

National Survey of Child and Adolescent Well-Being

No. 2: Collecting Saliva Cortisol Samples Among Young Children in the Child Welfare System



Findings from the NSCAW Study

methods brief



Understanding the immediate and long-term consequences of child maltreatment is crucial to helping children involved in investigations by the child welfare system (CWS). In recent years, research has focused on the neurobiological sequelae of child maltreatment in order to help explain maladaptive development. One area of study has been alterations in the biological systems that manage responses to stress. Maltreatment early in life may exert a critical influence on how stress is managed, creating the biological conditions for childhood and adult depression, anxiety, post-traumatic stress symptoms, eating disorders, substance abuse, and other chronic conditions.¹⁴

The body and brain adapt to acute stress through the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Survival depends on the ability to mount an appropriate response to stress.⁵ By means of a complex chain of hormones activated by stress, the hormone cortisol is released, mobilizing the body and brain for fighting or fleeing. Learning to manage stress is an important and necessary part of healthy development; nevertheless, strong and prolonged activation of the body's stress response can produce chronic dysregulation of the HPA axis, which can in turn have negative effects on physical development, the immune system, and cognitive functioning.⁶ This type of stress can be produced by recurrent child maltreatment, family violence, maternal depression, and parental substance abuse.⁷

Cortisol follows a daily rhythm; the highest levels appear around the time of awakening and then decline to low levels at bedtime.⁸ Elevated cortisol levels in the morning prepare humans for their daily activities by metabolizing stored energy, stimulating appetite, and promoting the processes involved in learning.⁹ In studies with children, levels of cortisol higher than expected for a given time of day are often considered synonymous with acute stress. Levels of cortisol lower than expected in the morning are viewed as a likely consequence of chronic stress. Children living under chronic conditions of maltreatment tend to present low early-morning levels of cortisol.¹⁰ Chronically high levels

of cortisol may leave children more susceptible to illnesses and infections. In contrast, chronically low cortisol levels may result in increased vulnerability to autoimmune disorders and asthma.

Purpose of the Brief

This methods brief examines the daytime cortisol levels of 5- to 6-year-old children who were infants when they were first involved in CWS investigations of maltreatment. The brief explores the feasibility of collecting salivary cortisol samples in the context of a national survey and examines the relationships between daytime cortisol levels, age, and gender. To assess this method, this methods brief answers the following questions:

- Can caregivers of young children investigated by the CWS successfully collect saliva samples according to the provided salivary cortisol sampling guidelines?
- Do the daytime cortisol levels assessed over 3 days show expected relationships in this sample of children?
- Do expected and significant age and gender differences in daytime cortisol levels emerge in this sample of children?

National Sample of Children Involved in Allegations of Child Maltreatment

In this methods brief, data from the National Survey of Child and Adolescent Well-Being (NSCAW) are used to describe daytime cortisol levels of 5- to 6-year-old children who were infants when first involved in CWS investigations. NSCAW is a national longitudinal study of the well-being of 5,501 children aged 14 years or younger who had contact with the CWS within a 15-month period starting October 1999.¹¹ To date, five waves of data collection have been completed, the final one having concluded in 2006. The data collection consisted of interviews or assessments conducted with the children and their current caregivers, teachers, and CWS caseworkers. Both children who remained in the CWS and children who left the system were followed for the entire study period. In the sample, 1,186 children met the criteria of having been younger than

12 months old at Wave 1 and having been 5 or 6 years old at Wave 5. From this sample of children, 440 children were selected for salivary cortisol sample collection, and 293 families agreed to collect the samples.

Characteristics of the Children in the Sample

Of the 293 families who agreed to collect salivary cortisol samples, 187 returned at least one saliva sample. Because only a small subsample of children was included, the weighted data and complex survey design typically used in analyses involving the NSCAW sample were not appropriate for the analyses reported here; therefore, all current analyses were conducted with unweighted data.

At Wave 5 the average child age was 69 months, with an age range of 61 to 81 months. In this sample 54% of the children were girls, and 46% of the children were boys. In terms of race/ethnicity, 39% of the children were White; 33%, African American; and 19%, Hispanic. Approximately 81% of the children were in kindergarten; 14% were in first grade. According to the baseline interviews with the children's caseworkers, the most serious form of reported maltreatment was physical neglect for 46% of the children, supervisory neglect for 26%, and physical abuse for 15%. At Wave 5, 46% of the children were living with a biological parent, 33% were living with an adoptive parent, and 6% were living in foster or kinship care.

Salivary Cortisol Collection and Assay Procedures

The caregivers were trained by the NSCAW field representatives to collect saliva samples from their children. The saliva samples were obtained in the morning (30 minutes after waking but before eating breakfast; mean time 7:31 a.m.) and in the evening (30 minutes before going to bed but before brushing teeth; mean time 8:30 p.m.) on 3 consecutive, typical weekdays. To stimulate salivation, the children chewed Trident Original sugarless gum, which previous research has shown to not affect cortisol levels.¹² Salivettes (Sarstedt, Newton, NC) were then placed in the children's mouths. Once saturated, the Salivettes were placed in prelabeled plastic vials.

The caregivers were instructed to refrigerate the saliva samples until they were mailed to the laboratory. Previous research has suggested that the conditions experienced during the mailing process do not influence cortisol levels.¹³ After shipment the saliva samples were stored at -5°F until examined. The saliva samples were assayed for cortisol determination with the High

Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, State College, PA). All saliva samples from each child were included in the same assay batch to minimize within-subject variability. The saliva samples were assayed in duplicate and were averaged. Any duplicates varying by more than 15% were reassayed. The intraassay coefficient of variance (or variability in the assay results within an assay batch) and interassay coefficient of variance (or variability in the assay results across multiple assay batches) were 2% and 9%, respectively.

Certain medications and collection procedures have been shown to affect cortisol levels.¹⁴ Caregivers therefore completed a brief questionnaire about their children's use of medication and saliva sampling times on each of the saliva sampling days. The questionnaires were inspected to ensure compliance with salivary cortisol collection guidelines; saliva samples that failed to comply were excluded from analyses.

Feasibility of Saliva Sample Collection

Saliva samples were successfully collected according to the salivary cortisol collection guidelines for 76% of the morning and 77% of the evening saliva samples. Of the 187 children who returned at least one saliva sample, 113 children successfully collected three morning saliva samples, 34 successfully collected two morning saliva samples, and 18 successfully collected one morning saliva sample. Furthermore, 118 children successfully collected three evening saliva samples, 32 successfully collected two evening saliva samples, and 13 successfully collected one evening saliva sample (Table 1).

Table 1. Number of successful and excluded collections for the morning and evening saliva samples

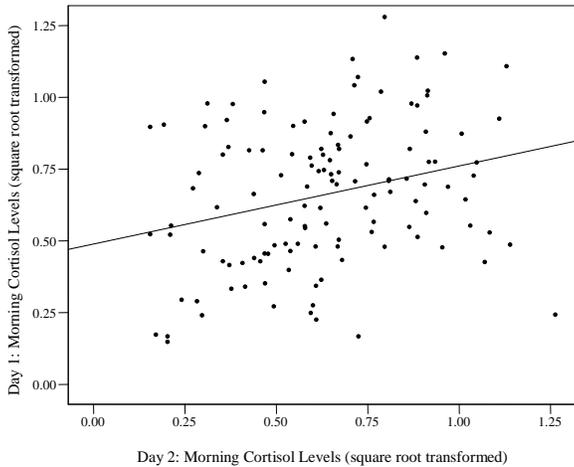
	Morning	Evening
Successful collections	425	431
Excluded collections		
No saliva sample or inadequate saliva	29	30
Invalid cortisol level	1	2
Issue regarding questionnaire	25	26
Issue regarding usage of medication	38	36
Issue regarding saliva sampling time	43	36

Relationships Among the Daytime Cortisol Levels for 3 Days

Although daytime cortisol levels vary from day to day, it was expected that cortisol levels across the 3 saliva sampling days would be significantly related. For all analyses, the morning and evening cortisol levels were subjected to square root transformations, and outliers were censored to normalize the distribution. Morning cortisol levels were modestly correlated across the 3

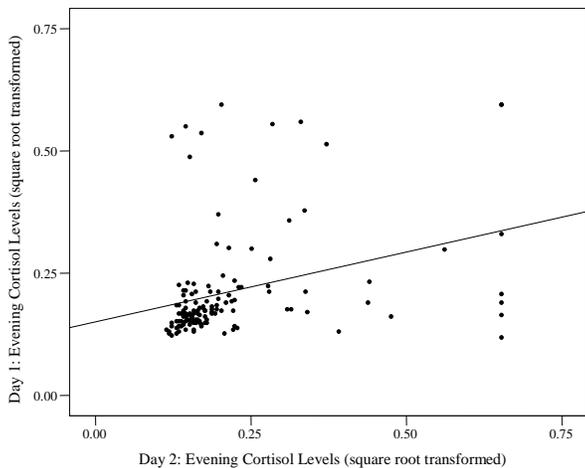
days: Children who had higher morning cortisol levels on one day tended to have higher morning cortisol levels on another day (Figure 1). Similarly, evening cortisol levels across the 3 days were comparable (Figure 2).

Figure 1. Relationship between morning cortisol levels on Day 1 and morning cortisol levels on Day 2



Note: Pearson product-moment correlations showed that morning cortisol levels across 3 days were significantly associated: Day 1 with Day 2, $r(124) = .27, p < .002$; Day 1 with Day 3, $r(121) = .32, p < .001$; and Day 2 with Day 3, $r(122) = .38, p < .001$.

Figure 2. Relationship between evening cortisol levels on Day 1 and evening cortisol levels on Day 2



Note: Pearson product-moment correlations showed that evening cortisol levels across 3 days were significantly associated: Day 1 with Day 2, $r(129) = .33, p < .001$; Day 1 with Day 3, $r(123) = .26, p < .003$; and Day 2 with Day 3, $r(128) = .36, p < .001$.

To create more stable measures of the children’s daytime cortisol levels, each child’s morning cortisol levels were averaged to create a composite morning cortisol level; likewise, each child’s evening cortisol levels were averaged to create a composite evening cortisol level. The mean cortisol levels were 0.47 micrograms per deciliter ($\mu\text{g}/\text{dl}$) in the morning and 0.09 $\mu\text{g}/\text{dl}$ in the evening (Table 2).

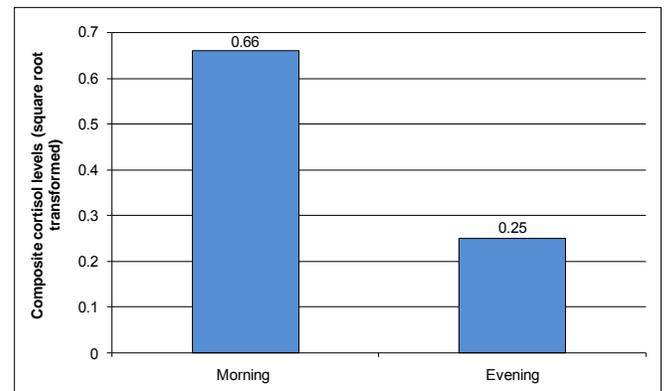
Table 2. Composite cortisol levels and saliva sampling times

	Morning	Evening
Mean cortisol level ($\mu\text{g}/\text{dl}$)	0.47	0.09
Mean saliva sampling time	7:31	20:30
Mean latency between sampling time and wake/bed time (min.)	17	26

Daily Rhythm in Daytime Cortisol Levels

Children showed the expected daily rhythm in their daytime cortisol levels, with significantly higher composite morning cortisol levels than composite evening cortisol levels (Figure 3). Additionally, 94% showed the expected decrease from morning to evening.

Figure 3. Composite morning and evening cortisol levels

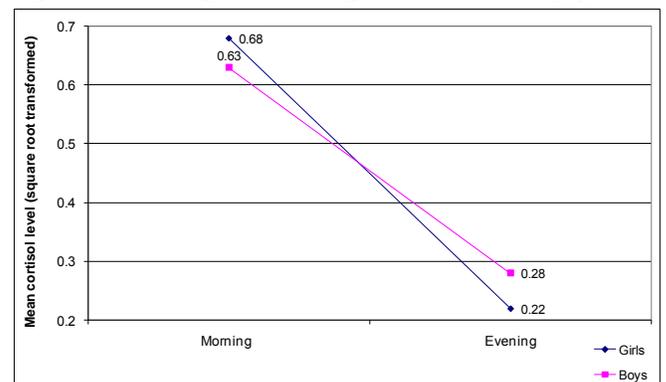


Note: A one-way ANOVA showed that the composite morning cortisol levels were significantly higher than the composite evening cortisol levels, $F(1, 157) = 479.61, p < .001$.

Age and Gender Differences in Daytime Cortisol Levels

Neither the composite morning cortisol levels nor the evening levels were significantly correlated with child age; however for the children’s daytime cortisol levels, boys showed a significantly higher evening cortisol level than girls (Figure 4).

Figure 4. Morning and Evening Cortisol levels by gender



Note: A one-way ANOVA showed that boys had significantly higher composite evening cortisol levels than girls, $F(1, 161) = 7.52, p < .007$. No significant difference emerged for morning levels, $F(1, 163) = 3.11, ns$.

Summary

One of the goals of this methods brief has been to report on the feasibility of collecting salivary cortisol samples from a national sample of children investigated for maltreatment and followed for several years. The careful collection of salivary cortisol samples was difficult with this at-risk population and as part of a national survey study. Of the 440 children selected to participate in the current study, 293 families (67%) consented to participate, 187 families (43%) collected at least one saliva sample, and 170 families (39%) collected at least one saliva sample according to the salivary cortisol collection guidelines. Only 92 families (21%) collected all six saliva samples according to the salivary cortisol collection guidelines.

These results suggest that the following additional training and supervision (e.g., in-person demonstrations) may be required to ensure the careful collection of saliva samples with young children in the CWS: emphasis on the critical collection procedures, regular contact on the saliva sampling days to remind families to collect the saliva samples, reiteration of critical collection procedures, response to any questions; and perhaps continued contact after the saliva samples are returned (to clarify any issues and to request the collection of additional saliva samples).

For families that complied with salivary cortisol collection guidelines, patterns of daytime cortisol levels approximated expectations established by previous research. The daytime cortisol levels among 5- to 6-year-old children were significantly related across the 3 days. Even though daytime cortisol levels fluctuate to manage each day's challenges, children's cortisol levels showed moderate stability from day to day. Specifically, children with higher morning cortisol levels on one day tended to have higher morning cortisol levels on another day.

The children's daytime cortisol levels also displayed the expected daily rhythm. Specifically, morning cortisol levels were significantly higher than evening cortisol levels, with 94% of the children displaying this pattern. The results of the families completing the cortisol collection procedures here were consistent with previous research showing high cortisol levels at the time of awakening and then decline to low levels at bedtime.^{8, 15} This daily rhythm is an important indicator of regulation of the HPA axis.

Analysis of cortisol levels by gender showed significant differences between girls and boys. Boys presented more flattened morning-to-evening cortisol activity because of

their higher levels of cortisol production in the evening. This gender difference in morning-to-evening cortisol levels is interesting because of the differing patterns of cortisol production in children with internalizing disorders, like depression and anxiety.^{16, 17} It will be important for future research to explore gender differences in cortisol levels.

The mean cortisol levels obtained among 5- to 6-year-old children who were infants when first involved in CWS investigations were 0.47 µg/dl in the morning and 0.09 µg/dl in the evening. These daily cortisol values are within the range of those reported for at-risk preschool-aged children in two previous studies.^{18, 19} This comparison must be undertaken with caution, however. Many factors impact cortisol values, including the sampling schedule, collection procedures, and type of assay. The sampling and assay procedures were similar across the Dozier, Fisher, and NSCAW studies, but with important differences. For example, the Fisher and NSCAW studies used Salivettes and Trident gum for collection, whereas the Dozier study used cotton dental rolls and flavored beverage crystals. Additionally, although all three studies used Salimetrics assay, the NSCAW study used the newest assay, which results in slightly lower cortisol values than the original assay used for the Dozier and Fisher studies.

Results of this brief showed that challenges to measuring cortisol in a national study remain, and substantial effort is required to obtain reliable measures. This effort may be worthwhile because the careful assessment of the daytime cortisol levels may well inform an understanding of the developmental outcomes of young children in the CWS. Previous research suggests that early adverse experiences, such as neglect or abuse, profoundly impact the development and functioning of the HPA system.^{18, 20} For example, Dozier and colleagues found that maltreated children placed in foster care as infants had significantly lower morning cortisol levels than nonmaltreated children reared in their biological families. These alterations in the HPA system might leave young children in the CWS vulnerable to a host of difficulties. Lower daytime cortisol levels have been observed in adolescent males with externalizing behavior problems and in adults with post-traumatic stress disorder.^{21, 22}

Current research with young children in the CWS is under way to help caregivers improve their parenting skills in order that they may help regulate the HPA axis of their children. Changes in caregivers as a result of such interventions include improvements in their

abilities to follow the child's lead in interactions, holding and touching the child, being responsive to the child, and in general maintaining a warm, consistent environment in which positive behavior is encouraged and problem behavior is limited.^{18, 19} Evidence suggests, after all, that alterations in the HPA system are amenable to therapeutic interventions that may help children develop regulatory capabilities ultimately to prevent the negative outcomes associated with child maltreatment.

Notes

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Available at: National Data Archive on Child Abuse and Neglect (NDACAN), Cornell University, ndacan@cornell.edu

Administration for Children and Families (ACF, OPRE)
http://www.acf.hhs.gov/programs/opre/abuse_neglect/nscaw/

This is the second in a series of NSCAW methods briefs focused on children who have come in contact with the child welfare system. Additional methods briefs address computer-mediated tasks to assess children's executive functioning, among other topics.