

# Effects of a Psychosocial Family-Based Preventive Intervention on Cortisol Response to a Social Challenge in Preschoolers at High Risk for Antisocial Behavior

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**Context:** Salivary cortisol levels during social challenge relate to adaptive functioning in children and adults. Low cortisol levels have been related to conduct problems and antisocial behavior. Although studies in rodents implicate early-life social experience in cortisol regulation, no studies with humans have examined the effects of an experimentally manipulated early-life social experience on cortisol regulation.

**Objective:** To examine the effects of experimental manipulations of social experience on cortisol response to a social challenge in preschoolers at risk for antisocial behavior.

**Design:** Randomized controlled trial.

**Setting:** Department of Child and Adolescent Psychiatry, New York University School of Medicine.

**Participants:** Ninety-two preschool-age siblings of youths adjudicated for delinquent acts.

**Intervention:** Family-based intervention included 22

weekly group sessions for parents and preschoolers and 10 biweekly home visits conducted during a 6- to 8-month period.

**Main Outcome Measures:** Salivary cortisol levels before and after a social challenge (entry into an unfamiliar peer group).

**Results:** Relative to controls, children in the intervention condition had increased cortisol levels in anticipation of the peer social challenge. Increases were relative to both preintervention cortisol levels during the challenge and cortisol levels in the home, which were not altered by the intervention.

**Conclusions:** A family-based preventive intervention for children at high risk for antisocial behavior alters stress response in anticipation of a peer social challenge. The experimentally induced change in cortisol levels parallels patterns found in normally developing, low-risk children.

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**S**TRESS REGULATION IN CHILDREN is important for understanding the development and prevention of psychiatric disorders.<sup>1</sup> Environmental factors that operate at key points in development may shape affective and behavioral regulation and hypothalamic-pituitary-adrenal (HPA) axis function in children, much as environmental factors have been shown to shape HPA regulation in rodents and nonhuman primates.<sup>2</sup> Early experiences in rodents exert lifelong organizing effects on stress responsivity. For example, individual differences in maternal licking and grooming during the first week of life produce long-term alterations in rat pup behavioral and endocrine responses to stress.<sup>3,4</sup> Maternal care during the first weeks of life appears to program rat pup stress re-

sponse,<sup>2,5</sup> presumably through a transactional process between mothers and their pups.

This work in animals has stimulated studies with young children.<sup>6-12</sup> Initial findings underscore the complexity of relations among context, development, behavior, and HPA axis function in young children. Children with or at risk for disruptive disorders fail to show expected stress responses, as measured by salivary cortisol levels.<sup>13,14</sup> Although findings are not entirely consistent, at least some subgroups of children with conduct disorders have been shown to exhibit a reduced cortisol response, and this abnormal response may be specific to or more easily detected in stressful or challenging conditions<sup>15-18</sup> and may be complicated by the presence of comorbid internalizing problems.<sup>19</sup> Children at risk for psychiatric dis-

orders, such as children with familial antisocial behavior, maltreated children, or those raised in orphanages, also exhibit lower or flattened cortisol responses to novel or challenging situations and to circadian changes.<sup>14,20,21</sup>

It is hypothesized that suboptimal caregiving environments lead to both abnormal stress response and conduct disorders. Consequently, an intervention that enhances early social experiences in children at risk for antisocial behavior should result in a more normalized stress response and the prevention of conduct disorders. Some studies<sup>22,23</sup> also suggest that a genetic predisposition toward stress or threat hyporesponsivity or a lack of emotional reactivity facilitates the development of aggressive behavior. Through the effects of genes or the environment, prior findings suggest that some children develop chronic aggression because of poor processing of specific emotional cues that contributes to faulty socialization. An emerging body of literature<sup>24,25</sup> shows that a subgroup of chronically aggressive children exhibit marked difficulty processing social cues that signal the need to inhibit aggressive impulses.<sup>26</sup> These findings have been attributed to reduced responding in specific neural architecture associated with stress responses and HPA axis regulation. Accordingly, by acting on this substrate, an intervention that alters early stress responses may result in the prevention of later conduct disorders.

Research studies that use an experimental approach may clarify the precise nature of the stress response in high-risk children and its association with psychiatric disorders.<sup>1</sup> Fisher et al<sup>20</sup> suggest that a family-based intervention delivered to maltreated preschoolers might normalize perturbed diurnal cortisol patterns. However, because this pilot study did not rely on randomization, the degree to which changes in cortisol levels reflected intervention effects remains unclear. In a preliminary study<sup>27</sup> of cortisol and family-based treatment effects in children with behavior disorders, cortisol response to stress predicted intervention outcome on aggression, suggesting a relationship between cortisol response and behavior change. However, this study did not examine intervention effects on cortisol, and therefore the malleability of the stress response in children remains unknown.

In the current study, we examine HPA activity within the context of a randomized intervention experiment aimed at preventing conduct problems in preschoolers at high risk for antisocial behavior. Although this randomized controlled trial was designed to evaluate intervention outcomes on aggression, we take advantage of the experiment to test whether intervention that alters parenting and child risk factors for aggression also alters the cortisol response. We examine intervention outcomes on children's anticipation of and response to a social challenge, as well as diurnal patterns, because considerable evidence suggests that abnormal HPA activity in children with or at risk for conduct disorders may be context specific.<sup>7,9,11,13,28</sup>

A large body of literature<sup>7,9,11,13</sup> characterizes normally developing preschoolers' HPA stress response in unfamiliar, socially challenging situations. Normally developing children show increased cortisol levels in novel or challenging situations; increased levels are not exhibited in response to familiar or nonstressful situations. For

preschoolers, peer interactions are particularly salient, and entry into new peer situations is especially challenging and stressful.<sup>13</sup> Cognitive, social, and emotional capacities all influence children's anticipation of and response to peer entry, a developmental task that typically taxes existing resources of preschoolers, as reflected in behavior and the HPA axis.<sup>7,9,11,13</sup>

In the current study, we examine intervention-induced changes in HPA axis activity immediately before and after a peer entry social challenge, as well as diurnal patterns under typical daily conditions in a sample of 92 preschool-age siblings of adjudicated youths. We have documented that these children have been exposed to a multitude of risk factors for psychiatric disorders, including poverty, high rates of maternal depression and anxiety, suboptimal parenting practices, high levels of parental stress and negative life experiences, relatively low cognitive functioning, and poor prenatal environments.<sup>29</sup> The current study examines cortisol response within the context of a randomized controlled trial of an intervention aimed at the prevention of aggression in these high-risk youth. The prevention model involves altering parenting practices and child social competence during the preschool period as a means of preventing aggressive behavior by school entry. We have previously reported the immediate effects of this psychosocial family-based intervention on independently observed aspects of the caregiving environment and child social competence during unfamiliar peer entry, including approach and regulatory behaviors.<sup>30</sup> Thus, we have demonstrated the efficacy of the intervention in altering the targeted risk factors. We have also found longer-term intervention effects on child aggression during parent-child interactions observed by independent, blinded raters in home and laboratory settings (L.M.B., K.K.G., K.-Y.H., et al, unpublished data, 1997-2003). In the current study, we take advantage of this prevention trial to test the hypothesis that an intervention that enhances the caregiving environment and socioemotional and behavioral competencies of preschoolers at high risk for antisocial behavior also produces changes in the HPA axis.

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## METHODS

### SAMPLE

Study participants were identified during a 5-year period from court records of adjudicated youths (N=6909). Families were eligible if a sibling was between 33 and 63 months old. Families were ineligible if the caregiver had a *DSM-IV* substance use or psychotic disorder determined by clinical interview or if the preschooler had a pervasive developmental disorder or severe or profound mental retardation. Eligibility and interest were established with a telephone screen. Informed signed consent was obtained during a visit at the study center or in the home.

Eighteen percent of court records indicated the presence of a young sibling. Of these, we contacted 47% of families; 33% had age-appropriate siblings living at home and met other study requirements (eg, location, language). Ninety-two families (48% of eligible families) completed assessments and entered the study. Eighty-three percent of adjudicated youths were male, with a mean  $\pm$  SD age of 15.6  $\pm$  1.3 years. Twenty-five percent were adjudicated for personal crimes, 31% for property crimes, 16%

for personal and property crimes, and 29% for substance-related crimes. Eighty-three percent of caregivers were the preschooler's biological mothers. Parent mean  $\pm$  SD age was 36.30  $\pm$  9.2 years. Sixty-four percent were African American, 28% Latino, and 8% other. Preschoolers were, on average, 3.94  $\pm$  0.7 years old. Fifty-three percent of preschoolers were female. The average IQ score<sup>31</sup> was 83.27  $\pm$  12.79, and 23% exhibited clinically elevated behavior problems at baseline (age of 4 years) according to parents.<sup>32,33</sup>

## PROCEDURES

Participants were assessed at baseline (before random assignment to condition) and approximately 9 months later (immediately after the intervention). The length of time between assessments did not differ between experimental conditions. The study was approved by the institutional review board of the New York University School of Medicine. Informed consent for cortisol assessments was obtained separately from and after consent procedures for the prevention trial and psychosocial assessments.

## PREVENTION PROGRAM

Details of the prevention program are presented elsewhere<sup>30,34</sup> and are briefly described herein. The program was an adapted version of an efficacious program for parents and preschoolers<sup>35,36</sup> aimed at improving parenting practices and preschoolers' social competence with the goal of preventing later conduct problems. The program consisted of 22 weekly, 90-minute concurrent groups for parents and preschoolers, 30 minutes of guided parent-preschooler interactions, 10 biweekly 90-minute home visits, and up to 6 additional family visits provided during the 6- to 8-month period of intervention.

Using a videotape modeling intervention, the parenting groups encouraged parents to use nonharsh, consistent, and appropriate disciplinary strategies, be less critical, and use positive reinforcement and play interactions to promote children's social competence. In the preschool groups, leaders taught social skills, reinforced positive behaviors, and provided consequences for negative behaviors. Following the groups, parents and preschoolers participated in guided parent-preschooler activities during which leaders coached parents to use specific parenting skills and modeled and reinforced effective parenting strategies.<sup>37,38</sup> Home visits were designed to help parents implement skills at home.

Of the 47 families assigned to intervention, the average attendance at groups was 13 sessions, and the average number of home visits was 6. The control condition consisted of assessments and monthly telephone calls.

## OUTCOME VARIABLES

Neuroendocrine functioning was measured by collecting salivary cortisol samples before and after entry into an unfamiliar peer situation and at home 4 times during a 12-hour period on a different day, approximately 1 week later. Saliva collection was chosen because it is the most frequently used procedure in studies in young children.<sup>11,13,14,17,25</sup> This convention has emerged because of the ease of collection for assessments at multiple time points, the stress-free nature of the procedures, and the observation that salivary cortisol levels reflect levels of the biologically active hormone available to central nervous system receptors.<sup>39</sup>

Cortisol assessments were collected at baseline (before randomization) (T1) and repeated in an identical fashion 9 months later (after intervention) (T2). Samples were collected with a

sterile cotton roll held by dental floss. The child mouthed the roll until it was wet. This method of collection is better tolerated by young children than traditional vial collection methods. The sample was then placed in a vial and kept in a freezer until transported on ice to the laboratory. Food intake was controlled during the social challenge collection but not for collection at home.

## CORTISOL LEVELS BEFORE AND AFTER A SOCIAL CHALLENGE

The social challenge included entry into an unfamiliar peer group for 30 minutes of play at a nursery school several blocks from the study center. Observations were scheduled with the school to correspond with planned free play. Approximately 40% of assessments took place during morning free-play activities (10:00 to 11:30 AM), and 60% took place during afternoon free play (2:00 to 4:00 PM). No significant differences were found in time of day or length of assessment by condition. Approximately 40 children (34 to 60 months old) from diverse socioeconomic and ethnic backgrounds were enrolled in the school. During free play, children were free to move from station to station (eg, blocks, pretend house, table art) in a large room designed for this purpose. At least 1 teacher and 3 assistants from the school staffed free play.

At both the preintervention and postintervention assessments, before leaving the study office for the school, a research assistant blinded to intervention status and study hypotheses explained to the child (in front of the parent) that he or she was "going to a school to play with some other children." Thus, children were informed about an impending stressful experience before cortisol sampling. Two or 3 blinded research assistants escorted the child and parent to the school (a 10- to 15-minute walk). All personnel associated with the social challenge were blinded to intervention assignment status. Hence, there is no plausible way in which messages communicated to children by staff could have differentially influenced response to the challenge across the 2 intervention groups. On arrival and in the presence of the parent, a salivary cortisol sample was collected. The sample was collected approximately 15 to 20 minutes after the child was told about the challenge task. Because cortisol release in preschoolers occurs as early as 10 minutes from the stimulus presentation (M. Gunnar, PhD, oral communication, October 2006), we consider this cortisol sample to be an index of the child's anticipation of the challenge.<sup>2</sup> After the cortisol sample was obtained, the child was required to separate from the parent and accompanied to the play area by 1 or 2 research assistants who observed the child during 30 minutes of free play. Immediately after the play period and just before reuniting with the parent, a second salivary cortisol sample was collected. This sample was meant to index the child's response to the social challenge. Assessment procedures, including timing of cortisol collection, were identical for intervention and control conditions. Further details of the procedure and intervention effects on behavior observed during this procedure are presented elsewhere.<sup>30,40</sup> Of note, others have shown that similar peer entry paradigms are stressful for preschoolers.<sup>7,13</sup> Consistent with previous reports, observations of child peer entry and play behavior<sup>40</sup> in the current study sample confirmed the stressful nature of the task. Specifically, observed behaviors indicative of stress included hovering outside the group, hesitation in approach, crying, and mild disruptive behavior. At baseline, behavioral signs of stress reactivity in children's peer entry behavior were significantly associated with parent ratings of child internalizing behavior ( $r=0.37$ ,  $P<.001$ ), child dysregulation ( $r=0.30$ ,  $P=.004$ ), and 2 different indices of disruptive behavior during parent-child play interactions ( $r=0.45$ ,  $P<.001$ ;  $r=0.35$ ,

**Table 1. Descriptive Statistics of Log-Transformed Cortisol Values and *t* Test by Intervention Status at Baseline<sup>a</sup>**

Cortisol Assessment	Full Sample		Control		Intervention		Difference by Intervention			<i>P</i> Value
	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	MD (SE)	<i>t</i>	<i>df</i>	
Before challenge	0.09 (0.29)	69	0.11 (0.28)	34	0.08 (0.30)	35	0.03 (0.07)	0.39	67	.70
After challenge	0.02 (0.31)	70	-0.002 (0.31)	33	0.05 (0.31)	37	-0.05 (0.07)	-0.66	68	.51
Home										
7 AM	0.32 (0.43)	56	0.34 (0.50)	27	0.30 (0.38)	29	0.05 (0.12)	0.38	54	.70
12 PM	0.20 (0.41)	58	0.25 (0.42)	27	0.16 (0.41)	31	0.09 (0.11)	0.79	56	.44
4 PM	-0.01 (0.49)	56	0.02 (0.56)	26	-0.03 (0.43)	30	0.05 (0.13)	0.40	54	.69
8 PM	0.08 (0.58)	57	0.03 (0.59)	26	0.12 (0.58)	31	-0.09 (0.15)	-0.57	55	.57
Mean of 12 PM and 4 PM values	0.16 (0.41)	60	0.24 (0.42)	28	0.10 (0.39)	32	0.14 (0.10)	1.35	58	.18
Slope	-0.15 (0.33)	58	-0.19 (0.38)	27	-0.11 (0.28)	31	-0.09 (0.09)	-0.99	56	.33

Abbreviation: MD, mean difference between intervention and control after log transformation.

<sup>a</sup>Data are nonimputed raw values. Similar findings emerged with imputed data.

$P = .008$ ).<sup>40</sup> The peer entry procedure appears to be stressful in general and to elicit more signs of stress in children with mental health problems. In addition, we previously reported that the intervention led to enhanced social competence during this peer entry paradigm. Specifically, relative to controls, children in the intervention condition were observed to be less anxious or withdrawn, more socially skilled, and better able to “fit in” during this peer entry task after intervention.<sup>30</sup>

### CORTISOL LEVELS AT HOME (DAILY DIURNAL PATTERN)

Approximately 1 week after the social challenge, parents collected salivary cortisol samples 4 times (7 AM, 12 PM, 4 PM, and 8 PM) at home on a single day. Parents were given detailed verbal and written instructions after a demonstration at the study center (and after participation in the challenge assessment) to obtain saliva samples. Parents were instructed to place the vials in the freezer and to bring the samples on ice to the next assessment visit (typically within a week). We consider the average of home cortisol samples at 12 PM and 4 PM as corresponding to the timing of the social challenge assessment.

### CORTISOL ASSAYS

The samples were processed through 3 freeze-thaw cycles and then centrifuged. The supernatant was used for assay. The cortisol radioimmunoassay procedure has an intra-assay and inter-assay coefficient of variation of 2.95% and 6.0% at a concentration of 3.1  $\mu\text{g/dL}$ . The lower limit of detection is 0.1  $\mu\text{g/dL}$ .

### STATISTICAL ANALYSES

Because cortisol data were skewed (skewness statistic ranged from 2.91 to 3.29 for challenge data and from 1.47 to 6.46 for home data, where 0 to 1 represents a normal distribution and values  $> 1$  represent skewness), we log-transformed values to normalize the distributions. After the transformation, cortisol variables were normally distributed (skewness  $\leq 0.77$ ). Three children had 7 extreme values (log-transformed data  $> 3$  SDs above the mean). Therefore, we excluded these 7 values, leaving the 3 children with partial cortisol data. We compared the equivalence of intervention groups at baseline for child race/ethnicity, sex, age, and school enrollment and for 7 cortisol variables. We evaluated effects of demographics and cortisol measures on attrition that might limit generalization and differential group attrition that might bias estimates of intervention ef-

fects. We conducted a series of 2-way analyses of variance (group  $\times$  attrition status), examining main effects of attrition and the interaction between attrition and intervention status.

Seventy-nine children (86%) and 67 children (73%) had at least partial cortisol data before and after intervention, respectively, and 9 children (10%) had no cortisol data (but had other study data) at either time. We assumed that these data were missing at random,<sup>41</sup> which means that the “missingness” is random after taking into account known values on cortisol; child sex, race/ethnicity, age, and school status; cognitive stimulation at home<sup>42</sup>; and child social competence.<sup>43,44</sup> We used multiple imputation methods<sup>45</sup> according to the SAS Multiple Imputation procedure<sup>46</sup> with 10 replicated imputations. This imputation made use of the joint distribution of the variables listed herein. As per Little and Rubin,<sup>45</sup> imputations were conducted separately for the control and intervention groups to account for the possibility of different missing data patterns by group. We used SAS PROC MIANALYZE to combine the results for the final inference.<sup>46</sup>

Intervention effects on cortisol values were evaluated via repeated-measures analyses of variance with the intervention condition as the between-subjects factor and time (T1 vs T2) as the within-subjects factor. To examine intervention effects on change in cortisol values at home, an additional 4-level, within-subject factor was included to represent time of day. Planned contrasts examined intervention effects on the linear trend (a 3-way interaction among intervention status, time [T1 or T2], and time of day). Analyses were conducted with an intent-to-treat design. Planned contrasts examined the direct effects of time and group and the interaction between time and group. The main study hypothesis was tested based on group  $\times$  time interactions; a significant interaction indicates an intervention effect. Post hoc comparisons assessed change for each outcome within groups. Estimates of effect sizes were examined via  $\eta^2$  (percentage of variance explained) and interpreted with the following conventions: small, 0.01 or higher; medium, 0.06 or higher; and large, 0.14 or higher.<sup>47</sup>

## RESULTS

### BASELINE DESCRIPTIVE STATISTICS AND GROUP EQUIVALENCE

As indicated in **Table 1**, no baseline differences were found between the intervention and control groups for any of the cortisol values, indicating that randomization

**Table 2. Intervention Effects on Challenge and Home Cortisol Levels**

Cortisol Assessment	Mean (SE)				ANOVA Group × Time			
	Control		Intervention		df	F	$\eta^2$	P Value
	Time 1	Time 2	Time 1	Time 2				
Before challenge	0.13 (0.06)	0.001 (0.09)	0.04 (0.05)	0.31 (0.08) <sup>a</sup>	1,90	9.62	0.10	.02
After challenge	-0.003 (0.06)	0.05 (0.09)	0.07 (0.06)	0.08 (0.08)	1,90	.91	0.01	.82
Home								
7 AM	0.26 (0.15)	0.45 (0.22)	0.28 (0.08)	0.24 (0.13)	1,90	.85	0.02	.47
Slope	-0.26 (0.15)	-0.21 (0.10)	-0.12 (0.06)	-0.07 (0.06)	1,90	.94	0.01	.98

Abbreviation: ANOVA, analysis of variance.

<sup>a</sup>Change from baseline score is different from zero ( $P = .02$ ).

led to equivalent groups at baseline. At baseline, a linear effect was found for home cortisol samples, with levels decreasing throughout the day ( $F_{1,48}=9.49$ ,  $P = .003$ ,  $\eta^2=0.17$ ), consistent with a typical diurnal pattern. A trend was found for prechallenge levels to be higher than postchallenge levels ( $F_{1,66}=3.18$ ,  $P = .08$ ,  $\eta^2=0.05$ ). Neither prechallenge nor postchallenge cortisol levels differed from the average of the home cortisol levels at 12 PM and 4 PM ( $F_{1,49}=0.99$ ,  $P = .33$ ;  $F_{1,51}=2.81$ ,  $P = .10$ ).

#### ATTRITION ANALYSES

Sixty-seven children (73%) had at least 1 postintervention cortisol value. Retention rates between intervention (79%) and control (67%) children were not different ( $\chi^2 = 1.69$ ,  $P = .19$ ,  $n=92$ ). No differences were found between those who were retained and those who were missing at T2 for any of the baseline variables. Furthermore, no significant attrition × intervention status interactions were found (effect sizes ranged from  $\eta^2=0.00$  to  $\eta^2=0.03$ ).

#### INTERVENTION EFFECTS ON CHILD CORTISOL LEVELS

As indicated in **Table 2**, a significant group × time interaction was found for prechallenge cortisol levels ( $F_{1,90}=9.62$ ,  $P = .02$ ,  $\eta^2=0.10$ ). The effect of intervention on anticipatory cortisol levels can be considered a medium effect. Post hoc analyses of change within groups indicated that there was an effect of time within the intervention group, reflecting an increase in prechallenge cortisol levels over time ( $t_{46}=2.53$ ,  $P = .02$ ); no such effect was found in controls. Thus, the group × time interaction reflects an increase in prechallenge cortisol levels after the intervention among children in the experimental group but not in the control group. Furthermore, the increase in cortisol levels in anticipation of the social challenge after intervention represents an increase relative to home cortisol levels (12 PM and 4 PM) at baseline for the intervention group ( $t_{46}=2.28$ ,  $P = .03$ ) but not for controls ( $t_{44}=-0.60$ ,  $P = .56$ ). This pattern suggests that the intervention resulted in an increased anticipatory cortisol response relative to cortisol levels in typical nonchallenge conditions. No intervention effects were found for postchallenge cortisol or home cortisol values (Table 2).

#### POST HOC ANALYSES OF CORTISOL LEVELS AND BEHAVIORAL CHANGE

Post hoc analyses of relations between anticipatory cortisol levels after the intervention and observed behavior during the challenge (behavior that changed as a result of the intervention<sup>30</sup>) indicate no clear associations ( $P = .21-.75$ ). This finding suggests that intervention-induced changes in cortisol levels in anticipation of the social challenge and changes in observed behavior during the challenge (eg, withdrawn behavior or socially competent behavior) are independent.

#### COMMENT

To our knowledge, the current study provides the only data from a randomized controlled trial that examines changes in children's stress response to a social challenge. The primary finding that family-based intervention affects the stress response in preschoolers at high risk for antisocial behavior contributes to the literature with regard to the breadth of possible preventive intervention effects. Specifically, in the current sample, we have shown previously that the intervention studied herein produces robust changes in independently observed parenting practices, child social competence, and child aggression during the preschool period<sup>30,34</sup> (see also L.M.B., K.K.G., K.-Y.H., et al, unpublished data, 1997-2003). The current study extends these intervention outcomes to HPA function. Thus, this study adds to the small body of literature in children that demonstrates both biological and behavioral outcomes from early intervention with preschoolers at risk for psychiatric disorders.<sup>20,48</sup> Together these studies underscore the plasticity of the HPA system in young children and suggest the potential for early intervention across biological and behavioral domains.

The intervention effects on the HPA system observed in this study were specific to children's anticipation of the social challenge paradigm. For various reasons, this study does not allow for a precise exploration of mechanisms that contribute to the change in anticipatory cortisol levels. One major limitation in the research literature arises from the incomplete knowledge concerning cortisol regulation in preschoolers. For example, the de-

gree to which social challenges reliably elicit similar cortisol responses over time and the degree to which relatively subtle procedural factors, such as changes in instructions, might influence responses remain unclear. Such complications should tend to reduce the power to detect such effects, making the findings in this study even more intriguing. Moreover, this limitation in the literature is compounded by the absence of data in the current study for a healthy group of preschoolers, studied in the same conditions as the high-risk preschoolers. As a result, it is impossible to know whether cortisol regulation was normal or abnormal in the high-risk preschoolers, either before or after the intervention. Nevertheless, despite these caveats, if the current findings are to generate future advances in this area, the observed relationships must be placed in a cogent theoretical context. A rich body of literature on HPA axis function provides some insights.

It has been argued that the cortisol system is activated in conditions in which central goals are threatened.<sup>49</sup> This motivational perspective assumes that situations characterized by social evaluation are expected to elicit a significant cortisol response as a result of the salient threat it poses to the goal of maintaining the social self. For preschoolers, the anticipation of separating from a parent and approaching an unfamiliar group of peers for play would be a highly salient threat. Appropriate garnering of the stress response in anticipation of this social challenge would be expected based on previous data.<sup>8,13,50</sup> In the current study, this was observed only after the intervention, possibly because of intervention-induced changes in cognitive appraisal, including the value placed on peer interactions, attention to instructions about the peer entry task, self-assessment of skills to be used during the pending task, and emotion and behavior regulation during the stimulus presentation. Any one of these factors may have changed as a result of the psychosocial intervention and may account for the altered cortisol response. On the basis of this demonstration that anticipatory cortisol response, but not postchallenge cortisol response, is altered by a psychosocial family-based intervention, future studies should carefully evaluate these possible explanatory processes.

Prior studies on social processes, aggression, and stress regulation provide a further contextual framework against which to place the current findings. Conduct disorder represents a heterogeneous construct, an observation that might contribute to inconsistencies in the prior literature relating aggression and HPA axis activity.<sup>25</sup> One relatively distinct subgroup of children with conduct disorders may be particularly likely to show reduced HPA axis activity. This subgroup exhibits reduced arousal on diverse measures of stress reactivity, a finding attributed to a specific neural deficit that causes poor processing of social reinforcers and faulty socialization.<sup>26,48</sup> Through this deficit, such children may fail to learn the rules that signal the need to inhibit aggression, particularly in circumstances in which rules are conveyed through social signals.<sup>26</sup>

This neural deficit is thought to involve a reduced capacity to engage the amygdala and associated circuitry when encountering social threat cues, consistent with data

implicating amygdala activation in autonomic arousal more generally and cortisol secretion specifically.<sup>25</sup> Although to our knowledge no brain imaging studies have examined relationships among social processing, cortisol levels, and amygdala activation in young children, a recent study<sup>51</sup> in adolescents found that elevations in cortisol levels associated with Cushing syndrome were associated with enhanced amygdala activation during the processing of social cues. Thus, in the current study, increased cortisol levels and reduced aggression after intervention may reflect adaptations in the amygdala-based architecture involved in diverse capacities, including regulation of the HPA axis, modulation of aggression, and processing of social cues.

Intervention did not alter diurnal patterns in these high-risk children. Previous work suggests that conduct disorders may be specifically associated with abnormal social challenge cortisol response. Although the lack of a normally developing comparison group in this study precludes conclusions about baseline diurnal and challenge patterns, it is possible that this group of high-risk children were hyporesponsive in anticipation of a social challenge but had relatively normal diurnal patterns. Flattened diurnal patterns might be seen only in children living in highly neglectful or chaotic environments and not necessarily in children at risk based on sibling antisocial behavior. Future developmental and experimental studies should consider cortisol response to social challenge as well as basal levels and diurnal patterns, because they may be differentially associated with risk status.

This study does not allow us to make conclusions about the clinical implications of the increased cortisol response in anticipation of social challenge. Given previous findings from this sample that indicate intervention-induced increases in socially competent behavior and decreases in withdrawn behavior during the challenge, as well as literature on hyporesponsive HPA function in at-risk children, these findings suggest that the intervention allowed children to develop the expected HPA axis response, as well as appropriate behavioral adaptation, in anticipation of a stressor. However, this interpretation remains only 1 possibility, considering our lack of directly comparable data in a sample of typically developing children. The lack of association between intervention-induced changes in behavior during the challenge and anticipatory responses suggests that these 2 intervention effects are independent and may reflect change in 2 distinct areas of functioning. In other words, some high-risk children may have benefited from the intervention by enhancing their social behaviors and others may have benefited by developing a more adaptive stress response in anticipation of a social stressor. Alternatively, no direct relationship may exist between the changes in behavior and HPA axis activity, with the intervention producing effects on both behavioral and neuroendocrine regulation, both of which are unrelated. As we follow up this sample into preadolescence, we will be able to evaluate the clinical relevance of intervention-induced changes in the HPA system, child social behavior, and the family environment in the preschool period for later psychiatric disorders. The demonstration of mediation, as reflected in associations between intervention-

induced changes in the HPA axis and aggression over time, would support perspectives that suggest that reactivity in brain circuits that regulate the HPA axis modulates aggression.<sup>24,25</sup> The failure to find such associations would suggest independent effects of the intervention on behavior and the HPA axis.

Several limitations to our study should be noted. The randomized controlled trial was designed to evaluate intervention effects on parenting practices, child social competence, and aggression. Although this trial provided an opportunity to evaluate cortisol outcomes in 2 contexts, certain design features, such as data from a low-risk group, would have been informative for placing the experimental findings into context. Specifically, in the absence of findings from a comparison group at low risk for antisocial behavior, our findings could alternatively be interpreted as suggesting either that the intervention produced compensatory but abnormally high elevations in cortisol levels before peer entry or that the intervention normalizes an already perturbed HPA axis. However, the absence of such data does not influence the central conclusions of the current study, which demonstrate psychosocial intervention effects on cortisol response in high-risk children.

Another limitation is that we did not collect a baseline, "unstressed" measure of cortisol before the social challenge. In this study, the prechallenge measure was interpreted to reflect children's anticipatory response to the instructions given describing the social challenge. Although we used the home cortisol measure as an index of resting cortisol levels during the same period of the day, it would have been preferable to have a measure of cortisol at home on the same day, or in the study center, before assessment procedures.

A final limitation involves differences in the collection of cortisol samples at home and for the social challenge. Collection of home cortisol samples was conducted by parents and therefore was performed less systematically than challenge collections performed by research staff. It is possible that parents did not adhere to suggested times and methods of collection. Therefore, an additional possibility for not detecting intervention effects in the home is that the data include more measurement error than challenge cortisol samples.

This study makes important contributions to the literature on children's stress responses. In preschoolers at high risk for antisocial behavior, a psychosocial intervention led to differences in the stress response in anticipation of a social challenge. This effect was demonstrated within the context of an intervention previously shown to produce robust effects on the caregiving environment and behavior in high-risk preschoolers. These findings provide the first evidence, to our knowledge, in humans that clinically relevant experimental manipulations to the family environment and child social behavior lead to alterations in the neuroendocrine system. Future studies will examine whether these immediate changes in the stress response relate to meaningful differences in the development of psychiatric disorders and adaptive functioning.

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