

Original Investigation

Intervention Effects on Diurnal Cortisol Rhythms of Child Protective Services–Referred Infants in Early Childhood Preschool Follow-up Results of a Randomized Clinical Trial

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IMPORTANCE A number of interventions for at-risk children have shown benefits for children's hypothalamic-pituitary-adrenal axis activity immediately after treatment. It is critical to examine whether such changes are maintained over time, given that physiological regulation is implicated in later mental and physical health outcomes.

OBJECTIVE To examine whether differences in diurnal cortisol production between children receiving the active parenting intervention and children in the control group persisted at a preschool follow-up (approximately 3 years following intervention).

DESIGN, SETTING, AND PARTICIPANTS Between-subject comparison of cortisol patterns among 2 groups of children (experimental and control groups) involved with Child Protective Services following allegations of neglect. The participants included 115 children (43.5% female) between 46.5 and 69.6 months of age (mean [SD], 50.73 [4.98] months) who had been previously randomly assigned to either the Attachment and Biobehavioral Catch-up (ABC) intervention (n = 54) or the control intervention (n = 61).

INTERVENTIONS The experimental ABC intervention focused on 3 aims: increasing parental nurturance to child distress, increasing synchronous interactions, and decreasing frightening parental behavior. The control intervention provided educational information about child development to parents. Both interventions were manualized and involved 10 sessions implemented by a trained parent coach in the families' homes or other places of residence.

MAIN OUTCOMES AND MEASURES Salivary cortisol samples collected at waking and bedtime for children on 3 separate days.

RESULTS Analyses revealed significant differences in cortisol production at the preschool follow-up, such that children in the ABC intervention group showed more typical patterns of cortisol production than children in the control intervention group. Specifically, children in the ABC group exhibited higher mean (SD) log-transformed morning levels than children in the control group (-0.87 [0.45] vs -1.05 [0.43] $\mu\text{g/dL}$, respectively [to convert to nanomoles per liter, multiply by 27.588]; $\beta = 0.18$; $P = .03$). Bedtime cortisol levels did not differ significantly between the ABC and DEF groups (mean [SD], -1.19 [0.49] vs -1.17 [0.48] $\mu\text{g/dL}$, respectively; $\beta_{01} = -0.01$; $P = .87$). Those in the ABC group showed a steeper decline in cortisol across the day (mean, -0.31 $\mu\text{g/dL}$) than those in the control group, who showed a blunted cortisol rhythm (mean, -0.12 $\mu\text{g/dL}$) ($\beta = -0.19$; $P = .02$).

CONCLUSIONS AND RELEVANCE Differences in cortisol production between the experimental and control groups persisted at the preschool follow-up and resembled differences initially observed 3 months following intervention. This is encouraging evidence that the ABC intervention for Child Protective Services–referred children may have long-lasting effects on a physiological stress system critical for health and adjustment.

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Children experiencing parental maltreatment face dual harm due to frequent stressful interactions with their family¹ and lack of access to the stress-reducing benefits of high-quality parental care.² Thus, exposure to parental maltreatment can disrupt normative developmental processes and is a major risk factor for psychological and physical health problems.^{1,3} Chronic activation of physiological stress systems, such as the hypothalamic-pituitary-adrenal (HPA) axis, is considered one of the mediating mechanisms for the unfolding of some of these disease processes.⁴ Indeed, there is accumulating evidence that HPA axis functioning is altered in children experiencing adverse care.⁵⁻⁹ Disrupted patterns of HPA activity are associated with numerous behavioral and emotional problems in children, including both internalizing and externalizing symptoms.¹⁰⁻¹³

In humans, the end product of the HPA axis is cortisol, a steroid hormone that follows a diurnal rhythm—increasing early in the morning, peaking approximately 30 minutes after waking, and declining throughout the day, reaching near-zero levels at night.¹⁴ This diurnal pattern is not present at birth but begins to emerge around 3 months of age^{15,16} and is fully entrained to daylight cycles by age 2 years.¹⁷ Children experiencing social deprivation or maltreatment show departures from this typical profile of diurnal HPA activity, suggestive of chronic stress. For instance, a flattened diurnal cortisol slope with blunted morning cortisol levels is increasingly recognized as a hallmark signature of chronic stress in children and adults experiencing adversity.¹⁸ This pattern has been noted across a wide range of adverse early-life exposures, including child maltreatment, foster care placement, and institutional (ie, orphanage) rearing.^{5,6,8,13} Meta-analytic reviews of the literature suggest that the initial response to severe, acute stress is often a heightened cortisol level¹⁸; however, as adversity becomes more chronic, negative feedback mechanisms can lead to downregulation at various levels of the HPA axis (eg, reduced synthesis of one of its secretagogues or decreasing number of receptors reading their signal¹⁹), which manifests as blunted cortisol levels and flattened diurnal slopes.¹⁸⁻²⁰ This phenomenon has been referred to as *hypocortisolism*^{13,19,20} and is a potential marker of developmental risk.¹³ Despite these average meta-analytic results, dysregulation of the HPA axis after chronic stress can also take the form of elevated cortisol levels or heightened cortisol reactivity compared with nonaffected controls, especially in adults who also develop depression.²¹ Much of this literature is based on adult studies, with findings in pediatric samples being scarcer and more mixed. For instance, some studies have reported an elevated morning cortisol level or a greater total diurnal cortisol level among subgroups of children who experience early life stress relative to comparison children.^{8,22,23} These children may later develop hypocortisolism, as some longitudinal studies have suggested.²⁴

There is emerging evidence that interventions with at-risk children and their parents can normalize children's diurnal cortisol production following initially blunted profiles.^{7,10,25-27} Such parenting interventions have also improved child socioemotional outcomes and reduced parental stress.^{25,28,29} In other studies, early parenting interventions have prevented the progressive blunting of morning cortisol levels

observed over time in the untreated or control group^{30,31} or reduced daily cortisol output in children at risk for externalizing disorders who carried the dopamine receptor D4 (*DRD4*) 7-repeat allele.³² Regardless of whether adversity is initially associated with elevated or blunted diurnal cortisol slopes in children, parenting interventions result in cortisol patterns in at-risk samples that approximate those of low-risk samples after treatment (for a review, see the article by Slopen et al²⁶).

One of the interventions designed to enhance behavioral and biological regulation in young children at risk for parental neglect is the Attachment and Biobehavioral Catch-up (ABC) intervention.^{25,27} This 10-week program helps parents become more synchronous and nurturing as well as less frightening. Recent randomized clinical trials have shown that this intervention improves child attachment security²⁹ and normalizes diurnal cortisol production in children at risk for parental neglect when assessed within a few months of the intervention.²⁷ However, the extent to which this normalizing effect persists later in development is currently unknown and represents a major gap in this intervention literature.

A critical question about any intervention's success is whether its effects are long lasting or simply transient. Sustained effects of the ABC intervention on cortisol regulation would reinforce inferences regarding the effects of parenting on child stress physiology. This is important given that correlational studies cannot disentangle effects of parenting from gene-environment correlations (ie, maltreating parents may also transmit genes predisposing to abnormal HPA functioning to their children). Additionally, some studies with institutionalized children experiencing neglect and transitioning into nurturing homes through adoption show an initial normalization of diurnal cortisol slopes,¹⁰ but other studies show that years later dysregulated cortisol patterns are present again when compared with nonadopted children.³³ This raises the possibility that early adversity may have programming effects on the HPA axis that become apparent with time and development, similar to what has been observed in experimental studies in primates and rodents.³⁴ The follow-up assessment in the present study is ideally suited for testing the possibility of long-lasting reversals in HPA functioning.

For these reasons, the goal of this study was to test the lasting effects of the ABC intervention by conducting a preschool follow-up. This assessment compared diurnal cortisol rhythms in children who had been randomly assigned to receive the 10-week ABC intervention with those of children in the control intervention condition.

Methods

Participants

Primary analyses included 115 children with a history of Child Protective Services (CPS) involvement in infancy. All families had been reported to CPS due to allegations of neglect and were referred to receive services as part of a city-level program designed to divert children from foster care. Children were between 46.5 and 69.6 months of age at the time of the preschool follow-up of cortisol regulation (mean [SD] age, 50.73

Table 1. Child Demographic Characteristics

Characteristic	ABC Intervention (n = 54)	DEF Control Intervention (n = 61)
Sex, No. (%)		
Male	32 (59)	33 (54)
Female	22 (41)	28 (46)
Race/ethnicity, No. (%)		
White	4 (7)	6 (10)
African American	37 (69)	38 (62)
Hispanic	1 (2)	11 (18)
Biracial	12 (22)	6 (10)
Age, mo		
Mean (SD)	51.5 (5.4)	50.1 (4.5)
Range	46.5-66.9	46.6-69.6
Postintervention follow-up, mo		
Mean (SD)	35.4 (8.7)	36.1 (6.66)
Range	15.9-53.9	20.9-46.7

Abbreviations: ABC, Attachment and Biobehavioral Catch-up; DEF, Developmental Education for Families.

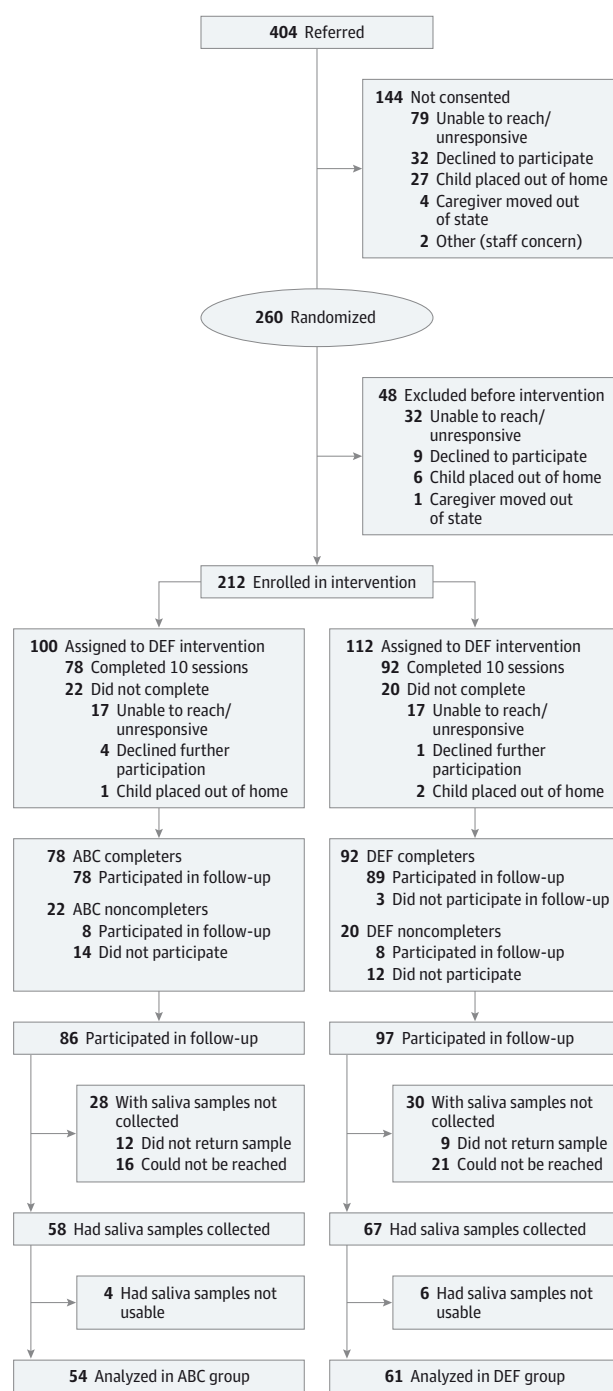
[4.98] months). **Table 1** shows demographic information about the sample. The sample included 3 pairs of siblings, with both children within the targeted age range at the time of referral. The study was approved by the University of Delaware Institutional Review Board. Written informed consent was obtained from parents.

Procedures

Agency workers referred parents to the research study if children met the following inclusion criteria at the time of CPS involvement: (1) younger than 2 years, and (2) living with a birth parent. Following referral, parents were contacted and invited to participate. On enrollment, a project coordinator randomly assigned participants to the experimental or control intervention condition using a randomly generated number sequence (with group assignment based on even vs odd digits). Following preintervention research visits, families completed the intervention and then participated in yearly follow-up visits. Recruitment began January 6, 2006, and preschool follow-up visits were conducted through July 15, 2012.

Figure 1 displays the CONSORT flow diagram. As shown in Figure 1, 183 participants (86.3% of those enrolled in the intervention phase) were retained during the postintervention phase of the study. To obtain cortisol data, 6 saliva samples were collected from each of 125 children at the time of the preschool follow-up, described later. Of these 125 children, 10 provided samples that were not usable (7 had insufficient volumes of saliva for all samples, 1 had insufficient volumes of saliva for 5 samples and the other sample was excluded as an outlier, and 2 had all samples excluded as outliers), resulting in a sample size of 115 children for this study. For the remaining 58 children (of the 183 retained for follow-up), cortisol data were not available because parents did not return the samples (n = 21) or because parents could not be reached to schedule a follow-up visit at that time (n = 37).

Figure 1. CONSORT Flow Diagram



Numbers of children enrolled in the Attachment and Biobehavioral Catch-up (ABC) intervention (n = 100) and in the Developmental Education for Families (DEF) control intervention (n = 112) following completion of preintervention baseline visits are reported. However, participants were randomly assigned to a group on consenting (N = 260; ABC intervention, n = 129; DEF intervention, n = 131), at which time the intervention group sample sizes were more similar. Follow-up numbers include participants seen for any postintervention visits. Of those enrolled in the ABC group, 86.0% were retained for follow-up; of those who completed the ABC intervention, 100% were retained for follow-up. Of those enrolled in the DEF group, 86.6% were retained for follow-up; of those who completed the DEF intervention, 96.7% were retained for follow-up.

Table 2. Descriptive Statistics

		Mean (SD) [Range]		
Intervention	No.	Time of Sample ^a	Cortisol, µg/dL	Log-Transformed Cortisol, µg/dL
ABC intervention (n = 54)				
Waking				
Day 1	42	8:12 (1:12) [5:44-11:00]	0.21 (0.18) [0.004-0.89]	-0.85 (0.44) [-2.40 to -0.05]
Day 2	43	8:25 (1:15) [6:30-11:32]	0.19 (0.19) [0.004-0.96]	-0.91 (0.44) [-2.40 to -0.02]
Day 3	43	8:12 (1:10) [6:15-11:14]	0.19 (0.15) [0.010-0.73]	-0.88 (0.41) [-2.00 to -0.14]
Bedtime				
Day 1	47	9:14 (1:16) [7:00-12:54]	0.11 (0.13) [0.004-0.67]	-1.24 (0.56) [-2.40 to -0.18]
Day 2	40	9:02 (1:01) [7:00-12:00]	0.13 (0.19) [0.004-1.00]	-1.21 (0.63) [-2.40 to 0.00]
Day 3	42	9:10 (1:05) [7:15-12:54]	0.13 (0.13) [0.004-0.47]	-1.13 (0.53) [-2.40 to -0.33]
DEF control intervention (n = 61)				
Waking				
Day 1	52	8:34 (1:20) [6:18-11:45]	0.20 (0.24) [0.004-1.04]	-0.94 (0.50) [-2.40 to 0.02]
Day 2	52	8:18 (1:06) [6:00-10:02]	0.17 (0.16) [0.004-0.83]	-1.02 (0.58) [-2.40 to -0.08]
Day 3	54	8:33 (1:16) [6:30-11:59]	0.14 (0.14) [0.004-0.68]	-1.10 (0.56) [-2.40 to -0.17]
Bedtime				
Day 1	55	9:17 (1:08) [6:30-12:00]	0.12 (0.10) [0.004-0.40]	-1.11 (0.45) [-2.40 to -0.40]
Day 2	52	9:16 (0:47) [7:45-11:00]	0.12 (0.12) [0.004-0.48]	-1.16 (0.55) [-2.40 to -0.32]
Day 3	52	9:17 (1:02) [7:30-12:00]	0.12 (0.12) [0.004-0.58]	-1.16 (0.56) [-2.40 to -0.23]

Abbreviations: ABC, Attachment and Biobehavioral Catch-up; DEF, Developmental Education for Families.

SI conversion factor: To convert cortisol to nanomoles per liter, multiply by 27.588.

^a Time of sample is expressed as AM for all waking samples and PM for all bedtime samples.

Interventions

Both interventions were manualized. They involved 10 sessions implemented by trained parent coaches in families' homes or other places of residence.

ABC Experimental Intervention | The ABC intervention had 3 primary aims: increasing nurturance to distress, increasing synchronous interactions, and decreasing frightening parental behavior. Sessions were guided by a manual with each focusing on 1 of the 3 ABC targets. Specifically, sessions 1 and 2 focused on nurturance, 3 and 4 on synchrony (or following the lead), and 5 and 6 on intrusive and frightening behavior. Sessions 7 through 10 were tailored to address parents' individual strengths and weaknesses and incorporated a focus on how parents' own histories of care influenced their parenting behaviors. Besides guiding discussions about the session topic, parent coaches provided feedback to parents about their interactions during the sessions both in the moment (ie, live coaching) and using videos. This feedback served to focus attention on the intervention targets and support parents in practicing those behaviors. In recent studies, this in-the-moment feedback has emerged as a key component of intervention effectiveness.³⁵

Developmental Education for Families Control Intervention | The Developmental Education for Families (DEF) intervention was adapted from a home-visiting program³⁶⁻³⁸ and focuses

on parent education about children's motor, cognitive, and language development. The DEF parent coaches provided general information about developmental milestones and suggested developmentally appropriate activities for parents to engage in with their child.

Saliva Sampling

The procedures used for collecting and assaying cortisol followed established protocols (for a description, see the article by Bernard et al⁵). Parents collected saliva samples from children twice per day (within 30 minutes of waking and right before bedtime) during a 3-day period. **Table 2** shows descriptive statistics of sampling times.

The saliva samples were stored in a freezer at -20°C prior to assay procedures. Samples were assayed using a high-sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, LLC). All samples from a child were assayed in duplicate on the same plate to minimize variability. The intra-assay and interassay coefficients of variation fell below 3.7% and 6.4%, respectively.

Cortisol Data Preparation

Following established procedures,⁷ biologically implausible cortisol values (ie, defined as values >2.0 µg/dL [to convert to nanomoles per liter, multiply by 27.588]) and cortisol values greater than 3 SDs above the mean were excluded from analy-

Table 3. Multilevel Modeling Coefficients of Intervention Effects on Diurnal Cortisol Production

Effect ^a	Log-Transformed Cortisol			
	Coefficient (SE)	t	df	P Value
Intercept, β_{00}	-1.05 (0.06)	-18.95	112	<.001
ABC intervention, β_{01}	0.18 (0.08)	2.18	112	.03
Child age, β_{02}	-0.02 (0.01)	-2.46	112	.02
Sample slope, β_{10}	-0.12 (0.06)	-2.13	112	.04
ABC intervention, β_{11}	-0.19 (0.08)	-2.33	112	.02
Child age, β_{12}	-0.00 (0.01)	0.58	112	.56
Time slope, β_{20}	-0.03 (0.03)	-0.84	112	.40
ABC intervention, β_{21}	-0.01 (0.05)	-0.20	112	.84
Child age, β_{22}	-0.01 (0.01)	-1.49	112	.14

Abbreviation: ABC, Attachment and Biobehavioral Catch-up.

^a β_{00} and β_{10} represent the waking level of cortisol and the slope of cortisol production across the day, respectively, for children in the Developmental Education for Families control intervention group. β_{01} and β_{11} represent the difference in the waking level of cortisol and slope of cortisol production across the day, respectively, between children in the Developmental

Education for Families control intervention group and children in the ABC intervention group. β_{02} , β_{12} , and β_{22} represent the effect of child age on the waking level of cortisol, slope of cortisol, and time of cortisol collection, respectively. β_{20} represents the effect of time of sample collection on cortisol level, and β_{21} represents the effect of intervention group on time of sample collection.

ses as outliers. Each child could have up to 6 cortisol values (ie, 3 waking and 3 bedtime samples). Of 690 possible samples, 574 were included in analyses, with 2.2% removed as outliers and 14.6% missing owing to an inadequate volume of saliva or because no sample was collected. Seventeen samples (2.5%) had cortisol levels below the detectable limit of the assay; these samples were replaced with a value of 0.004 $\mu\text{g/dL}$. Log₁₀ transformation was used to normalize the distribution of cortisol values owing to a positive skew. Table 2 shows descriptive statistics of cortisol values.

Statistical Analysis

Hierarchical linear modeling³⁹ was used to examine intervention group differences in cortisol levels at waking and bedtime as well as in slope cortisol levels across the day, following analytic procedures used previously.⁵ Hierarchical linear modeling accounts for the nonindependence of repeated measures by modeling multiple data points as nested within individuals, which further allows for missing data.

The following level 1 within-individual model was specified:

$$\log(\text{cortisol}_{it}) = \pi_{0i} + \pi_{1i}(\text{sample}) + \pi_{2i}(\text{time}) + e_{ti},$$

with log-transformed cortisol values as the dependent variable, π_{0i} as the estimated intercept of cortisol at waking, π_{1i} as the estimated slope of cortisol from waking to bedtime (with “sample” representing whether the sample was collected at waking [0] or bedtime [1]), π_{2i} as the regression coefficient representing the effect of the time-varying covariate (with “time” representing the sample collection time), and e_{ti} as the within-individual error.

Level 2 (ie, between-subject) variables were included to examine whether there were intervention group effects on cortisol levels at waking or bedtime and in change across the day. Child age was included as a covariate given that it was associated with cortisol levels in preliminary analyses. The following level 2 model was specified:

$$\pi_{0i} = \beta_{00} + \beta_{01}(\text{ABC}) + \beta_{02}(\text{child age}) + r_{0i},$$

$$\pi_{1i} = \beta_{10} + \beta_{11}(\text{ABC}) + \beta_{12}(\text{child age}) + r_{1i},$$

$$\pi_{2i} = \beta_{20} + \beta_{21}(\text{ABC}) + \beta_{22}(\text{child age}) + r_{2i},$$

with β_{01} and β_{11} representing the intervention effect (with DEF coded as 0 and ABC coded as 1) on the waking log-transformed cortisol value and the cortisol slope, respectively, and β_{02} and β_{12} representing the effect of child age (grand centered at the mean) on the waking log-transformed cortisol value and the cortisol slope, respectively.

Results

Waking cortisol levels differed significantly between children in the ABC group (mean [SD], -0.87 [0.45] $\mu\text{g/dL}$) and children in the DEF group (mean [SD], -1.05 [0.43] $\mu\text{g/dL}$), controlling for time of sample collection and age ($\beta_{01} = 0.18$; $P = .03$). Specifically, children in the ABC group showed a higher waking level of cortisol than children in the DEF group (Table 3). Intervention effects on bedtime cortisol levels were examined by rerunning the model with the bedtime sample as the intercept. Bedtime cortisol levels did not differ significantly between the ABC and DEF groups (mean [SD], -1.19 [0.49] vs -1.17 [0.48] $\mu\text{g/dL}$, respectively; $\beta_{01} = -0.01$; $P = .87$). There was a significant intervention effect on the change in cortisol level across the day, with children in the ABC group showing a steeper waking to bedtime pattern (ie, more negative slope) (mean, -0.31 $\mu\text{g/dL}$) than children in the DEF group (mean, -0.12 $\mu\text{g/dL}$) ($\beta_{11} = -0.19$; $P = .02$). Thus, children in the DEF group showed a more blunted diurnal cortisol pattern than children in the ABC group (Figure 2).

To estimate effect sizes, Cohen d was computed by dividing the unstandardized coefficients for intervention effects (accounting for level 1 and level 2 covariates) by the within-group standard deviation.^{40,41} Estimates of within-group

standard deviation were computed using the raw data for waking cortisol level (to examine the intervention effect on the intercept) and raw data for waking to bedtime change in cortisol level (to examine the intervention effect on the slope). Based on conventions, the effect sizes for group differences in waking cortisol level and diurnal slope were approximately medium ($d = 0.41$ and -0.43 , respectively).

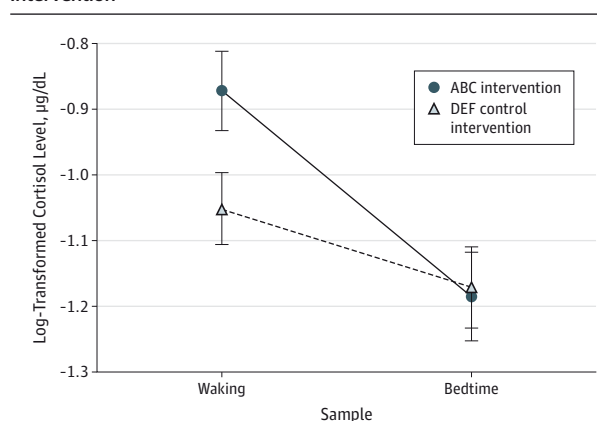
We examined whether findings held if (1) children who did not complete the interventions as intended were excluded, and (2) children who were part of a sibling pair were excluded. Six children in the DEF group and 6 in the ABC group who provided follow-up cortisol data did not complete the full 10 sessions (considered noncompleters but retained for follow-up visits). When these 12 noncompleters were excluded from analyses, findings held for the effect of the ABC intervention on waking cortisol level ($\beta_{01} = 0.20$; $P = .02$) and the diurnal slope ($\beta_{11} = -0.24$; $P = .01$). There were 3 pairs of siblings in the sample (2 in the ABC group, 1 in the DEF group). When 1 sibling from each pair was randomly excluded from analyses, the intervention effect held for waking cortisol level ($\beta_{01} = 0.20$; $P = .03$) and for the diurnal slope ($\beta_{11} = -0.20$; $P = .02$). Finally, we examined the within-person stability in cortisol patterns observed within months of the intervention²⁷ and cortisol patterns examined in the present study approximately 3 years later. We found modest stability in children's cortisol levels between these 2 times ($r = 0.43$; $P = .001$).

Discussion

Most previous studies that have found effects of parenting interventions on diurnal cortisol production have not yet reported long-term follow-up assessments, with a recent review of these studies concluding that long-term follow-ups are greatly needed in this literature.²⁶ The present study examined whether the effects of the ABC intervention on children's diurnal cortisol rhythms are long lasting and can still be observed during the preschool age range (approximately 3 years following intervention). Initial assessments conducted roughly 3 months after this 10-week attachment-based parenting program showed a normalization of diurnal cortisol slopes in CPS-referred children who were randomly assigned to the experimental group, but not in those in the control group.²⁷ Our analyses revealed a similar pattern of results at the preschool follow-up, such that children in the ABC arm showed a more typical pattern of cortisol production, with higher morning levels and a steeper decline across the day, than children in the control condition. The results suggest that the intervention led to persistent, long-term effects on the functioning of the HPA stress system, which may have implications for preventing child psychological and physical health problems given previous reports linking cortisol disruptions to these deleterious child outcomes.¹⁰⁻¹³

Blunted cortisol levels, often termed *hypocortisolism*, are increasingly recognized as a biomarker of chronic stress and may result from downregulation of the HPA axis subsequent to chronic cortisol elevations.¹⁸⁻²⁰ There are a few possible interpretations for the normalization of these diurnal rhythms observed with the intervention. Given that neglect is chronically

Figure 2. Log-Transformed Cortisol Levels for Children Who Received the Attachment and Biobehavioral Catch-up (ABC) Intervention vs Children Who Received the Developmental Education for Families (DEF) Control Intervention



Error bars indicate standard error. To convert cortisol to nanomoles per liter, multiply by 27.588.

stressful for children, the intervention may prevent or minimize exposure to neglectful parenting, directly preventing stress. Additionally, the 3 intervention targets may support children's regulation in a number of ways. First, helping parents respond with nurturance when children are distressed may lead to quicker and more effective soothing following stress responses, thus preventing prolonged exposure to an elevated cortisol level. Second, synchronous interactions may help children develop a sense of control over their environment and thus support their independent self-regulatory skills.^{42,43} Such enhanced biological and behavioral regulation may persist over relatively long periods. Third, coaching parents to avoid engaging in frightening behaviors toward their children may further serve to prevent dysregulation.

One limitation of this study is that the possible mediating pathways for the effects obtained were not directly tested. Future studies should explore parent behaviors (eg, maternal sensitivity) as they change throughout the intervention and afterward to better understand the processes underlying the intervention's effectiveness. Second, the 2 cortisol samples collected per day did not allow us to examine the cortisol awakening response (the difference in cortisol levels from waking to 30 minutes later) or to obtain a sensitive measure of total area under the curve across the day, both of which add unique information about the functioning of the HPA axis. To date, intervention studies with children have not specifically assessed the cortisol awakening response²⁶ and few have assessed the area under the curve³²; thus, future research should include a more frequent sampling schedule to capture these indices. Third, we did not have information about children's experiences of stress on the days of sample collection, preventing us from accounting for effects of daily experiences on cortisol patterns. Finally, our study would have benefited from a low-risk comparison group to assess whether the steeper slopes observed during follow-up were within the normative range for this age.

Conclusions

This study provides encouraging evidence that the ABC intervention has long-lasting implications for enhancing

children's cortisol regulation. Future research will have to clarify the extent to which these persistent biological patterns may contribute to or be accompanied by reductions in physical or mental health problems for children experiencing neglect.

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