Salivary Cortisol Responsivity to an Intravenous Catheter Insertion in Children with Attention-Deficit/Hyperactivity Disorder

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Objective To compare salivary cortisol baseline levels and responsivity as well as behavioral distress to intravenous (IV) catheter insertions in 4- to 10-year-old children with ($n = 29$) and without ($n = 339$) attention-deficit/hyperactivity disorder (ADHD). **Methods** This is a secondary data analysis from a sample of 542 children who participated in a multisite study on distraction. Data included were demographic variables, Pediatric Behavior Scale-30, Observational Scale of Behavioral Distress-Revised, and four salivary cortisol samples. **Results** Home samples from the ADHD group revealed nonsignificant but higher cortisol levels than the non-ADHD group. However, on the clinic day, the ADHD group had significantly lower cortisol levels before (0.184 vs. 0.261, $p = .040$) and 20–30 min after IV insertion (0.186 vs. 0.299, $p = .014$) compared with the non-ADHD group. **Conclusions** Cortisol levels in children with and without ADHD differ in response to the stress of an IV insertion.

Key words attention; children; hyperactivity and ADHD; stress.

Children with health problems often need stress provoking or painful procedures to diagnose or treat their illness. In order to test an intervention's effectiveness to decrease children's procedural distress, measures are needed to assess multiple aspects of the stress response. For example, with an intravenous (IV) insertion, behavioral responses may include crying, flailing, or verbal complaints, and physiological responses may include increased blood pressure, muscle tension, and sweating. Responses to stress may be voluntary or involuntary and engaging or disengaging as described by others (Compas, Conor-Smith, Saltzman, Thomsen, & Wadsworth, 2001; Conner-Smith, Compas, Wadsworth, Thomsen, & Saltzman, 2000). Multiple measures may be needed to capture the range of stress responses. While behavioral observation scales assess behavioral responses and self-report measures assess perceptions of pain, physiologic responses are not captured by these measures.

Physiological responses are controlled by the HPA axis, and the sympathetic and parasympathetic components of the peripheral nervous system. The HPA axis interacts with the central stress activation centers within the brain stem and hypothalamus and releases corticotrophin-releasing hormone (CRH), which is one of the primary effectors of the stress reaction (Charmandari, Tsigos, & Chrousos, 2005). CHR stimulates the release of additional chemicals that impact cortisol and adrenocorticotropic hormone (ACTH) secretion, which are the crucial neurotransmitters in the acute stress reaction. Salivary cortisol, a physiological marker of stress, has emerged as an easy to collect and useful measure of the physiological response to stress in children.

Cortisol levels generally follow a circadian (24 hr) pattern that develops during infancy (Lewis & Ramsay, 1995). Levels are lowest on awakening, quickly reach peak, and gradually decrease during the afternoon and evening.

While the typical circadian pattern occurs in most people, both levels and patterns may vary among certain groups. Individuals typically have increased cortisol levels in response to stressful events. Researchers have reported atypically high cortisol baseline levels and low responsivity associated with extreme physiological, emotional, and social stressors in some children. Overstimulation of the HPA system resulting in chronic cortisol elevation can cause chronic health problems such as hypertension and adrenal fatigue leaving the body unable to mount the typical stress response. If cortisol is used as a measure of distress in research with children, it is important to identify factors that may impact cortisol baseline levels and responsivity in children. Information on the impact of specific medical diagnoses or medications on cortisol levels is emerging.

Atypical baseline and response patterns have been identified in children and adolescents with a range of psychological disorders, including attention deficit hyperactivity disorder (ADHD). One of the most common psychiatric disorders of children (Biederman, 2005; Kendall, Leo, Perrin, & Hatton, 2005), ADHD affects an estimated 5–10% of children worldwide (Faraone, Sergeant, Gillberg, & Biederman, 2003). Because of the high prevalence of ADHD, it is likely that many children having medical procedures have this diagnosis.

Children with ADHD appear to respond differently to psychological stress when compared to peers without ADHD (Kaneko, Hoshino, Hashimoto, Okano, & Kumashiro, 1993). For example, children with internalizing disorders such as depression (Mannie, Harmer, & Cowen, 2007) and posttraumatic stress disorder (PTSD) in children with autism (Marinović-Curin et al., 2008) have been found to exhibit persistently high cortisol levels, which increase in response to stress. In contrast, children with externalizing disorders such as oppositional defiant disorder (ODD), conduct disorder (CD), and ADHD often display blunted cortisol levels when measured on waking, throughout the day, and in response to a stressor (Kaneko et al., 1993; Kariyawasam, Zaw, & Handley, 2002; McBurnett, Lahey, Rathouz, & Loeber, 2000; Randazzo, Dockray, & Susman, 2008; Snoek, Van Goozen, Matthys, Buitelaar, & van Engeland, 2004).

Temperament traits associated with ADHD, such as poor effortful control and high surgency, have also been associated with lower cortisol responsivity in response to stressors such as peer rejection (Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003). Researchers have noted a strong negative correlation between cortisol response and the aggressive behaviors often associated with externalizing conditions such as ADHD or ODD (King, Barkley, & Barrett, 1998; McBurnett et al., 2000; Randazzo et al., 2008; van de Wiel, van Goozen, Matthys, Snoek, & van Engeland, 2004; van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000). Snoek and others (2004) studied four groups of children: children with comorbid ADHD and ODD, children with ADHD but without ODD, children with just ODD and a normal control group. There were no differences in baseline cortisol levels among the four groups. Children with just ODD, and ADHD with ODD, displayed a significantly weaker cortisol response to stress compared to children in the other two groups (ADHD alone and controls), suggesting differences in the cortisol-stress relationship among children with externalizing behaviors, particularly in children with ODD.

In a recent study, baseline and postvenipuncture salivary cortisol samples were obtained from 178 elementary school boys with ADHD and comorbid anxiety or behavioral disorders (Hastings, Fortier, Utendale, Simard, & Robacy, 2009). These authors hypothesized that cortisol reactivity would vary according to the type of comorbidity (anxiety or disruptive behavior disorder) as well as by ADHD subtype (inattentive, hyperactive/impulsive, or combined type). Boys with ADHD and comorbid anxiety disorders showed the cortisol responsivity to the IV stressor. In contrast, children with ADHD and comorbid disruptive behaviors showed diminished levels. In addition, the dampened response was most marked in boys with either inattentive or hyperactive subtypes of ADHD and a comorbid behavioral disorder, but not in boys with the combined subtype and a comorbid behavior disorder.

Van Goozen and colleagues (Van Goozen et al., 2000) conducted a study comparing cognitive and physiological measures of stress in response to a psychological stressor in children with ODD alone ($n = 14$), ODD and comorbid ADHD ($n = 12$) and controls ($n = 36$). The ODD and ADHD groups had lower baseline cortisol levels as well as lower stress responsivity than controls. Similarly, Blomqvist et al. (2007) reported lower cortisol levels in children with ADHD undergoing clinical dental examinations when compared with controls. Eighteen 13-year-old children with ADHD and 71 controls underwent a routine annual dental examination and completed a standard tool used to measure anxiety associated with dental care. In the ADHD group, 13 of the 18 (72.2%) were diagnosed with the hyperactive/impulsive subtype of ADHD. Four saliva samples were obtained: on awakening on the pre-exam day, immediately following the exam, and on awakening and 30 min afterward on the morning after the exam. The children with the ADHD subtype of hyperactivity/impulsivity had significantly higher anxiety about the dental exam than controls and lower cortisol levels at all

four sample times. These authors suggested that while many children are anxious about dental procedures, children with the impulsivity/hyperactivity subtype of ADHD may have lower baseline cortisol levels and cortisol response to the stress of having a dental exam.

These findings, however, are not consistent; other studies suggest that there are no significant differences in cortisol baseline levels and responsivity among children with various types of childhood behavioral disorders. For example, Schulz, Halperin, Newcorn, Sharma, and Gabriel (1997) conducted a study to determine baseline plasma cortisol levels in 50 school-aged boys who fit DSM-III criteria for the diagnosis of ADHD. Each subject was categorized as ''aggressive'' ($n = 23$) or ''nonaggressive'' ($n = 27$). Participants underwent an IV insertion, and salivary cortisol was collected at 105 and 115 min after the procedure. These two values were then used to obtain an average baseline cortisol level for each child. The authors reported no significant differences between the groups. Jansen and others (Jansen et al., 1999) reported that in a sample of children with ADHD, dysthymia, ODD/conduct disorder, or pervasive developmental disorder ($n = 52$), a physical, but not a psychological, challenge resulted in an overall increase in cortisol.

It is possible that variations in these study findings may be at least partially due to differences across samples, stressor type and duration, and factors such as lack of a control group, not considering comorbid diagnoses or developmental issues (Shirtcliff & Essex, 2008). In a meta-analysis of cortisol in children and adolescents with externalizing behavior disorders, no association was found between cortisol reactivity and externalizing behaviors, although there was a small but significant association between baseline cortisol and externalizing behaviors which varied by the age of the child (Alink et al., 2008). Baseline cortisol levels were high in preschool children with externalizing behaviors, lower in school age children, and there was no significant relationship found in adolescents.

When considering the impact of ADHD and other health conditions on cortisol levels in children, it is also important to note that certain medications including amphetamines, steroids, and antidepressants are known to affect cortisol levels (King & Hegadoren, 2002). Kariyawasam et al. (2002) compared salivary cortisol levels in 32 children with ADHD/ODD and in 25 controls. In the group with co-morbid ADHD/ODD, 14 children had taken their usual dose of methylphenidate or D-amphetamine; the remaining subjects were not medicated at the time of saliva collection. Cortisol levels were significantly lower in the group with ADHD/ODD compared to controls ($p < .05$). However, this difference was highly significant between the 18 subjects with ADHD/ODD that were not medicated compared to controls ($p < .001$). These results suggest treatment with amphetamine may normalize cortisol levels in children with ADHD.

In summary, many studies of cortisol levels in children with externalizing behavioral disorders such as ADHD have found atypical cortisol baseline levels and levels in response to stressful conditions, including the emotional and physiological stress associated with common medical procedures (e.g., IV insertion). However, while there are a number of studies, the findings are often contradictory and inconsistent. Studies are needed to document children's cortisol patterns in a natural, although stressful setting. Furthermore, considering the prevalence of ADHD in children, it is important to understand cortisol baseline levels and responsivity in this population when using cortisol as a research measure of an individual's response to a stressor.

Study Purpose

A randomized study of the use of distraction with children undergoing an IV insertion provided a unique opportunity to assess cortisol levels in children with and without ADHD while undergoing a stressful procedure in a natural setting (McCarthy et al., 2010a, 2010b).This secondary data analysis compared baseline cortisol levels and cortisol responsivity of children with and without ADHD undergoing a common medical procedure. Since children with ADHD often display hyperactive behaviors, differences in their distress behaviors were also compared in order to explore the value of multiple measures of distress when evaluating an intervention.

The following research questions were addressed:

1. Are there differences in baseline cortisol levels in children with and without ADHD on a typical day?
2. Are there differences in cortisol responsivity in children with and without ADHD on a day they undergo IV insertion?
3. Are there differences in children with and without ADHD in observable distress behaviors in response to an IV insertion?

Methods
Participants

Subjects in this study were from a sample of 542 children who participated in a multisite research study to identify factors that explain children who benefit from distraction and parents who are able to successfully coach their

children in the use of distraction during an IV insertion. Included were children aged 4–10 years old, English speaking, having a planned IV insertion for diagnostic testing (e.g., endoscopy, bronchoscopy, radiographic imaging test), and no major cognitive impairments (i.e., in regular classes and able to answer the study questions).

For this secondary analysis, children were categorized into two groups: children with ADHD and children without ADHD. Groups were determined by parental report of (a) the child's ADHD diagnosis or (b) the child taking medications typically prescribed for ADHD (e.g., methylphenidate). A total of 54 children comprised the ADHD group and 488 the non-ADHD group.

For the analyses described, only children with at least two cortisol samples (i.e., minimum of two clinic, two home, or pair of clinic and home) were included (Table I). Children in the ADHD group ($n = 29$) were significantly older ($p = .003$) with a median age of 8 years vs. 7 years for the non-ADHD group ($n = 339$). There were more boys in the ADHD group compared to the non-ADHD group (66 vs. 48%, $p = .072$). Of the 29 children, 28 had a parent reported diagnosis of ADHD (one parent did not answer the question, but the child was on medication for ADHD) and 22 (75.9%) were on medications for ADHD (e.g., Concerta®, Ritalin, Adderrall). The ADHD group was compared to the non-ADHD group with respect to the subscales of the parent report on the Pediatric Behavior Scale (PBS) (Lindgren & Koeppl, 1987). The ADHD group had significantly higher scores in the areas of impulsivity, inattention, and hyperactivity (Table II), confirming that the two groups differed on key diagnostic criteria for ADHD.

Instruments

Demographic Questionnaire
Parents completed a demographic questionnaire for the primary study. The demographic variables examined for this subproject included child age, gender, race, medical diagnoses, and history of previous IV insertions.

PBS-30
The parents of all subjects completed the PBS-30, which includes 30 questions about their child's typical behavior. The PBS was designed specifically to evaluate behavior problems in children with health problems on a 0 to 3-point scale ranging from a zero indicating no problems to three indicating that specific behaviors are very often a problem (Lindgren & Koeppel, 1987). The PBS-30 is an abbreviated measure that evaluates four major areas of behavioral/emotional adjustment: aggression/opposition, hyperactivity/inattention, depression/anxiety, and physical complaints. The internal consistency for those general items is 0.83, 0.87, 0.80, and 0.73, respectively (McCarthy, Lindgren, Mengeling, Tsalikian, & Engvall, 2002). In this particular study, internal consistencies of the of PBS domains ranged from 0.74 (depression/anxiety) to 0.94 (hyperactivity/inattention).

Cortisol
Four salivary samples were collected from each child: two in the clinic, one before IV insertion (C1), the other after 20–30 min of the IV insertion (C2), and two at home on a typical day (H1 and H2) at times that corresponded with the times cortisol samples were collected in the clinic. For example, if the C1 sample was obtained from a subject at 9 a.m. (measuring the stress of being at the clinic) and C2 sample was obtained at 11:00 a.m. (20–30 min after the IV was inserted, measuring the stress of having an IV inserted), then the home samples would be collected within 30 min of 9 a.m. (H1) and 11 a.m. (H2) on a typical day.

A standardized collection protocol assured consistency and control in samples collected in the clinic and at home. Screening questions were asked and saliva was not collected from children who had taken oral steroids within 1 month of the clinic visit. Home cortisol samples were collected and returned to the researchers between 1 week and 3 months following the procedure. A full explanation of the cortisol collection procedures is described elsewhere

Table I. *Characteristics of Children by ADHD groups*

	ADHD $n = 29$	Non-ADHD $n = 339$	p-value[a]
Age, median (25th–75th percentile)	8 (7–9)	7 (6–9)	.003
Gender (male, %)	19 (66)	163 (48)	.072
Race (white, %)	23 (79)	276 (81)	.780
Previous IV? (yes, %)	($n = 28$, 1 missing) 24 (86)	($n = 335$, 4 missing) 263 (79)	.368

[a]Wilcoxon rank sum test and chi-square, as appropriate.

Table II. *PBS Median (25th–75th percentile) by ADHD groups*

	ADHD $n = 28$ (1 missing)	Non-ADHD $n = 338$ (1 missing)	p-value[a]
Impulsivity	7 (4.0–8.5)	3 (2.0–5.0)	<.0001
Inattention	6 (5.0–9.0)	2 (1.0–4.0)	<.0001
Hyperactivity	6 (5.5–9.0)	3 (1.0–5.0)	<.0001

[a]Wilcoxon rank sum test.

(Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006). Samples were sent to an outside laboratory for analysis. The lab used in this study routinely monitors and reports intra-assay coefficient of variation (CVs) between 4.0% and 6.7% and inter-assay CVs between 7.1% and 9%. For this specific data set, duplicate analysis and controls were used to further establish reliability of the data set. The overall CV, this study sample duplicates, was 8.18% (McCarthy et al., 2009).

Observational Scale of Behavioral Distress-Revised

All IV insertions were videotaped and child behavioral distress was measured using the Observational Scale of Behavioral Distress-Revised (OSBD-R) behavioral coding system. Videotapes of the child's behavior 2 min before and 2 min after the procedure were divided into 10 s intervals and coded on the occurrence of eight behaviors: information seeking, cry, scream, restraint, verbal resistance, emotional support, verbal pain, and flail (Jay, Elliott, & Varni, 1986). Scores for each behavioral category were weighted to indicate the intensity of distress (e.g., scream is weighted more than cry). Finally a single, overall behavioral distress score was calculated by adding together the weighted values of each of the behaviors, with higher scores reflecting more distress.

Jay and colleagues (Jay et al., 1986) reported that internal consistency for the eight behavioral categories is 0.72 and for this study it was 0.75. Each tape was scored by one of three trained members of the research team, and 20% of the tapes were scored by all three for validity. Inter-rater CV for the total OSBD score was 15% [95% confidence interval (CI): 14–17%]. Inter-rater reliability as measured by the intraclass correlation coefficient was 0.98.

Procedure

Following approval by the Institutional Review Board of the three study hospitals, potential participants were contacted by letter prior to the child's scheduled appointment and were approached by research staff upon arrival at the clinic. Written informed consent was obtained from one or both parents and assent was obtained from children aged 7 years and older. All subjects received a topical anesthetic cream prior to IV placement. During the 30–60 min needed for the anesthetic cream to take effect, subjects responded to questions asked by the research assistant and the parents completed a questionnaire booklet that included demographic information and the PBS-30. Parents remained with their children during the procedure and were randomized to receive instruction in the use of distraction (treatment group) or to provide support to their child as usual during the procedure (control group, routine care).

The two clinic salivary cortisol samples were collected as described. Parents were provided with collection materials, and return mailing instructions for the two home cortisol samples. All IV procedures were videotaped and the 2 min prior to and 2 min following the IV insertion were scored later in 10 s intervals using the OSBD-R.

Data Analysis

Differences in cortisol levels in children with and without ADHD were analyzed using linear mixed model analysis for repeated measures. The fixed effects in the model included diagnosis group (ADHD vs. non-ADHD), distraction intervention, gender (male, female), and age group (4–6, 7–10 years), as the between subject effects, and location (clinic or home), and Time (1 or 2), as the within subject repeated measures effects. Subject within diagnosis group was the random effect. The model also included the two- and three-factor interactions of diagnosis group, location, and time as fixed effects. In addition, interaction effects of gender and age with ADHD were initially included, but were not statistically significant and were dropped from the model ($p > .48$ for gender; $p > .38$ for age).

Specific between-group comparisons of interest included testing for differences in mean cortisol between the ADHD and non-ADHD groups at each time point. Within-group comparisons included testing for changes in cortisol from Time 1 to Time 2 at clinic and home, and for differences in cortisol levels from clinic to home at each time point. These were performed using test of mean contrast, with the t-test statistic for the mean contrast computed from estimates of the fitted linear mixed model. For each set of hypotheses tested, p-values were adjusted using Bonferroni's method to account for the number of tests performed. For the comparison of mean cortisol level between ADHD and non-ADHD groups, which was done for home and clinic at each of the two times, Bonferroni adjustment was applied by multiplying the unadjusted p-value by 4 (test at 2 locations × 2 time points = 4 tests). The same adjustment was used in the test for the difference in cortisol levels between home and clinic (or between Times 1 and 2), within ADHD and non-ADHD at each of the two times (or at each location). For comparing cortisol responsivity (home to clinic change) between ADHD and non-ADHD groups at Times 1 and 2, adjustment was made for two tests. The p-values given in the Result section (and Table) corresponding to these tests are the Bonferroni adjusted p-values.

This analysis assumes that the data have a normal distribution. This was not the case for cortisol, which has a right skewed distribution. To normalize the data distribution, the log transformation was applied to the cortisol

values, and those data were used in the analysis. Estimates of the mean cortisol levels were calculated by back transformation of the mean of the log-transformed values from the fitted linear mixed model to the original scale. The differences in observable distress behaviors in response to an IV insertion between the ADHD and non-ADHD groups were analyzed by comparing the mean OSBD-R scores using the Wilcoxon rank sum test.

Results
Cortisol Baseline Levels and Responsivity

The fixed effects and the p-value for the test of the fixed effects from the fitted linear mixed model are presented in Table III. This fitted model showed that after adjusting for child's age, gender, and distraction intervention, there was a significant diagnosis * location interaction effect (p = .0001). This means the effect on cortisol levels from

home to clinic (i.e., cortisol responsivity) differed significantly between the ADHD and non-ADHD groups. The estimates from the fitted mixed model of the adjusted mean home and clinic cortisol levels for the ADHD and non-ADHD groups at Times 1 and 2 are shown in Table IV. Estimates of the linear contrasts corresponding to the research questions were computed and tested, and are summarized below for home samples, clinic samples, and home versus clinic samples. They are also presented in Table IV as percent changes in cortisol levels between home and clinic and Times 1 and 2.

Home Baseline Cortisol Samples
Baseline cortisol levels collected at home trended downward between Times 1 and 2, but were not statistically significantly, different in both the ADHD group (0.286 vs. 0.224; p = .858) and the non-ADHD group (0.202 vs. 0.188; p = .692). The children with ADHD had higher cortisol levels than the children without ADHD at both Time 1 (0.286 vs. 0.202) and Time 2 (0.224 vs. 0.188), although the differences were not statistically significant (p = .261 and p > .90, respectively).

Clinic Cortisol Samples
On the clinic day, children without ADHD had cortisol levels that increased significantly from Times 1 to 2 (14.5% change, p = .005). Children with ADHD had cortisol levels that essentially remained the same from Times 1 to 2 (0.7% change, p > .99). The children with ADHD had significantly lower cortisol levels compared to children without ADHD at Time 1 (0.184 vs. 0.261, p = .040) and Time 2 (0.186 vs. 0.299, p = .014).

Table III. *Test of Fixed Effects from the Fitted Linear Mixed Model for Cortisol*

Effect	Degrees of freedom	F-value	p-value
Diagnosis	(1, 345)	0.40	.527
Location	(1, 490)	0.07	.797
Time	(1, 278)	0.52	.473
Diagnosis *Location	(1, 490)	14.81	.0001
Diagnosis * Time	(1, 278)	1.50	.221
Location *Time	(1, 367)	3.08	.080
Diagnosis*Location* Time	(1, 367)	0.03	.873
Distraction intervention	(1, 353)	6.03	.014
Gender	(1, 354)	0.90	.344
Age	(1, 356)	0.03	.867

Table IV. *Clinic versus Home Cortisol Change by ADHD Groups, Adjusted for Distraction Intervention, Gender and Age*

	ADHD (n = 29)[†]			Non-ADHD (n = 339)[†]			ADHD versus non-ADHD Compare percent change clinic–home
	Clinic (IV)	Home	Percentarge change clinic–home	Clinic (IV)	Home	Percentage change Clinic–Home	
Time 1[a]	0.184 ± 0.024	0.286 ± 0.052	−35.6 ± 12.7% p = .105	0.261 ± 0.010	0.202 ± 0.010	29.1 ± 7.2% p < .0001	p = .002
Time 2[b]	0.186 ± 0.029	0.224 ± 0.044	−17.2 ± 18.5% p > .99	0.299 ± 0.014	0.188 ± 0.010	59.0 ± 9.7% p < .0001	p = .010
Percentage change Time 2–Time 1	0.7 ± 14.5% p > .99	−21.6% ± 15.3% p = .858		14.5 ± 4.8% p = .005	−7.1 ± 5.0% p = .692		

[†]For home–clinic changes, where there were paired observations for both home and clinic, the ADHD group had n = 16 at Time 1 and n = 14 at Time 2; the non-ADHD group had n = 205 for Time 1 and n = 197 for Time 2. For Times 1–2 changes, where there were paired observation for both Times 1 and 2, the ADHD group had n = 28 for clinic and n = 14 for home; the non-ADHD group had n = 334 for clinic and n = 181 for home.
[a]Time 1, clinic 0.184 versus 0.261, p = .040; Time 1, home 0.286 versus 0.202, p = .261.
[b]Time 2, clinic 0.186 versus 0.299, p = .014; Time 2, home 0.224 versus 0.188, p > .9.

Home Versus Clinic Cortisol Samples

Children without ADHD had higher cortisol levels on the clinic day when compared to the home day at both Time 1 (29.1% change, $p < .0001$), in response to the clinic visit, and Time 2 (59.0% change, $p < .0001$) in response to the IV insertion. In contrast, children with ADHD had lower cortisol levels on the clinic day when compared to the home day at both Time 1 (-35.6% change, $p = .105$) and Time 2 (-17.2% change, $p > .99$). The change in cortisol level from home to clinic differed significantly between ADHD and non-ADHD groups at both Time 1 ($p = .002$) and Time 2 ($p = .010$). This result was reflected in the significant test for diagnosis * location interaction ($p = .0001$). The ADHD children's cortisol levels decreased from home to clinic while the non-ADHD children's cortisol levels showed significant increases from home to clinic.

Observable Distress Behaviors, OSBD-R

The OSBD-R mean scores for children with and without ADHD were not significantly different (median of 1.7 for ADHD vs. 1.4 for non-ADHD; $p = .871$). To control for group age and gender differences, matched analysis of OSBD-R mean scores for boys aged 7 years and older were examined separately but were also not significant ($p = .402$).

Discussion

Results of this secondary data analysis suggest that baseline cortisol levels and cortisol responsivity to a stressful medical procedure such as an IV insertion differ between children with and without ADHD. On the baseline day, the non-ADHD group showed the typical pattern of cortisol levels that decreased over daytime hours. Children with ADHD did not display statistically significant differences in their cortisol levels compared to the non-ADHD children, although their cortisol levels appeared to be trending higher on the baseline day compared to children without ADHD. In response to the stress of attending clinic and having an IV inserted, the non-ADHD group displayed the expected increase in cortisol levels. In contrast, children with ADHD showed an atypical response to the stress of the clinic visit and IV insertion, with lower cortisol levels on the clinic day compared to the home day and essentially no change in cortisol levels in response to the stress of an IV needle stick on the clinic day. The results of this study are consistent with some previous studies that found different cortisol responses to stress in children with ADHD compared to children without ADHD (Kaneko et al., 1993; King et al., 1998; McBurnett et al., 2000; Randazzo et al., 2008; van de Wiel et al., 2004; van Goozen et al., 2000).

While children with and without ADHD differed in their cortisol levels, they did not differ in their behavioral responses to the IV insertion. Using the OSBD-R, it was found that there were no significant behavioral differences between the children with and without ADHD in response to the IV insertion suggesting that cortisol is measuring a difference in physiological responsivity as opposed to the behavioral response captured by the OSBD-R. These findings demonstrate the importance of using multiple measures in research to better understanding of the full range of the stress response in children and differences based on diagnoses.

The findings from this study add to the growing literature on cortisol baseline levels and responsivity in children with DBDs in general and more specifically children with ADHD. While there have been inconsistent findings (Alink et al., 2008; Shirtcliff & Essex, 2008), these results support findings that children with ADHD and other externalizing disorders to have blunted or hyporesponsivity in response to stressful situations (Blomqvist et al., 2007; Hastings et al., 2009; van Goozen et al., 2000). The unique contribution of this study, to the overall literature on cortisol in children with ADHD, is that this study measured cortisol response *in vivo*, in a clinical setting where an IV insertion occurs for a scheduled medical procedure and not during researcher-induced physical (i.e., running on a treadmill or jumping rope) or mental (i.e., academic testing or playing videogames) stress (Blomqvist et al., 2007; Hastings et al., 2009). Based on these results, hyporesponsivity to a stressor in children with ADHD occurs both in lab and natural settings. The children with ADHD in this study not only had a blunted cortisol response to a stressor compared to the other children, but also appeared to have higher cortiosl levels on nonstressful days compared to other children. Further research is needed to explain why these differences in cortisol levels occur in children with ADHD compared to other children, and what the impact is on children with ADHD.

Strengths of this study include the natural setting that it occurred in, the inclusion of both children with and without ADHD for comparison, and cortisol levels obtained on both the day of the stressor and a baseline day. Limitations of this study are primarily related to secondary data analyses. The diagnosis of ADHD was based only on parent report of the child's diagnosis and current use of medications. While scores on the PBS-30 supported the diagnoses, there was no way to confirm the diagnoses by medical criteria. It is possible that some children assigned to the ADHD group may not have met DSM-IV-TR diagnostic criteria, and some children in the comparison group may have had undiagnosed or unreported ADHD.

While previous studies have reported that blunted cortisol response is particularly evident in children with DBDs and ADHD, it was not possible to identify children in this study with both ADHD and comorbid conditions such as ODD, a limitation that has been noted in other studies (Shirtcliff & Essex, 2008). Another limitation is the small sample size of children with ADHD. Future studies specifically addressing the question of cortisol responsivity in children with ADHD would include a larger, more clearly defined sample and would allow for documenting co-morbid diagnoses.

While the findings of this study are valuable in further understanding the stress response in children with ADHD, these findings also provide important information for investigators and clinicians using cortisol as a measure of stress. These results, along with the results of other studies (Blomqvist et al., 2007; Hastings et al., 2009), indicate that the patterns of cortisol responsivity in children are not consistent, but can vary based on factors such as medical diagnoses and certain medications. In the primary study, to control for well-documented factors that impact salivary cortisol levels in all individuals, the sample collection process was standardized; factors such as recent dietary and medication intake were documented for consideration in analyses (Hanrahan et al., 2006). Initial data analyses in the primary study identified ADHD as the strongest explanatory variable of cortisol responsivity. When children with ADHD were removed from the analyses, other variables emerged explaining cortisol responsivity related to the stress of an IV insertion, such as parent perception and the timing of telling the child about the IV procedure (McCarthy et al., 2010b). Researchers who include cortisol as a measure of stress in pediatric research need to be aware of the potential for contradictory cortisol responses in these children. A process for the careful identification of children with ADHD and related comorbidities such as ODD is a critical step in data collection, and data may need to be analyzed controlling for these diagnoses.

Funding

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