = 1.7$; Table 2).

**Assayable samples and maltreatment status.—**The maltreatment groups also differed in the number of assay-able cortisol samples returned $t(164) = 1.977$, $p = .05$ (see Table 2). Specifically, non-maltreating dyads provided a greater number of assay-able samples ($M = 11.5$, $SD = 1.3$) than the maltreating dyads ($M = 10.7$, $SD = 3.1$). Maltreating mothers in particular ($M = 5.4$, $SD = 1.5$) seemed to provide the fewest number of assay-able samples compared to non-maltreating mothers ($M = 5.9$, $SD = 0.3$). Examining the maltreating and non-maltreating dyads for differences in the number of assay-able samples on day one compared to day two, $F(1,164) = 1.972$, $p = .162$ (see table 3) revealed no difference, nor did the number of assay-able samples returned based on collection time differ, $F(2, 328) = 0.616$, $p = .541$ (see Table 3).

## Discussion

The current study addresses a significant gap in the literature by providing data on adherence to in-home sampling of salivary cortisol among maltreating and demographically similar nonmaltreating mother-child dyads. Analyses revealed that on mothers' first attempt at collection of diurnal cortisol from themselves and their children, maltreating mothers were significantly less likely than nonmaltreating mothers to be adherent to the collection protocol, with nearly half of the maltreating sample demonstrating nonadherence. Importantly, the use of electronic monitoring caps as a surveillance technology provided critical information by signaling nonadherence in the vast majority of cases. Having an objective measure of the timing of each cortisol sample that could be compared with participants' self-reported collection times was essential for identifying multiple problems that could not have been otherwise detected. For researchers interested in analyzing the diurnal slope, it is especially noteworthy that on the first collection, 22% of the maltreating families, compared to 6% of nonmaltreating families, provided at least one cortisol sample with a self-reported log time that did not have a corresponding MEMS cap opening. Missing cap openings could reflect a number of problems including not closing the cap following a bottle opening or a cap malfunction. Most importantly, it may signal that multiple cortisol samples were provided at the same time of day, which if undetected, could lead to overestimations of flat diurnal slopes among maltreating families. As such, the electronic monitoring caps provide vital information for in-home collection of diurnal cortisol among maltreating families.

Although maltreating mothers had difficulty with adherence on their first collection attempt, analyses of all participants' best collection attempt revealed comparable adherence between maltreating and nonmaltreating mothers for both their own diurnal cortisol collection and their children's on most indices. Specifically, maltreating and nonmaltreated mothers returned the same total number of samples, and same number of samples using both adherence windows. Moreover, the majority of all samples returned were of high enough quality to be assayed.

Nonetheless, a few significant differences emerged between groups. When using large and small adherence windows to account for discrepancies in time between the objective and self-reported collection times, the number of adherent samples returned by the nonmaltreating families' was highly stable across both days of diurnal cortisol collection; in contrast, the maltreating families returned fewer samples within the large and small adherence window on day 2 compared to day 1. Given the greater amounts of unpredictability and instability generally found among maltreating families (Cicchetti & Valentino, 2006), it is not entirely surprising that maltreating families had more difficulty maintaining adherence to the strict collection protocol across both days of collection compared to the nonmaltreating families. Decreased adherence on day 2 may also reflect participant burden; thus, it may be most feasible use the minimum sampling protocol of two consecutive days with maltreating families as adherence may decrease further across additional days of sampling.

The only other significant difference to emerge between maltreating and nonmaltreating groups on participants best attempt at saliva collection was that maltreating mothers provided fewer assay-able samples (M= 5.4) than nonmaltreating mothers (M = 5.9). This difference represents less than a one-sample difference between groups; thus, although statistically significant it does not result in any practical differences between groups. Overall, these data reveal that obtaining valid diurnal cortisol data from maltreating and nonmaltreating families via home-based collection is feasible, though it may require resampling to achieve adequate adherence. Rates of adherence from participants' best collection are similar to those reported in other samples with preschool aged children (i.e., Smith & Dougherty, 2013). It is important to note, however, that adherence on participants' best collection was not perfect. Thus MEMS caps continue to be useful to identify nonadherent samples for cleaning cortisol data before analysis.

The current study has some limitations that should be addressed. For budgetary reasons, we only assayed participants' best cortisol collections. Thus, when families were asked to re-do their cortisol collection because of nonadherence, we did not send the original (first collection) samples for assay. As such we are able to report on less information about the quality of those samples. Additionally, although we coded official child welfare records and interviewed mothers about child maltreatment, it is possible that unreported child maltreatment occurred within our nonmaltreated sample. We also coded maltreatment records for maltreatment subtypes but did not analyze adherence as a function of maltreatment subtype, which may be important for future research. Finally, participants' opinions regarding the acceptability and feasibility of the cortisol protocol were not

collected; future research might also consider surveying participants for this information including their ideas on how to enhance feasibility.

The results of the current study have a number of implications for researchers interested in collecting diurnal cortisol from children in maltreating families. First, although maltreating families are initially poor at adhering to the sampling protocol, it is possible to obtain adherent data from them on subsequent attempts. To do so, objective measures of adherence and surveillance technology, such as electronic monitoring caps are very important so that adherence can be monitored in real time. Objective data is essential for identifying problems that would otherwise go undetected. Importantly, research with other populations has demonstrated that merely telling participants that their collection is being monitored (regardless of whether or not this is true), enhances participant adherence (Stalder et al., 2015); however, our data suggest that this does not seem to apply to maltreating mothers. All mothers in the current study were informed that their sample collection times were being monitored but that alone was not sufficient to obtain adherence. Electronic monitoring caps can be sterilized and re-used among participants; thus with a modest initial investment ($108 per MEMS cap + $720 software), it may be feasible to obtain objective electronic monitoring data for a relatively large sample depending on how many participants will be collecting diurnal cortisol simultaneously. If electronic monitoring for the whole sample is not feasible, doing so with a random subsample may be a useful alternative to check for adequate adherence.

Other technologies may also be valuable to enhance adherence. In the current study we provided all mothers with cell phones and used texts for collection reminders and to answer questions during home collection. It may be helpful to consider other ways cell phones can be utilized, such as asking families to take photo of each sample (with sample ID number visible) as soon as it is provided, which would both confirm which tube was used at which time and provide another time stamp for each sample; preprograming phones with alarms to signal collection times; and uploading a video with sample collection instructions. Additionally, those interested in the cortisol awakening response may consider providing participants with actigraphs or accelerometers to wear overnight, so that an objective measure of waking time is available.

Another practical suggestion is for researchers to budget for extra sample supplies. Extra supplies are necessary so that all families may practice obtaining samples from themselves and their children during training and to account for re-sampling among nonadherent families. We suggest that researchers budget to purchase 30% more supplies than the collection design necessitates. Each family will need an additional tube and swab per family member so that the participants can practice collecting, and researchers can ensure sufficient samples. Further, approximately 20% of our families were asked to restart the protocol. In most cases this required a complete new set of supplies to be given to the family. Encouragingly, the high percentage of samples that could be assayed from the current study suggests that the practice samples were effective in training mothers on how to obtain high quality samples that were adequate for assay.

The results of this study also have implications for the child maltreatment and neuroendocrinology fields. Extensive recommendations for best-practices in diurnal cortisol collection have emerged from the psychoneuroendocrinology literature (i.e., Stalder et al., 2015). Many of these recommendations include very stringent sample exclusion criteria (e.g., sample collection within a 5 min. window of the expected time) that while useful and important in highly controlled settings or with low risk samples, are not entirely practical with high risk samples. Some degree of trade-off between the incorporation of stringent methodological designs and the flexibility that may be necessary to obtain diurnal cortisol data from high risk samples, such as maltreating mothers and their children, should be expected and acknowledged by the field. After all, even with more flexible adherence criteria, examination of diurnal cortisol and physiological regulation in the context of child maltreatment and the extent to which physiological dysregulation may be remediated through interventions, are arguably among the most valuable data to inform both the child maltreatment and psychoneuroendocrinology fields.

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**Table 1.**

Sample characteristics by maltreatment group.

| | Nonmaltreated (n =64) | Maltreated (n = 102) |
|---|---|---|
| **Variable** | **M (SD)/%** | **M(SD)/%** |
| Maternal Age | 29.5(6.3) | 28.8(5.0) |
| Child Age | 4.49(1.03) | 4.52(1.12) |
| Child Gender (Male) | 45.3% | 52.0% |
| Child Ethnicity ** | | |
| African American | 39.7% | 40.6% |
| Caucasian | 19.0% | 26.7% |
| Mixed Race | 17.4% | 27.7% |
| Hispanic | 23.8% | 5.0% |
| Maternal Education | | |
| Up to 11th grade | 20.8% | 34.2% |
| HS grad or GED | 27.1% | 32.9% |
| Some trade school or college | 33.3% | 23.3% |
| Associates Degree | 14.6% | 5.5% |
| Bachelors or higher | 4.2% | 4.1% |
| Maternal Employment (Employed) | 40.6% | 39.6% |
| Family Income (≤$12,000/year) | 53.1% | 57.8% |
| Maternal Receptive Language | | |
| PPVT-4 score | 85.40(11.8) | 84.33(12.7) |

Note:

* p<.05,

** p<.01;

PPVT-4 is the Peabody Picture Vocabulary Test, 4th Edition,

**Table 2.**

Means and standard deviations of the number of samples the dyads returned across the two days, split by maltreatment status.

| | Non-Maltreating | | Maltreating | | Total | |
|---|---|---|---|---|---|---|
| | **M** | **SD** | **M** | **SD** | **M** | **SD** |
| **Total returned** | 11.9 | (0.5) | 11.4 | (2.3) | 11.6 | (1.9) |
| **Large adherent** | 10.1 | (3.3) | 9.5 | (3.3) | 9.7 | (3.3) |
| **Small adherent** | 9.8 | (3.3) | 9.1 | (3.4) | 9.3 | (3.4) |
| **Assayable** | 11.5 | (1.3) | 10.7 | (3.1) | 11.0 | (2.5) |

**Table 3.**

Means and standard deviations of the number of samples the dyads returned across the three times, split by day and dyad maltreatment status.

| | Non-Maltreating | | Maltreating | | Total | |
|---|---|---|---|---|---|---|
| | ***M*** | ***SD*** | ***M*** | ***SD*** | ***M*** | ***SD*** |
| **Day 1** | | | | | | |
| **Returned** | 5.9 | 0.4 | 5.8 | 1.1 | 5.8 | 0.9 |
| **Large adherent** | 5.0 | 1.9 | 5.0 | 1.7 | 5.0 | 1.8 |
| **Small adherent** | 4.8 | 1.9 | 5.0 | 1.7 | 4.8 | 1.8 |
| **Assayable** | 5.8 | 0.7 | 5.5 | 1.5 | 5.6 | 1.3 |
| **Day 2** | | | | | | |
| **Returned** | 6.0 | 0.4 | 5.7 | 1.3 | 5.8 | 1.1 |
| **Large adherent** | 5.1 | 1.8 | 4.4 | 2.0 | 4.7 | 1.9 |
| **Small adherent** | 4.9 | 1.8 | 4.2 | 2.0 | 4.5 | 2.0 |
| **Assayable** | 5.8 | 0.7 | 5.3 | 1.7 | 5.5 | 1.4 |

**Table 4.**

Means and standard deviations of the number of samples the dyads returned across the two days, split by collection time and dyad maltreatment status.

| | Non-Maltreating | | Maltreating | | Total | |
|---|---|---|---|---|---|---|
| | ***M*** | ***SD*** | ***M*** | ***SD*** | ***M*** | ***SD*** |
| **Time 1 (Waking)** | | | | | | |
| **Returned** | 4.0 | 0.3 | 3.8 | 0.8 | 3.9 | 0.6 |
| **Large adherent** | 3.4 | 1.2 | 3.2 | 1.2 | 3.3 | 1.2 |
| **Small adherent** | 3.1 | 1.3 | 3.0 | 1.3 | 3.0 | 1.3 |
| **Assayable** | 3.9 | 0.5 | 3.7 | 1.0 | 3.8 | 0.9 |
| **Time 2 (Midday)** | | | | | | |
| **Returned** | 4.0 | 0.3 | 3.8 | 0.8 | 3.9 | 0.6 |
| **Large adherent** | 3.4 | 1.2 | 3.2 | 1.2 | 3.3 | 1.2 |
| **Small adherent** | 3.4 | 1.2 | 3.1 | 1.2 | 3.2 | 1.2 |
| **Assayable** | 3.8 | 0.5 | 3.6 | 1.0 | 3.7 | 0.9 |
| **Time 3 (Evening)** | | | | | | |
| **Returned** | 4.0 | 0.3 | 3.8 | 0.9 | 3.8 | 0.7 |
| **Large adherent** | 3.3 | 1.3 | 3.0 | 1.4 | 3.1 | 1.3 |
| **Small adherent** | 3.3 | 1.3 | 3.0 | 1.4 | 3.1 | 1.3 |
| **Assayable** | 3.8 | 0.6 | 3.5 | 1.1 | 3.6 | 1.0 |