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Blunted stress cortisol reactivity and failure to acclimate to familiar stress in depressed and sub-syndromal children

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Abstract

Depressed adults have shown blunted or elevated cortisol reactivity in response to various forms of psychosocial stress. However, there have been few studies of cortisol reactivity in children who had early onset depression or a history of depression during the preschool-school period. The present study utilized a laboratory stress paradigm and collected salivary cortisol from preschoolers at baseline (age 3–5) and 24-month follow-up (age 5–7). Repeated-measures MANOVAs were used to compare cortisol reactivity to mild stress between children with major depressive disorder (MDD), elevated symptoms of depression (sub-syndromal MDD), and healthy controls. For healthy children, a quadratic cortisol reactivity curve was found at baseline ($n=73$), which appeared flatter under similar stressful situations at follow-up ($n=14$), which may reflect acclimation to the paradigm. In contrast, children with MDD ($n=46$) and sub-syndromal MDD ($n=76$) showed a peak cortisol response to the novelty of lab arrival and then reduced and blunted responses to stressors at baseline. These cortisol responses persisted at follow-up in children with any history of MDD ($n=41$) or sub-syndromal MDD ($n=73$). These results suggest that the hypothalamic-pituitary-adrenal (HPA) axis shows a blunted response to stress and failed to acclimate to familiar stressful situations in depressed and sub-syndromal depressed children.

Keywords

Hypothalamic-pituitary-adrenal axis; Salivary cortisol; Child; Major depressive disorder; Preschool; High-risk

1. Introduction

Excessive secretion of cortisol (hypercortisolemia) is a well-established biological correlate of major depressive disorder (MDD) (Nestler et al., 2002; Pariante and Lightman, 2008), although its diagnostic utility has been minimized by its lack of specificity (Braddock, 1986; Gillespie and Nemeroff, 2005). Depressed individuals (at ages 8–78) show higher cortisol levels throughout circadian periods (Lopez-Duran et al., 2009; Stetler and Miller, 2011), at sleep onset (Kaufman et al., 2001), at pre-stress (Gotthardt et al., 1995; Young et al., 2000), and/or at post-stress (Heim et al., 2000b), compared to healthy controls. Alternatively,

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constant cortisol levels following stressful situations (blunted cortisol reactivity to stress), as opposed to a dynamic change in cortisol, have been reported in several studies of depressed adults, adolescents, and children (Burke et al., 2005; Lopez-Duran et al., 2009).

However, the issue of whether or not stress cortisol reactivity is altered in childhood/adolescent MDD has been less straightforward (Kaufman et al., 2001; Burke et al., 2005; Stetler and Miller, 2011). Only a few studies have examined cortisol reactivity to stress in young children (Lopez-Duran et al., 2009). Examining a pattern of cortisol reactivity in preschool onset MDD (PO-MDD) is particularly important, given recent findings illustrating long-term and deleterious psychological, biological, and social trajectories associated with PO-MDD (Luby et al., 2009b; Luby et al., 2009c). PO-MDD is a psychiatric disorder characterized by DSM-IV depressive criteria (except 2-week duration criterion) that are developmentally adjusted for age appropriate manifestations in some cases that occurs during the preschool ages (i.e., under the age of 6). A growing body of empirical data provides validation for PO-MDD (Luby et al., 2003b; Luby et al., 2006; Luby et al., 2009a; Luby et al., 2009c; Dougherty et al., 2011a), and several epidemiologic studies have detected depressed preschoolers (Egger and Angold, 2006; Lavigne et al., 2009; Wichstrom et al., 2011; Bufferd et al., 2012). (Luby and colleagues 2003a, 2004) reported that depressed preschoolers at ages 3–6 showed blunted cortisol reactivity, with elevations in cortisol levels, in contrast to healthy controls who exhibited a dynamic cortisol reactivity curve. Note that, unlike healthy adults, healthy preschoolers (Luby et al., 2003a; Dougherty et al., 2010; Dougherty et al., 2011b) and adolescents (Rao et al., 2008) showed a quadratic reactivity curve with two peaks of cortisol levels - upon arrival to the novel laboratory environment and at the end of a laboratory stress paradigm. Nevertheless, this quadratic reactivity curve was disrupted in PO-MDD (Luby et al., 2003a; Luby et al., 2004).

Cortisol levels among the children of depressed mothers, a group deemed to be at high risk for MDD, have also been examined. This high-risk group showed high circadian cortisol levels, or heightened cortisol reactivity to stress (Essex et al., 2002; Brennan et al., 2008; Feldman et al., 2009), depending on other psychological factors (Ashman et al., 2002; Dougherty et al., 2011b). Maternal MDD may impact an offspring's cortisol secretion through genetic mechanisms, as suggested by evidence that preschoolers with genotypes known to confer high risk for MDD (i.e., the short-5-HTTLPR and the Met-BDNF allele carriers) have been associated with elevations in cortisol under stress (Dougherty et al., 2010).

Therefore, cortisol reactivity appears to be blunted and/or heightened not only in children with MDD but also in those at high familial/genetic risk for MDD. However, it is unclear whether non-depressed children who are at a high risk for MDD due to their expression of sub-clinical symptoms (*sub-syndromal MDD*) show alterations in cortisol reactivity. In depressed adolescents and adults, blunted cortisol reactivity is more apparent as MDD severity increases (Burke et al., 2005; Harkness et al., 2011), suggesting that cortisol reactivity may be altered in sub-syndromal MDD, compared to non-depressed controls. There is a gap in the literature pertaining to cortisol reactivity in sub-syndromal MDD in childhood.

Furthermore, it also remains unclear how cortisol reactivity in children changes as a consequence of acclimation to stressful situations. According to allostatic load model, healthy individuals are expected to optimize a physiological response to adapt to the demands of the environment (Juster et al., 2010; McEwen and Gianaros, 2010). Based on this model, it is possible that healthy children may initially show a quadratic reactivity curve to new stressors at baseline assessment (as discussed earlier) but then show a diminished its reactivity to identical stressors at follow-up assessment due to acclimation to stressful

situations. In contrast, this pattern may depend on depression severity, such that depressed children may fail to optimize and adjust their cortisol response to the familiar stressful situations (i.e., lack of adaptation) (McEwen, 1998; Juster et al., 2010). To our knowledge, no studies have comprehensively examined a potential effect of MDD severity on cortisol reactivity at baseline and follow-up assessments. To clarify this issue, it is necessary to use a similar stress paradigm and assess cortisol reactivity across different waves.

Therefore, the present study aimed to address how stress cortisol reactivity over a 24-month period differed among children with current or past history of MDD, sub-syndromal MDD, and healthy status. To achieve this goal, cortisol reactivity to similar mild laboratory-induced stress was repeatedly assessed at two periods: baseline and 24 months later. We hypothesized that healthy children would initially show a quadratic cortisol reactivity during a baseline stress paradigm because of its novelty (Luby et al., 2003a; Luby et al., 2004; Rao et al., 2008) but show no cortisol reactivity during a follow-up stress paradigm because they have become familiar with it. In contrast, children with MDD and sub-syndromal MDD would show blunted or heightened cortisol reactivity at the baseline (Luby et al., 2003a; Luby et al., 2004; Burke et al., 2005; Lopez-Duran et al., 2009) and would show similar cortisol reactivity at the follow-up because of lack of adaptation (McEwen, 1998; Juster et al., 2010).

2. Methods

2.1. Participants

This investigation is a multi-method and multi-informant longitudinal study designed to examine the nosology, course, and psychophysiological correlates of PO-MDD. Children between 3.0 and 5.11 years old were recruited from pediatricians' offices, daycares, and preschools in the St. Louis metropolitan area, using the Preschool Feelings Checklist (PFC) (Luby et al., 1999; Luby et al., 2009c). Preschoolers with depressive symptoms were oversampled to obtain a large sub-sample of this group. Children with chronic medical illnesses, neurological problems, pervasive developmental disorders, language and/or cognitive delays were excluded. A total of $N = 306$ caregiver-child dyads agreed to participate at baseline (T1), $N = 271$ dyads completed the 12-month follow-up assessment (T2), and $N = 257$ dyads completed the 24-month assessment (T3); that is, $N = 257$ children had longitudinal data at T1, T2, and T3. The Institutional Review Board (IRB) at the Washington University School of Medicine approved all study procedures.

Salivary cortisol was obtained from children at T1 and T3: collection rates at both waves were 99%. As a result of our diagnostic criteria, exclusion criteria, and outlier assessment of children's cortisol data (discussed below), cortisol samples included in our analyses were: (1) $N = 195$ in the analysis of T1 cortisol within T1 group status, (2) $N = 122$ in the analysis of T3 cortisol within T3 group status, (3) $N = 194$ in the analysis of T1 cortisol within a history of group status, and (4) $N = 127$ within a history of group status.

2.2. Diagnostic assessment

The Preschool Age Psychiatric Assessment (PAPA) was used to assess age-appropriate symptom manifestations of Axis-I psychiatric disorders at T1, T2, and T3 (Egger et al., 1999, 2003). The PAPA is a semi-structured diagnostic parent informant interview designed for children aged 2.0–6.0, with established test-retest reliability (Egger et al., 2006). All interviews were administered by trained interviewers and audiotaped for later review and calibration by master coders. Primary caregivers were asked about their children's depressive symptoms within the last 6 months using 31 items in the PAPA MDD module. The total number of symptoms endorsed in this module was summed to provide a

measurement of children's MDD severity. All of the items in the MDD module map onto the nine 'core' DSM-IV MDD symptom criteria. MDD status was determined using a diagnostic algorithm that was consistent with DSM-IV criteria, with the exception of the 2-week duration criterion. The 2-week duration criterion was excluded from the MDD algorithm, based on data suggesting that it may not be applicable to preschoolers (Luby et al., 2003b; Gaffrey et al., 2011). Information about the durations in our MDD sample has been reported previously (Luby et al., 2009c). Diagnosis of co-morbid psychiatric disorders (i.e., mania, general anxiety disorder, posttraumatic stress disorder, separation anxiety disorder, attention deficit/hyperactivity disorder, oppositional defiant disorder, and/or conduct disorder) was also assessed using the PAPA.

2.3. MDD status

Children were grouped based on *T1 MDD status*, *T3 MDD status*, and *a history of MDD status*. For T1 and T3 status, children were categorized into the following groups: (1) *healthy* if they did not meet DSM-IV criteria for any psychiatric disorder and showed fewer than two DSM-IV MDD symptoms, (2) *sub-syndromal MDD* if they did not meet DSM-IV MDD criteria (with/without other psychiatric disorders) but showed two or more DSM-IV-defined MDD symptoms, or (3) *MDD* if they meet DSM-IV MDD criteria. Seven children in the analysis of T1 group status and nine children in the analysis of T3 group status were excluded because they met DSM-IV criteria for any psychiatric disorders without co-morbid MDD and showed fewer than two DSM-IV MDD symptoms.

For a history of MDD status classification, children were categorized into (1) *always-healthy* if they had remained healthy across the study waves (T1, T2, and T3), (2) *ever sub-syndromal MDD* if they had ever shown sub-syndromal MDD status (but not MDD) across any of the waves, or (3) *ever MDD* if they had ever developed MDD across any of the waves. Children who had not met criteria for sub-syndromal MDD or MDD but had met diagnostic criteria for other psychiatric disorders ($n = 8$ in the analysis of T1 cortisol; $n = 4$ in the analysis of T3 cortisol) were excluded from the analyses.

2.4. Procedure

Salivary samples were collected at T1 and T3 during the course of the laboratory assessment. Assessments started either in the morning (approximately 9:00 AM) or afternoon (approximately 1:00 PM), based on family availability. To obtain saliva, children were instructed to chew on a sterile cotton roll without salivary stimulant. Saliva was then frozen and stored in a vial under -20°C . Salivary cortisol levels were later measured using the Gamma Coat Cortisol Radioimmunoassay kit (DiaSovin, Stillwater, MN) at the Washington University General Clinical Research Center, St. Louis, MO.

The T1 and T3 assessments included three cortisol collections. The first cortisol collection took place upon laboratory arrival, which is thought to reflect stress in anticipation of the novel laboratory environment. Then, children were separated from their parent/caregiver to complete a comprehensive developmental, emotional, and diagnostic battery including multiple paradigms from the Laboratory Temperament Assessment Battery (Lab-TAB), an observational measure of temperament (Goldsmith et al., 1995). The Lab-TAB involves several tasks designed to be mildly stressful for children (Gagne et al., 2011). The second collection of cortisol occurred during the assessment, after children completed the following Lab-TAB tasks: "Transparent Box" task (at T1) or the "Storytelling" and "Picture Tearing" tasks (at T3). The third cortisol collection took place at the end of the assessment, after the following Lab-TAB tasks: "Not Sharing," "Box Empty," "No Candy," and "Impossible Circles" (at T1) or the "Wrong Gift" (at T3) (see Supplementary Material 1 for description of each Lab-TAB task). Because similar Lab-TAB stress paradigm was administered across

the three annual waves, by T3, subjects had become familiar with our laboratory environment and these types of stress paradigms.

Primary caregivers' reports of the number of stressful/traumatic life events experienced by the child at T1, T2, and T3 were also obtained using the PAPA (Egger et al., 1999, 2003). Information on life events was obtained using dichotomous questions that asked whether or not the child experienced any of 15 stressful events (e.g., new siblings) and 12 traumatic events (e.g., death of a relative).

2.5. Exclusion criteria

We excluded children who had lost a tooth, experienced a major acute stressor (e.g., car accident) within 24 hours of the assessment, used steroids, and/or had fever (body temperature ≥ 99.5 °F) because these experiences are known to alter cortisol (Luby et al., 2003a; Schreiber et al., 2006; Fisher et al., 2011). At T1, 82 children were excluded because of tooth loss ($n = 7$), recent acute stress ($n = 21$), steroid use ($n = 15$), fever ($n = 5$), and/or missing data ($n = 39$). At T3, 106 children were excluded because of tooth loss ($n = 39$), recent acute stress ($n = 33$), steroid use ($n = 20$), fever ($n = 5$), and/or missing data ($n = 21$).

2.6. Statistical analyses

A common logarithm was used to normalize skewed cortisol values. Independent-samples t -tests showed that $\log_{10}$ -transformed cortisol levels in the morning were significantly higher than those in the afternoon at T1 ($t(196) = 3.28$ for the first, $t(196) = 2.17$ for the second, $t(196) = 2.11$ for the third, $ps < 0.05$) and at T3 ($t(126) = 2.80$ for the first, $t(126) = 2.00$ for the second, $t(126) = 2.91$ for the third, $ps < 0.05$). Because of the differences in cortisol between morning and afternoon, we examined outliers separately for children in the morning versus afternoon assessment, using Tukey boxplots. Any children whose $\log_{10}$ -transformed cortisol values were more than 3 interquartile ranges (IQRs) above the third quartile or more than 3 IQRs below the first quartile were considered outliers (Frigge et al., 1989).

After removing outliers ($n = 3$ in T1 cortisol analyses; $n = 8$ in T3 cortisol analyses), four repeated-measures MANOVAs were performed with $\log_{10}$ -transformed cortisol as the dependent variable, cortisol collection sequence (first, second, and third) as the within-subjects variable, MDD group status (T1, T3, or history) as the between-subjects variable, and time of day (morning/afternoon), food intake prior to the assessment (yes/no), co-morbid psychiatric disorders (presence/absence), and the total number of stressful and traumatic life events as the control variables. The first analysis examined T1 cortisol reactivity in relation to T1 group status; the second analysis examined T3 cortisol reactivity in relation to T3 group status; finally, the third and fourth analyses examined T1 or T3 cortisol reactivity in relation to a history of group status. If an interaction was significant, Bonferroni-adjusted post hoc comparisons were used to identify specific differences.

As an additional analysis, we also run full information maximum likelihood (FIML) to impute missing cortisol data and then ran a three-way repeated-measures MANOVA where an assessment wave (T1 or T3) was additionally included. The purpose of this analysis was to check our MANOVA results after controlling Type II error rate, given that relatively many subjects had missing cortisol data at T1, T3, or both assessments due to our diagnostic criteria, exclusion criteria, outlier, etc. For detailed methods and results of these imputed data, see Supplementary Material 2.

3. Results

3.1. Background characteristics

Table 1 shows demographic and clinical characteristics for our child participants who were classified into either the healthy, sub-syndromal MDD, or MDD group at only T1 and had valid T1 cortisol data. The MDD group was older and had caregivers with lower income and education levels than the healthy group ($ps < 0.05$). However, children's age was not significantly correlated with cortisol at T1 ($p > 0.05$), and repeated-measures MANOVAs revealed that there were no differences in T1 cortisol between household income levels or caregiver's education levels. Because children's age and caregivers' income and education levels did not affect cortisol at T1, these variables were not considered as additional covariates in the subsequent analyses. Table 2 describes background characteristics for all children who were categorized into the healthy, sub-syndromal MDD, or MDD group at only T3 and had valid T3 cortisol data. There were no group differences in any demographic characteristics. Thus, no additional variables were controlled for in the analysis of T3 cortisol.

Table 3 summarizes background characteristics for all children who were always healthy or had ever shown sub-syndromal MDD or MDD at T1, T3, and/or T5 and who had valid cortisol data at least T1 or T3. The ever MDD group had more traumatic life events, more unmarried caregivers, and lower caregiver education levels than the ever sub-syndromal MDD and the always-healthy groups ($ps < 0.05$). Nevertheless, the average number of traumatic life events was not significantly correlated with cortisol at T1 and T3, and repeated-measures MANOVAs yielded that there were no differences in T1 and T3 cortisol between caregiver's marital status or education levels. Based on this, the average traumatic life events and caregivers' marital status and education levels were not included as additional covariates.

Tables 1–3 also confirms that the MDD (or ever MDD) group scored higher on MDD severity than the other two groups, and the sub-syndromal MDD (or ever sub-syndromal MDD) group showed a higher MDD severity score than the healthy (or always healthy) group ($ps < 0.01$). Co-morbidity rates in the MDD group were 67% at T1, 75% at T3, and 72% through all three assessments.

Finally, Table 4 shows the attrition analysis comparing background characteristics within subjects who had valid T1 cortisol data. Overall, children were more likely to drop out at T3 follow-up if they had lower family income and increased MDD severity at T1 ($ps < 0.05$). However, within each of the healthy, sub-syndromal MDD, and MDD groups at T1, there were no differences in any background characteristics between subjects included at T3 and those excluded at T3. None of the T1 groups showed a change in, at least, age, gender, household income, caregiver's education, caregiver's marital status, life events, and MDD severity at the follow-up.

3.2. Group status and cortisol reactivity at T1 baseline

After controlling for time of day, food intake, co-morbidities, and stressful and traumatic life events, the main effect of T1 cortisol collection sequence was significant (Wilks' $\lambda = 0.91$, $F(2,187) = 8.77$, $p < 0.01$; identical to results of adjusted univariate F -test). Nevertheless, the main effect of T1 group status was not significant. More importantly, the effect of T1 cortisol collection sequence significantly interacted with T1 group status (Wilks' $\lambda = 0.92$, $F(4,374) = 4.05$, $p < 0.01$; identical to results of adjusted univariate F -test) in a quadratic ($F(2,188) = 3.28$, $p < 0.05$) fashion (see Figure 1 for means and standard errors). Post hoc tests yielded that, although there were no statistically significant group differences in cortisol levels at any collections, there were group differences in patterns of cortisol

reactivity across the collections. Specifically, the healthy group showed a U-shaped cortisol reactivity curve; as Figure 2 illustrates, this group showed a significant decrease in cortisol between the first and second collections and then a significant increase between the second and third collections ($ps < 0.01$). In contrast, although the MDD and sub-syndromal MDD groups also showed a significant decrease in cortisol between the first and second collections ($ps < 0.01$), their cortisol levels did not change between the second and third collections and remained significantly lower than their initial levels ($ps < 0.05$).

3.3. Group status and cortisol reactivity at T3 follow-up

After time of day, food intake, co-morbidities, and life events were controlled for, there were no main effects of T3 cortisol collection sequence and T3 group status, and there was no significant interaction between them, on log10-transformed cortisol values.

3.4. History of group status and cortisol reactivity at T1 and T3

The first analysis examined T1 cortisol reactivity in relation to a history of group status, with controlling for time of day, food intake, co-morbidities, and life events. The main effect of T1 cortisol collection sequence was significant (Wilks' $\lambda = 0.93$, $F(2,187) = 7.62$, $p < 0.01$; identical to the results of the adjusted univariate F -test) in a U-shaped curve ($F(1,188) = 6.47$, $p < 0.05$). However, the main effect of a history of group status and the interaction between T1 cortisol collection sequence and a history of group status were not significant.

The next analysis examined T3 cortisol reactivity in relation to a history of group status, with controlling for time of day, food intake, co-morbidities, and life events. Note that the sample size in this analysis was smaller than that in the previous analysis of T1 cortisol reactivity and a history of group status because the number of valid cases for T3 cortisol reactivity was fewer than that for T1 cortisol reactivity. The main effects of the T3 cortisol collection sequence and a history of group status were not significant, but there was a significant interaction between T3 cortisol collection sequence and a history of diagnostic group status (Wilks' $\lambda = 0.92$, $F(4,240) = 2.56$, $p < 0.05$; identical to the results of the adjusted univariate F -test) in a linear ($F(2,121) = 4.32$, $p < 0.05$) fashion (see Figure 3 for means and standard errors). Post hoc tests showed that there were no group differences in cortisol levels at any of the collections, whereas there were group differences in patterns of cortisol reactivity at T3. Figure 4 illustrates that the always-healthy group did not show a change in cortisol levels across the T3 collections. In contrast, the ever MDD and sub-syndromal MDD groups showed reduced cortisol levels between the first and second collections ($ps < 0.01$) and then did not change between the second and third collections; they exhibited lower cortisol levels at the third collection than their initial levels ($ps < 0.05$).

4. Discussion

The present study examined cortisol reactivity across two mildly stressful laboratory assessments one year apart, among the three groups of children: MDD, sub-syndromal MDD, and healthy. At T1 (baseline), all children showed decreased cortisol levels between the first and second collections, but the patterns of T1 cortisol reactivity differed between the second and third collections, depending on T1 group status. That is, healthy children showed increased cortisol responses between the second and third collections, whereas children with MDD or sub-syndromal MDD showed no change in cortisol between those collections (see Figures 1 and 2).

At the T3 follow-up, when children were familiar with the laboratory environment and the stress paradigms, the patterns of cortisol reactivity differed from those at T1 as a function of group status history. Children who were always healthy did not show changes in cortisol

levels over the collections at T3 (see Figures 3 and 4), which was different from cortisol reactivity patterns shown in the T1 healthy group. In contrast, children with ever MDD or sub-syndromal MDD status at any wave showed reduced cortisol levels between the first and second collections and then showed no change in cortisol between the second and third collections at T3 (see Figures 3 and 4), which was the same as cortisol reactivity patterns shown in the T1 MDD or sub-syndromal MDD groups.

4.1. Cortisol reactivity in healthy children

Healthy children initially showed a U-shaped cortisol reactivity curve in response to mild stress, which was consistent with prior findings in similarly aged cohorts (Luby et al., 2003a; Dougherty et al., 2010; Dougherty et al., 2011b). Therefore, for normally developing children, exposure to the novel laboratory environment was experienced as a stressor, as were exposures to the frustrating Lab-TAB tasks. Results of the follow-up analysis demonstrated that the cortisol reactivity curve became flattened (compared to baseline). Note that this was evident only in children who were always healthy across all of the study waves. It is possible that the change in cortisol reactivity between T1 and T3 within healthy children may reflect acclimation to the familiar stressful situations. That is, normally developing children may show that the hypothalamic-pituitary-adrenal (HPA) axis functioning responds to stress sensitively at T1 baseline, and then their HPA axis system adapts to the familiar stress at T3 follow-up. The rationale behind this idea has suggested that healthy individuals are capable of modulating their physiological response so that they can adapt to the demands of the environment (Juster et al., 2010; McEwen and Gianaros, 2010). In contrast, if this healthy capability is disturbed (e.g., due to MDD episodes), cortisol response under the familiar stressful situations may reflect what is called 'lack of adaptation,' reflecting a failure to habituate to stressors (McEwen, 1998; Juster et al., 2010). Nevertheless, our findings should be interpreted with caution due to the small size of the always-healthy group ($n = 14$), raising the possibility of a Type II error. In addition, longitudinal analysis of cortisol reactivity within healthy subjects is further needed.

4.2. Cortisol reactivity in children with MDD/sub-syndromal MDD

Depressed children also experienced stress from the novel environment and then decreased their cortisol levels. However, unlike healthy children, their cortisol did not increase following mild stressors, a pattern that has been referred to as blunted cortisol reactivity (Burke et al., 2005). The pattern of these cortisol responses again emerged at the follow-up in children with any prior history of MDD. Notably, this is different from the patterns of cortisol reactivity evident in healthy children, who showed changes between T1 and T3, possibly due to their acclimation to the stressors. Thus, childhood MDD may be characterized by (1) persistently blunted cortisol reactivity to mild stressors and (2) a failure to acclimate to familiar stressful situations. Interestingly, evidence for similarly altered cortisol responses was also found in children with sub-syndromal MDD, suggesting that blunted cortisol reactivity and acclimation failure may be manifest even in prodromal MDD.

Blunted cortisol reactivity to stress has been consistently reported in MDD across the age span (Burke et al., 2005; Lopez-Duran et al., 2009), especially in severe MDD (Harkness et al., 2011). The mechanism underlying blunted cortisol reactivity may result from down-regulation of the HPA axis (Gunnar and Vazquez, 2001). While individuals are capable of adapting to acute stress by activating HPA axis activity, chronic stress repeatedly over-activates and eventually impairs the HPA axis (McEwen and Stellar, 1993; Juster et al., 2010). As Table 3 illustrates, because depressed children often report more traumatic life events than healthy controls (Luby et al., 2009c), they may experience HPA axis down-regulations, resulting in altered cortisol responses to stressful situations, such as blunted responses and a lack of acclimation to familiar stressors.

While blunted cortisol reactivity in depressed children in our study was consistent with previous findings, our results showing that cortisol levels never heightened following stressors were not expected. In fact, prior studies employing the same stress paradigm showed that depressed preschoolers had persistently elevated cortisol levels in response to stressors (Luby et al., 2003a; Luby et al., 2004). The discrepancy in these findings may be the result of different methodologies. Compared to the prior studies assessing cortisol at 30-minute intervals, our study sampled cortisol at 70-minute intervals, which might be long enough to allow depressed children to recover from each stressor. Future research designs should assess multiple cortisol levels at shorter time intervals to clarify this discrepancy.

Moreover, children in our study did not show evidence of hypercortisolemia, often reported in MDD (Kaufman et al., 2001; Lopez-Duran et al., 2009; Stetler and Miller, 2011). Meta-analysis has suggested that stress cortisol reactivity levels are higher in MDD than healthy controls (Lopez-Duran et al., 2009), but this may be marked only in the afternoon (Burke et al., 2005). The present study collected cortisol samples in the morning and afternoon, which might have confounded this issue. While we adjusted all analyses for assessment time, mixing cortisol samples across different assessment times was a limitation to our study. Or, there may be developmental differences in cortisol reactivity because of delayed development of neurotransmitter content and synthetic activity (Kaufman et al., 2001). In fact, basal and diurnal cortisol levels vary between childhood, adolescence, and adulthood (Kaufman et al., 2001; Shirtcliff and Essex, 2008; Shirtcliff et al., 2012), which may affect developmental variations in cortisol levels in response to stress.

Alternatively, the extant literature suggests that hypercortisolemia may be specific to melancholic/anhedonic MDD in children (Luby et al., 2003a; Luby et al., 2004), whereas atypical MDD or MDD with co-morbid stress-related disorders may be characterized by low levels of cortisol (hypocortisolemia) (Heim et al., 2000a; Fries et al., 2005). Furthermore, adverse experiences in childhood may moderate the relationship between high MDD severity and low cortisol reactivity to stress in adolescents (Harkness et al., 2011), as well as school-aged children (Badanes et al., 2011). Future research should compare cortisol reactivity between these different subtypes of MDD accounting for key variables.

An important limitation of our study was that cortisol was not sampled at the same time of day across all subjects and all study waves. Assessing cortisol at different times of day across the study waves led to considerable within-subjects variability in longitudinal cortisol levels, making it necessary to conduct cortisol analyses at T1 and T3 separately. Future studies should be designed to collect saliva samples in the same manner across all subjects, as well as across all waves of the study, whenever feasible. Furthermore, another limitation of the present study was to sample cortisol only three times over 140 minutes at T1 and T3, which was less frequent than other cortisol studies (Rao et al., 2008; Kryski et al., 2011), and did not include a recovery period in our assessment. If, for instance, cortisol was assessed every 10 minutes after each stressful situation or assessed during a recovery period, the patterns of cortisol responses may have been different. Another study limitation was due to attrition between T1 and T3. In particular, the always-healthy group had only $n = 14$ in T3 analysis of cortisol, leading to low statistical power. While attrition in longitudinal studies is common, our results must be interpreted with caution due to the attrition. Finally, we did not include a psychiatric control group (e.g., anxiety disorders without MDD) or temperament control group (e.g., shyness without MDD) in order to clarify whether our findings in depressed children were specific to MDD. We controlled for comorbidities in all analyses, but future research should also compare cortisol reactivity between MDD and the additional control groups.

The present study suggests that blunted cortisol reactivity to stress might be evident in early childhood forms of MDD. Moreover, persistently blunted cortisol responses to mild stressors, as well as a failure of acclimation to familiar stressful situations, may be early markers of childhood MDD and sub-syndromal MDD although further longitudinal research is needed. As such, they may provide clues to early developmental pathophysiology process in the disorder and may also serve as future targets for early intervention or prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Patterns of baseline (T1) cortisol reactivity in children with baseline group status. Each group size was following: $n = 73$ healthy children, $n = 76$ sub-syndromal MDD children, and $n = 46$ MDD children. Error bars represent standard error of the mean.

Figure 2.

Baseline (T1) cortisol changes in children with baseline group status. Each group size was following: $n = 73$ healthy children, $n = 76$ sub-syndromal MDD children, and $n = 46$ MDD children. Error bars represent standard error of the mean. *Bonferroni-adjusted $p < 0.05$; **Bonferroni-adjusted $p < 0.01$.

Figure 3.

Patterns of 24-month follow-up (T3) cortisol reactivity in children with longitudinal group status. Each group size was following: $n = 14$ healthy children, $n = 73$ sub-syndromal MDD children, and $n = 41$ MDD children. Error bars represent standard error of the mean.

Figure 4.

24-month follow-up (T3) cortisol changes in children with longitudinal group status. Each group size was following: $n = 14$ healthy children, $n = 73$ sub-syndromal MDD children, and $n = 41$ MDD children. Error bars represent standard error of the mean. *Bonferroni-adjusted $p < 0.05$; **Bonferroni-adjusted $p < 0.01$.

Table 1

Characteristics of T1 Baseline Group Status ($N = 195$)

Variable	Healthy ($n = 73$)	Sub-syndromal MDD ($n = 76$)	MDD ($n = 46$)	F or χ^2
Age in years	4.29 (0.79)	4.43 (0.79)	4.69 (0.81)	3.56*
Gender [/]				
Girl	35	38	16	2.92
Boy	38	38	30	
Ethnicity [/]				
European American	47	42	22	5.62
African American	18	26	17	
Hispanic	0	1	0	
Mixed or other	8	7	7	
Total household income [/]				
\$20,000	12	15	13	16.15*
\$20,001–\$40,000	4	11	11	
\$40,001–\$60,000	13	14	6	
\$60,001	37	32	11	
Refused to answer	7	4	5	
Caregiver's education [/]				
High school diploma	8	12	9	18.36**
Some college	20	30	25	
4-year college degree	16	19	6	
Graduate or higher education	29	15	6	
Caregiver's marital status [/]				
Married	50	44	18	16.42
Widowed	1	0	0	
Separated	1	1	2	
Divorced	3	6	7	
Never married	17	25	19	
Refused to answer	1	0	0	
Number of life event types (<i>SD</i>)				
Stress	3.22 (1.99)	3.37 (1.86)	3.35 (1.85)	0.13
Trauma	1.26 (1.04)	1.29 (1.25)	1.61 (1.29)	1.40
Stress & Trauma	4.48 (2.55)	4.66 (2.45)	4.96 (2.62)	0.50

Variable	Healthy (<i>n</i> = 73)	Sub-syndromal MDD (<i>n</i> = 76)	MDD (<i>n</i> = 46)	<i>F</i> or ²
MDD severity (<i>SD</i>)	0.81 (0.97)	3.87 (1.56)	8.41 (3.71)	180.33**

Note: Subjects who exhibited a psychiatric disorder and less than two MDD symptoms at T1 (*n* = 7) were not shown in this table because they were not included in the analysis.

I Data are presented as frequency.

* *p* < 0.05;

** *p* < 0.01.

Table 2

Characteristics of T3 Follow-Up Group Status ($N=122$)

Variable	Healthy ($n = 52$)	Sub-syndromal MDD ($n = 54$)	MDD ($n = 16$)	F or χ^2
Age in years (<i>SD</i>)	6.50 (0.76)	6.31 (0.84)	6.81 (0.77)	2.51
Gender [/]				
Girl	24	27	4	3.16
Boy	28	27	12	
Ethnicity [/]				
European American	34	32	11	8.84
African American	14	15	2	
Hispanic	0	0	1	
Mixed or other	4	7	2	
Total household income [/]				
\$20,000	5	9	2	8.88
\$20,001–\$40,000	8	7	1	
\$40,001–\$60,000	10	8	4	
\$60,001	27	30	7	
Refused to answer	2	0	2	
Caregiver's education [/]				
High school diploma	5	5	2	1.64
Some college	13	19	5	
4-year college degree	17	14	4	
Graduate or higher education	17	16	5	
Caregiver's marital status [/]				
Married	34	30	8	2.89
Widowed	0	0	0	
Separated	0	0	0	
Divorced	5	4	2	
Never married	12	17	5	
Refused to answer	1	3	1	
Number of life event types (<i>SD</i>)	1.29 (0.89)	1.67 (1.64)	1.69 (1.40)	1.23
Stress				
Trauma	0.54 (0.64)	0.63 (0.85)	0.44 (0.63)	0.48
Stress & Trauma	1.83 (1.12)	2.30 (1.86)	2.13 (1.71)	1.21
MDD severity (<i>SD</i>)	0.87 (0.97)	3.63 (1.52)	8.13 (2.68)	144.99**

Note: Subjects who exhibited a psychiatric disorder and less than two MDD symptoms at T3 ($n = 9$) were not shown in this table because they were not included in the analysis.

Data are presented as frequency.

* $p < 0.05$;

** $p < 0.01$.

Table 3

Characteristics of a History of Group Status ($N = 195$)

Variable	Always healthy ($n = 27$)	Ever Sub- syndromal MDD ($n = 99$)	Ever MDD ($n = 69$)	F or χ^2
Age in years	4.32 (0.85)	4.34 (0.76)	4.62 (0.81)	2.99
Gender [/]				
Girl	16	48	27	3.43
Boy	11	51	42	
Ethnicity [/]				
European American	19	55	34	7.41
African American	6	35	22	
Hispanic	0	0	1	
Mixed or other	2	9	12	
Total household income [/]				
\$20,000	4	16	20	15.02
\$20,001–\$40,000	2	10	14	
\$40,001–\$60,000	4	20	10	
\$60,001	16	45	19	
Refused to answer	1	8	6	
Caregiver's education [/]				
High school diploma	3	15	11	16.49*
Some college	8	32	36	
4-year college degree	3	25	12	
Graduate or higher education	13	27	10	
Caregiver's marital status [/]				
Married	21	60	29	24.32**
Widowed	1	0	0	
Separated	0	1	3	
Divorced	1	5	10	
Never married	4	33	26	
Refused to answer	0	0	1	
Average number of life event types (SD)	2.19 (1.37)	2.31 (1.51)	2.52 (1.30)	0.69
Stress	0.68 (0.43)	0.82 (0.66)	1.12 (0.99)	4.40*
Trauma	2.87 (1.64)	3.13 (1.81)	3.63 (1.99)	2.25
Average MDD severity (SD)	0.43 (0.46)	2.59 (1.28)	6.38 (3.42)	90.37**

Note: Each group size indicates the maximum number of subjects in the group whose cortisol data were valid at least T1 or T3. Subjects who were not categorized into the healthy, sub-syndromal MDD, or MDD group at any assessment waves were not shown in this table because they were not included in the analysis. Values for all variables, except for life events and MDD severity, are based on T1 baseline assessment. Scores for life events and MDD severity were averaged over T1, T2, and T3.

I Data are presented as frequency.

* $p < 0.05$;

** $p < 0.01$.

Table 4Characteristics of Subjects Included and Excluded in T3 Cortisol Analysis ($N = 202$)

Age in years (<i>SD</i>)		Included at T3 (<i>n</i> = 89)	Excluded at T3 (<i>n</i> = 113)	<i>F</i> or ²
Age in years (<i>SD</i>)		4.51 (0.84)	4.36 (0.76)	1.72
Gender ^I	Girl	39	54	0.32
	Boy	50	59	
Ethnicity ^I	European American	55	58	2.83
	African American	25	39	
	Hispanic	0	1	
	Mixed or other	9	15	
Total household income ^I	\$20,000	10	31	11.47 [*]
	\$20,001–\$40,000	12	14	
	\$40,001–\$60,000	19	16	
	\$60,001	43	40	
	Refused to answer	5	12	
Caregiver's education ^I	High school diploma	10	22	5.56
	Some college	31	46	
	4-year college degree	19	22	
	Graduate or higher education	29	23	
Caregiver's marital status ^I	Married	53	61	2.88
	Widowed	0	1	
	Separated	2	2	
	Divorced	7	10	
	Never married	26	39	
	Refused to answer	1	0	
Life events (<i>SD</i>)	Stress	3.04 (1.73)	3.48 (1.99)	2.64
	Trauma	1.25 (1.03)	1.42 (1.29)	1.12
	Stress & trauma	4.29 (2.12)	4.90 (2.75)	3.00
MDD severity (<i>SD</i>)		3.12 (2.73)	4.18 (4.05)	4.44 [*]

Note: This table includes all subjects who had valid T1 cortisol data. Values for all variables are based on T1 baseline assessment.

^IData are presented as frequency.

^{*} $p < 0.05$;

^{**} $p < 0.01$.