A R T I C L E

INTERVENTION EFFECTS ON MORNING AND STIMULATED CORTISOL RESPONSES AMONG TODDLERS IN FOSTER CARE

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ABSTRACT: Toddlers in child welfare often have a dysregulated stress response. We tested whether toddlers with caregivers randomized to a 10-week attachment-based intervention, Promoting First Relationships (PFR; J.F. Kelly, D. Sandoval, T.G. Zuckerman, & K. Buehlman, 2008) would show postintervention change in stimulated salivary cortisol patterns during a research home visit involving a separation–reunion procedure, as compared to a condition including child development and resource advice, but no attachment strategies. At baseline and postintervention, toddlers with a caregiver change within 7 weeks of enrollment ($\underline{n} = 48$, age 10–25 months) provided four saliva samples during a $1\frac{1}{2}$hr research visit, and samples the next morning. The categorical dependent variable was the pattern of cortisol activity during the course of the postintervention research visit: flat, decreasing, or increasing. Multinomial logistic regression was used to test for postintervention group differences in cortisol patterns, controlling for time of day, child's age, morning cortisol level, and baseline cortisol pattern. At baseline and postintervention, 92% of children demonstrated atypically low morning cortisol (<.21 ig/dL); postintervention, flat, decreasing, and increasing patterns were exhibited by 70, 15, and 15% of the sample, respectively. Significantly more children in the PFR condition showed an increasing pattern. This may signal an intervention effect on separation-based stress response physiology.

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Caregiver sensitivity and predictability are crucial for a child to develop a well-regulated stress response (Gunnar & Donzella, 2002). For children who enter state dependency as infants, a dysregulated stress response is all too common (Dozier et al., 2006; Fisher, Gunnar, Dozier, Bruce, & Pears, 2006; Tarullo & Gunnar, 2006). This dysregulation places them at increased physiological risk for internalizing problem behavior (Cicchetti & Rogosch, 2001), externalizing behavior problems in adolescence (Shirtcliff, Granger, Booth, & Johnson, 2005), depression as adults (Wilkinson & Goodyer, 2011), and a compromised immune system (Segerstrom & Miller, 2004).

Difficult or challenging behaviors, some of which may themselves be correlates of a dysregulated stress response in young children, can lead foster caregivers to feel rejected and unneeded by these children. A harmful cycle ensues as caregivers are then not able to provide their children with the type of regulating and positive interactions that would be most beneficial (Dozier, Stovall, Albus, & Bates, 2001). Separations, the loss of attachment figures, and adapting to new caregivers' schedules and routines are such significant sources of stress that the buffering effect of a sensitive caregiver is an absolute requirement if young children in foster care are to avoid the consequences of toxic stress (National Scientific Council on the Developing Child, 2005). Recognizing this, the American Academy of Pediatrics (2012) has called for treatment programs that can help caregivers provide young children in the foster care system with the relationships necessary to protect them from the long-term consequences of the stressors inherent in their early experiences (Shonkoff & Garner, 2012).

There is presently a gap in the literature in understanding how occurrences common to maltreated children in foster care

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impact their stress response in the short-term (during home visits, removals, and placements) and how the repetition of separation experiences may impact cortisol reactivity over time. The length of time required to observe a significant impact could be fairly short for infants and toddlers in foster care due to the number of disruptions they experience. For example, the children in our sample, despite their young age, already had changed primary caregivers an average of 3.3 times prior to enrollment in the project. The psychobiology of early maltreatment has been conceptualized as creating vulnerability to psychosocial stress and adversity, and because of their early histories, young children in foster care may be unusually vulnerable to further dysregulation as a result of their involvement in the foster care system (De Bellis, 2005; Rutter, 2000).

Defining a poorly modulated stress response in young children is not simple or straightforward. A child's cortisol level in response to stress may be influenced by genes (Ising et al., 2008) or early experiences with caregivers, or reflect an interaction between the two (Luijk et al., 2010). If we think of a modulated stress response as one in which the child responds with developmentally appropriate emotional, behavioral, and physiological responses to the onset and cessation of stressful stimuli, then an unmodulated response could be one that is absent or weak, or unusually intense and long-lasting, and may or may not be evident in the child's behavior (Keenan, Jacob, Grace, & Gunthorpe, 2009). Insecurely attached toddlers are in fact less likely to show a correlation between behavioral distress and cortisol elevation in response to being left at daycare by their caregiver (Ahnert, Gunnar, Lamb, & Barthel, 2004). Examining cortisol reactivity in children who share a common context of separations, removals, and early trauma has the potential to provide important information about the range of impact these experiences can have on the developing stress response system of young children, and the potential long-term benefits of intervention (Cicchetti, Rogosch, Toth, & Sturge-Apple, 2011; Fisher et al., 2006; Tarullo & Gunnar, 2006).

Several studies that have focused on the relationship between attachment behavior patterns and cortisol reactivity have determined that children displaying insecure patterns experienced hypothalamic–pituitary–adrenal (HPA) arousal in response to a separation from their caregiver (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996; Spangler & Grossman, 1993); however, this response may be absent in foster children with caregivers they may have known for only a short time (Dozier, Peloso, Lewis, Laurenceau, & Levine, 2008). Other studies have suggested that repeated loss of attachment relationships results in a hyporesponsive stress response (Cicchetti, Rogosch, Howe, & Toth, 2010; Flinn, 2006; Gunnar & Vazquez, 2001; Ruttle et al., 2011). In such cases, improved attachment relationships may be associated with greater ability to mobilize a stress response.

Researchers seeking to define a poorly modulated stress response are confronted with apparent contradictions because both overarousal and underarousal to the same stressor are not uncommon in groups of young children; one approach to this problem is to focus on deviations from expected, typical responses to an event that is likely to be a stressor, such as the child's primary caregiver leaving the home. Patterns of HPA activation in that context may reveal characteristics of a child's stress regulatory functioning (Keenan et al., 2009). Thus far, there is no perfect agreement in the literature as to what different patterns of cortisol reactivity mean, although there are strong arguments made that developmental level, timing of separation, and a child's own physiology influence the course (Adam, Klimes-Dougan, & Gunnar, 2007; Bernard & Dozier, 2010; Spangler & Grossman, 1993). What is well-understood, however, is that young children in foster care are physiologically and emotionally engaged in constant adaptation to their environment; interventions should then demonstrate that they influence adaptation in ways that will promote healthy regulation and attachment and provide meaningful protection from further dysregulation and risk of psychopathology. This article reports on the results of a randomized controlled trial which tested the effects of a brief, relationship-based intervention on morning salivary cortisol levels and stimulated salivary cortisol patterns shown by fostered toddlers with a recent caregiver change.

THE STRESS RESPONSE SYSTEM

The stress response system (SRS) develops over the first years of life, and both internal and external factors influence individuals' baseline hormone levels and thresholds for activation, how sensitive they are to events, and how quickly they recover from exposure to a stressor (Boyce & Ellis, 2005; Gunnar & Donzella, 2002). The SRS consists of three components: the sympathetic and parasympathetic systems and the HPA axis. In the short-term, the SRS serves to assess and respond to an individual's environmental risks, challenges, and opportunities for engagement; over the life span, the functioning of the SRS has ramifications for self-regulation, attachment, immune functioning, and mental health (Beers & De Bellis, 2002; Del Guidice, Ellis, & Shirtcliff, 2011).

Diurnal Pattern and Morning Cortisol

The HPA axis maintains a diurnal pattern in which the highest levels of the hormone cortisol are produced early in the morning and decline throughout the day (Gunnar & Cheatham, 2003). The diurnal pattern of cortisol production begins to emerge in the first months of life, however, there is a great deal of individual variability in the age at which this pattern emerges and becomes stable (deWeerth, Zijl, & Buitelaar, 2003). While studies do not agree on the earliest emergence and stability of the diurnal pattern, most researchers agree that it is stable in normally developing infants by 12 months of age (Goldberg et al., 2003; also see Klug et al., 2000). Thus, the functioning of the HPA axis can be assessed by measuring the cortisol level of an early morning saliva sample and/or the cortisol pattern throughout the day.

Young children placed in foster care as infants have demonstrated low early morning cortisol (Bruce, Fisher, Pears, & Levine, 2009). However, high morning cortisol levels also have been associated with young children who have been maltreated (Cicchetti

& Rogosch, 2001) as well as foster infants under 24 months of age (Dozier et al., 2006). Fisher, Pears, and Levine (2009) found lower morning cortisol among preschoolers in foster care; in the same sample, foster children with severe emotional maltreatment demonstrated high morning cortisol. The length of time a young child has been exposed to a trauma or stressor may impact their HPA response; recent stressful or traumatic events have been connected to elevations in cortisol. On the other hand, chronic, long-term stress can result in downregulation of the HPA axis, as revealed by lower levels and flattened cortisol trajectories across the day (Cicchetti et al., 2010; Flinn, 2006; Gunnar & Vazquez, 2001; Ruttle et al., 2011). Fisher et al. (2006) found that four or more foster placements and being initially placed in foster care before the age of 2 years were both predictors of lower morning cortisol in preschoolers.

Response to Threat

Another way to assess the functioning of the HPA axis is to measure a stimulated stress response or the rise and fall pattern of cortisol reactivity to a stressor. The HPA axis is responsible for the physiological response to threat, with the end product of increased cortisol circulation. Through a feedback loop, cortisol acts to inhibit both the hypothalamus and the pituitary gland, decreasing the amount of cortisol when the threat has passed or, in the case of young children, in response to comforting or reassurance. Cortisol is secreted in a pulsitile fashion; this aspect of cortisol production can introduce error when multiple cortisol samples are taken from an individual. However, researchers have hypothesized that acute stressors may speed up the occurrence of what would have been a spontaneous cortisol pulse; thus, stress-induced and naturally occurring pulses are not completely independent of one another (Young, Abelson, & Lightman, 2004). In a stress response, the purpose of cortisol is to produce an alert and vigilant state; however, high levels of circulating cortisol also dampen the immune system and overtax both the physical and psychological coping resources of an individual (McEwen, 2008).

Individual differences also determine SRS reactivity within a given environmental and relationship context. Characteristics of a disrupted stress response have varied in the cortisol literature, and include unusual elevations in response to a stressor, a delay in return to baseline, or a flat cortisol response to a stressor (Adam et al., 2007). An additional pattern of dysregulation called "declivity" occurs when cortisol declines below pretest levels in response to a laboratory stressor (Shirtcliff et al., 2005), although there is no consensus in the literature as to what the decline indicates with regard to SRS regulation (Adam et al., 2007).

What constitutes a stressor, and elicits a stress response in a particular child, depends greatly on the child's experiences, SRS sensitivity, and available resources. Because caregiver sensitive availability is a crucial influence on the development of the SRS, researchers have looked at cortisol reactivity in the context of separation from a primary caregiver as measured in the Strange Situation (Ainsworth, Blehar, Waters, & Wall, 1978), a laboratory procedure involving two brief departures by the caregiver (Hane & Fox, 2006). In studies with young children, the child's age as well as concurrent or previous maltreatment and their attachment relationship with their current caregiver have been found to impact their cortisol response to separation (Bernard & Dozier, 2010; Hill-Soderlund et al., 2008; Spangler & Grossman, 1993; Spangler & Schieche, 1998). In general, the Strange Situation procedure has had mixed results in creating a stress response in young children. Other studies have found connections between maternal qualities impacting the mother–child relationship and a child's cortisol response; for example, the degree of maternal depression predicted a flatter cortisol response to a stranger entering the child's home and conducting cognitive testing (Fernald, Burke, & Gunnar, 2008). Risk factors such as interparental violence and maternal emotional unavailability also have predicted a flatter cortisol response in toddlers to a stressful laboratory task (Sturge-Apple, Davies, Cicchetti, & Manning, 2012).

Intervention Effects on Morning and Stimulated Cortisol Patterns

Improved caregiving may significantly increase foster children's morning cortisol levels over time (Cicchetti et al., 2011; Fisher et al., 2006). To our knowledge, however, the only study to examine intervention effects on very young foster children's stimulated cortisol response has found that the intervention, treatment control, or comparison group did not show a stimulated cortisol response to the Strange Situation, although the treatment group had lower initial cortisol values postintervention than did the treatment control group and were comparable to a non-foster care comparison group (Dozier et al., 2008).

In the present randomized trial examining toddler SRS regulation after a relationship-focused intervention, stimulated cortisol was collected in the child's home with their current caregiver (foster, birth, or kin). The study design followed the protocol of the parent study from which participants were recruited, and therefore required that both research visits and intervention take place in the natural environment of the foster child. In that context, we hoped to observe the child's cortisol response to some elements that are common in child welfare and often accompany short- or long-term separations from caregivers.

We also examined morning cortisol levels to gain a sense of the ongoing cortisol patterns present in the children. Thus, the first aim of this study was to test whether a brief, 10-week, relationship-based intervention affected foster toddlers' morning cortisol levels. We predicted that toddlers in the intervention condition who had low morning cortisol at baseline would show an increase postintervention. The second aim of this study was to determine whether toddlers in the intervention group would show a change in stimulated cortisol patterns from pre- to postintervention, as compared to toddlers in the comparison condition. We predicted that postintervention, toddlers in the Promoting First Relationships (PFR; Kelly, Sandoval, Zuckerman, & Buehlman, 2008) intervention would be more likely to show cortisol patterns reflecting a mobilization of a stress response, as demonstrated by their HPA axis response to

a stranger coming to the home and to their caregiver briefly leaving the home. We reasoned that for toddlers in child welfare who had experienced extended or permanent separations from one or more primary caregivers since birth, a healthy or adaptive stress response to a stranger's arrival and the caregiver's departure from the home would be a rise in cortisol because the event could be linked with past experiences of significant relationship stress. In this case, the alertness accompanying elevated cortisol also could indicate an investment in the caregiver's status. We anticipated this rise would be followed by a decline about 45 min later. A less optimal response would be flat or declining cortisol levels, especially in the context of overall low cortisol levels because these would reflect an unremediated hyporegulation from prolonged exposure to stress.

METHOD

Participants

This study involved a subsample of participants in a project funded by the National Institutes of Health (Spieker, Oxford, Kelly, Nelson, & Fleming, 2012) examining the impact of a relationship-based intervention with 210 infants and toddlers in the child welfare/foster care system and their caregivers. In that project, psychosocial outcomes in the areas of child development, caregiver sensitivity, and attachment were collected. Community providers of mental health services received training in the PFR and provided a 10-session, relationship-based intervention to participants randomly assigned to the experimental group. PFR utilizes reflective practice with caregivers, including discussion of videotaped interactions with their child, and focuses on building caregiver confidence and competence in three areas that research has shown to impact developmental outcomes for very young children: caregiver sensitivity, developmentally appropriate expectations, and reframing caregiver understanding of, and responses to, children's ambiguous cues and difficult behavior. An additional provider delivered the resource and referral comparison condition, Early Education Support (EES). The comparison condition was conducted as three 90-min home visits spread out over 10 weeks, in which a provider helped connect the caregiver to resources such as early intervention, child care, Early Head Start, and housing. In addition, the provider answered questions and made suggestions about early child development. In fidelity testing, we were able to confirm that virtually no PFR strategies were used in the EES home visits (Spieker et al., 2012).

After verbal consent was provided to participate in the parent study, 57 consecutive subjects and their caregivers were invited to also participate in the saliva-collection substudy. Of those invited, 54 agreed to participate. Saliva was collected from all 54 subjects at baseline. At the time of the postintervention research visit, 2 caregivers had dropped out of the study, and 4 children had a placement change. Those participants were excluded from the analyses; however, we did conduct a supplementary intent-to-treat analysis (ITT; Kazdin, 2009) including the 2 participants who dropped out (discussed later).

Primary participants included 48 children ranging in age from 10 to 25 months ($M = 17.10$, $SD = 4.31$). All children had been involved with Child Protective Services (CPS), were currently in state dependency, and had experienced a change in caregiver within 7 weeks of the baseline research visit. At baseline, 37.5% of the children in this subsample were living with a foster parent, 37.5% had been returned to their birth parent, and 25.0% were living with a family member. For 3 children in the subsample, this was a first placement since their involvement with CPS began; 45 children had experienced multiple placements. The average number of primary caregiver changes experienced by participants from birth to enrollment in the study was 3.31. Subsample participants reported their race as White (68.8%), Black (16.7%), American Indian (12.5%), and Hawaiian Native (2.1%).

Procedures

The study had institutional review board approval and informed consent regarding research participation. A research home visitor collected saliva samples with an absorbent, sterile cotton dental roll from the toddlers in the study using the passive drool method (collection of the saliva that naturally pools in the mouth). The cotton rolls were treated with approximately .16 g of sweetened cherry-flavored Koolaid. This method was demonstrated by Talge, Donzella, Kryzer, Gierens, and Gunnar (2005) to not affect the rank-ordering of cortisol values; these authors stipulated that the particular assay used in this study (Salimetrics, LLC, State College, PA) may return slightly higher values when using treated as opposed to untreated dental rolls. Thus, per their recommendation, the same preparation, collection, and assay procedures were used for all participants. The rolls were 6 in. long to ensure that an adult had a firm grasp on the roll to prevent choking. The child was assisted to place one end of the dental roll in his or her mouth for about 20 s; caregivers also were provided with a dental roll so that they could demonstrate the technique for the child. Upon removal, the research visitor made sure that the end of the roll was completely saturated with saliva and snipped off the saturated end into a prelabeled tube, which was then sealed. All cortisol samples were kept in a designated freezer until they could be transported to the University of Washington School of Nursing Biobehavioral Laboratory (within 48 hr), where they were kept frozen at -70°.

The first five saliva samples (S1–S5) were collected at baseline as follows: (a) on arrival (S1); (b) just before the brief separation conducted at the beginning of the research visit in which the caregiver left the house or apartment for 3 min (based on the 3-min separation used in the laboratory-based Strange Situation) and returned (S2); (c) 30 min after the separation (S3); and (d) 45 min after the separation (S4). The following morning, caregivers were instructed to obtain a fifth sample (S5) within 30 min of the child waking up and before eating or drinking. The second five saliva samples (S6–S10) were collected at postintervention in exactly the same manner. The morning samples were placed in the family's

freezer (not in the door) and collected by the research visitor the same day.

All assays were analyzed with an extended range, high-sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, LLC, State College, PA). The intraassay variation was 3.65%, and the interassay variation was 6.41%.

Cortisol Data Preparation

Cortisol values 3 *SD*s above or below the mean (baseline, $n = 3$; postintervention, $n = 2$) were examined in the context of between-subjects effects. All extreme values were consistent with cortisol levels for a particular child; knowing which child the value was for explained 45% of the variance while knowing which time point of 10 explained only 4% of the variance. For that reason, these values were retained in analysis. Each child in the study had 10 possible samples, five collected at baseline and five collected at postintervention. All samples were successfully collected at baseline. At postintervention, four morning samples were not collected because the caregiver forgot or did not follow the collection protocol, and two samples from the end of the research visit were not collected because the caregiver ended the visit early (One subject was missing both the postintervention morning and final samples.) Of the original 48 children in the study, 43 provided both baseline and postintervention samples that included morning cortisol. Of the five children not included in the final model, 4 were male, 3 were in the PFR intervention group, 3 were in foster care and 2 in kin care, and 3 were White, 1 was Black, and 1 was American Indian.

Exploratory analyses were conducted to look for patterns using the morning samples as well as all four samples collected at baseline and postintervention. Mean morning samples collected at baseline ($M = .121$, $SD = .11$ ig/dL) and postintervention ($M = .160$, $SD = .21$ ig/dL) were not significantly different than one another, $t = 1.12$, $SE = .035$, $p = .264$. The morning samples collected at baseline and postintervention were not significantly correlated with each other, $r = .099$, $p = .521$. Baseline morning cortisol levels were significantly correlated with all of the other four samples (S1–S4), S1: $r = .71$, $p = .001$; S2: $r = .63$, $p = .001$; S3: $r = .69$, $p = .001$; S4: $r = .66$, $p = .001$. Postintervention morning cortisol levels were significantly correlated with all of the other four samples, S6: $r = .47$, $p = .001$; S7: $r = .49$, $p = .001$; S8: $r = .72$, $p = .000$; S9: $r = .73$, $p = .000$. Mean elapsed time in minutes between samples at baseline were S1–S2: 7.06; S2–S3: 30.75; and S3–S4, 15.75. At postintervention, they were S6 –S7: 6.69; S7–S8: 30.31; S8–S9: 15.08.

There was a very close time interval between S1/S6 and S2/S7, and a significant correlation between S1 and S2, $r = .83$, $p = .001$; S6 and S7 were not significantly correlated, $r = .070$, $p = .635$. The actual length of time that research visitors spent with families between collection of the first sample and the preseparation sample was less than had been anticipated (~6.8 min, on average). Gunnar and White (2001) estimated that an interval of 15 to 32 min between samples is necessary to detect an increase in salivary cortisol. Because of the brief interval between the collection of S1/S2 and S6/S7, the latter samples were not included in analysis.

Exploratory analyses also were conducted using the samples collected upon arrival (S1, S6), 30 min after the separation task (S3, S8), and 45 min after the separation task (S4, S9), as some studies have found a peak cortisol response to a stressor in toddlers occurring between 15 and 40 min (Goldberg et al., 2003; Gunnar & White, 2001). We did not find evidence of this pattern in our sample (Our analysis and results are explained in detail later.) In the current sample, the near complete lack of this pattern may reflect a characteristic of our foster care sample or it may support the assertion that the peak-to-recovery period for some infants following a stressor might be longer than 45 min (Keenan et al., 2009).

We graphed individual trajectories of cortisol across Research Visits 1 and 2 and explored the use of regression models to describe variations in the levels of change; however, using raw or log-transformed data failed to tell us very much about our sample. Overall, the levels of cortisol in the sample both pre- and postintervention were quite low across all collection time points. Most subjects' cortisol levels remained flat across the research visits, and the models could not explain the few instances of definite upward or downward trajectories. In our sample, the direction of change rather than the amount of change appeared to be the relevant differentiating factor; for that reason, we next conducted nonparametric analyses where the results could be grouped in an interpretable way. To better understand the patterns of cortisol reactivity present in our sample, we examined change scores to assess the characteristics of those infants demonstrating patterns of increasing, decreasing, or flat cortisol.

For the final model, change scores from samples collected at the time of the data-collectors' arrival (S1/S6) and the final collection point 45 min later (S4/ S9) were calculated. It was evident from these change scores that three patterns of cortisol reactivity were present in the sample: Cortisol levels remained flat, increased, or declined over the course of the research visit. A conservative cut point of .05 ig/dL was determined based on average variability within the sample to allow us to capture meaningful changes in cortisol level across collection points; the average $\frac{1}{4}$ *SD* for collection points ranged from .051 (S9) to .018 (S4).

To ensure that this threshold for change was not too low, we compared the sample-to-sample variability of the cortisol assays. During the assay process, each of our derived samples was split into two and analyzed separately. The typical difference between assays for a given subject had a mean *SD* of .029 ig/dL, which was lower than the selected cut point of .05 ig/dL A categorical variable was created which designated whether the child's cortisol pattern was flat (did not change more .05 ig/dL), increasing, or decreasing. Time of day for each sample was documented and included as a covariate in the model.

Preliminary Analyses

Morning cortisol levels in this sample ranged from .007 to .447 ig/dL at baseline and from .002 to 1.11 ig/dL postintervention. In defining "low" morning cortisol in young children, care must be taken in examining values across studies due to differences in sampling and assaying techniques. However, as an informal comparison, "low" morning cortisol in 3- to 6-year-olds was defined by Bruce et al. (2009) using the lower quartile of their sample as <.30 ig/dL while Dozier et al. (2006) defined "low" as <.21 ig/dL in a sample of 20- to 60-month-olds, where "low" was designated as values 1 *SD* below the mean of comparison children who had not been in foster care. In our sample of 10- to 25-month-olds, average morning levels at both baseline ($M = .121$, $SD = .109$) and postintervention ($M = .159$, $SD = .212$) were below even the most conservative cut point of <.21 ig/dL.

We categorically explored the postintervention cortisol patterns in our sample, using chi-square analysis with Intervention Category (PFR, comparison) and Cortisol Pattern (flat, increasing, decreasing) as the two factors. A significant difference emerged between the two intervention categories, $\chi^2(N = 46) = 7.35$, $p < .05$, $\phi = .40$. The binomial probability of individual cells was analyzed with EXACON (Bergman & El-Khouri, 1987, 1998) to determine if the observed frequency in a cell was significantly greater or less than expected. The expected number in the PFR increasing cell was 3.2, and the observed number was 6. The expected number in the comparison group increasing cell was 3.8, the observed number was 1. Although the numbers in these cells were small, the binomial probabilities were significant at $p < .10$.

Chi-square tests comparing baseline cortisol patterns and postintervention cortisol patterns revealed that baseline pattern did not predict postintervention pattern, $\chi^2(N = 46) = 3.23$, $p < .52$, $\phi = .27$. Children who demonstrated the increasing pattern postintervention ($n = 7$) were all in the flat category at baseline except for 1 child (down); of the children in the down category at postintervention ($n = 7$), 6 were in the baseline flat category, and 1 child was down. Because of the small number of subjects in each category, we created a dichotomous variable designating baseline (preintervention) patterns as flat or not flat for the final model.

The direction of change in cortisol levels from S1 to S4 (Visit 1) and S6 to S9 (Visit 2) by intervention group are presented in Table 1.

TABLE 1. *Pre- and Postintervention Classifications of Stimulated Cortisol Patterns by Intervention Group*

	PFR			EES		
	$n = 21$			$n = 25$		
T2	Decrease	Increase	Flat	Decrease	Increase	Flat
T1						
Decrease	0	1	5	1	0	2
Increase	0	0	3	0	0	2
Flat	1	6	5	5	1	14
Total	1	7	13	6	1	18

TABLE 2. *Likelihood Ratio Tests on Individual Variables of Multinomial Logistic Regression Model Relating Cortisol Pattern With Intervention Category*

Variable	χ^2	*df*	*n*
Time of Day	4.81	2	43
Age in Months	6.57*	2	43
T1 Cortisol Pattern	3.50	2	43
Morning Level	4.78	2	43
Intervention Category	7.84*	2	43

*$p < .05$. **$p < .01$. ***$p < .001$.

Data Analytic Strategy

Morning cortisol levels. An analysis of covariance (ANCOVA) model was estimated to assess differences by experimental condition in the child's morning cortisol level postintervention. Covariates included child's age, time of day, and baseline morning cortisol level, transformed using a log transformation to reduce the impact of outliers.

Stimulated cortisol patterns. Multinomial logistic regression (Hosmer & Lemeshow, 1989) was used to examine the association between postintervention cortisol pattern and intervention group. The categorical dependent variable representing the pattern of cortisol activity during the course of the research visit (flat, increasing, or decreasing) was predicted from intervention group, controlling for time of day, child's age, morning cortisol level, and flat or not flat cortisol pattern observed at baseline. Morning cortisol levels were transformed using a log transformation to reduce the impact of outliers. All predictors were entered into the analysis at the same time.

RESULTS

Morning Cortisol Levels

The ANCOVA was not significant, $F(1, 43) = .001$, $p = .971$, allowing us to accept the null hypothesis that the population adjusted means are equal. No treatment effects were in evidence for morning cortisol levels in this sample.

Stimulated Cortisol Patterns

The overall multinomial logistic regression model was significant, $\chi^2(10, N = 43) = 26.11$, $p = .004$ (see Table 2), and likelihood ratio tests on the individual variables (see Table 3) revealed that the child's age and intervention group were significant predictors of cortisol pattern. Whether the child ate or drank during the course of the research visit ($n = 12$) did not increase or decrease the probability of a particular cortisol pattern after controlling for other

TABLE 3. *Parameter Estimates for Multinomial Logistic Regression Model Relating Cortisol Pattern with Intervention Category*

Significant Contrast	Wald χ^2	*B*	*SE*	Odds Ratio	Confidence Interval
Flat vs. Decreasing					
Time of Day	1.99	−.29	.21	.75	.49–1.13
Age in Months	.32	−.08	.14	.92	.70–1.22
Flat at Visit 1	1.03	1.41	1.39	4.09	.27–61.88
Morning Level	.55	.37	.50	1.45	.54–3.88
Random = PFR	.34	−.77	1.31	.46	.04–6.03
Flat vs. Increasing					
Time of Day	2.28	−.39	.26	.67	.40–1.13
Age in Months	3.56†	.42	.22	1.52	.98–2.35
Flat at Visit 1	2.20	2.26	1.5	9.55	.48–188.53
Morning Level	2.79†	1.09	.66	2.99	.83–10.80
Random = PFR	4.30*	3.33	1.60	27.79	1.20–643.47
Decreasing vs. Increasing					
Time of Day	2.91	−.10	.32	.90	.48–1.70
Age in Months	3.87*	.50	.25	1.65	1.00–2.71
Flat at Visit 1	.19	.85	1.94	2.34	.05–103.78
Morning Level	.88	.72	.77	2.06	.46–9.26
Random = PFR	4.46*	4.10	1.94	60.08	1.34–2691.40

*$p < .05$. †$p < .10$.

variables. Other variables tested that did not impact the model were gender, race, caregiver type (foster, birth, kin), number of placements, and age at first removal.

Parameter estimates for each of the significant contrasts are presented in Table 2. Older children and children of caregivers who received PFR were more likely to show the increasing cortisol pattern postintervention, as compared to flat and decreasing patterns, than were younger children and children in the comparison condition. The small sample size contributed to very large confidence intervals and a large odds ratio for the variables representing intervention group and dichotomous cortisol pattern at baseline. To obtain a more precise estimate would require a larger sample size. We also conducted an ITT (Kazdin, 2009) in which we included data from the 2 participants who dropped out of the study after Visit 1 and before intervention. We carried forward the last known values (in this case, cortisol data from the first research visit) and assumed no treatment effect. The overall multinomial logistic regression model was significant, $\chi^2(10, N = 43) = 21.56, p = .017$, and likelihood ratio tests on the individual variables revealed that PFR group remained a significant predictor of the postintervention increasing cortisol pattern.

Exploring Fluctuations in Cortisol Level at the 30-Min Collection Time Point

To be categorized as flat, a subject's cortisol level did not increase or decrease by more than .05 μg per dL between the first (S1 or S5) and final (S4 or S9) collection points. When cortisol levels were examined at the 30-min collection time points (S3 and S6), the majority of the subjects were already on the trajectory detected at the final (45-min) time point. There were 6 exceptions at baseline—five whose cortisol level decreased by more than .05 ig/dL, ranging from .06 to .21 ig/dL, and 1 whose cortisol level increased by .08 ig/dL. There were four exceptions at postintervention: Two subjects' cortisol increased by .08 and .06 ig/dL, respectively, and 2 subjects' cortisol decreased by .08 and .06 ig/dL, respectively. To account for the possibility that these exceptions experienced a meaningful change in cortisol followed by a rapid return to their earlier level, subjects whose cortisol levels increased or decreased by more than $\frac{1}{4}$ *SD* at S8 were reclassified from flat to increasing or decreasing at postintervention. The pattern variable at baseline was adjusted to reflect S3 fluctuations as well. The model was rerun with these adjusted variables. The overall multinomial logistic regression model was significant, $\chi^2(10, N = 43) = 19.66, p = .033$, and likelihood ratio tests on the individual variables revealed that PFR group remained a significant predictor of postintervention increasing cortisol pattern.

Other Associations with Flat, Increasing, or Decreasing Cortisol Patterns

Children who demonstrated a flat cortisol pattern at postintervention had significantly lower morning cortisol levels than did children who showed the increasing pattern, $F(2, 40) = .21, p = .007$. When the level of the first cortisol samples (S1 and S6) taken upon researcher arrival were compared across the cortisol patterns, at both baseline and postintervention children with the decreasing cortisol pattern had significantly higher initial cortisol levels than did children with either flat or increasing cortisol patterns, baseline: $F(2, 45) = 14.36, p = .001$; postintervention: $F(2, 43) = 9.24, p = .001$. We did not find significant associations between cortisol pattern pre- and postintervention on other observational measures of the children in the study, such as the security score provided by the Toddler Attachment Sort-45 (Andreassen, Fletcher, & Park, 2006) or parent-report measures such as the Child Behavior Checklist (Achenbach & Rescorla, 2000). We feel that this argues for the importance of physiological measures such as cortisol for providing information about the adaptation of young children that cannot be obtained through other means.

DISCUSSION

Toddlers in this study experienced risks in their birth homes which led to their involvement with CPS and an average of 3.31 caregiver changes prior to enrollment in this study. Ninety-two percent of them demonstrated atypically low morning cortisol levels at baseline, and 89% had atypically low morning cortisol levels at postintervention. We expected our slightly younger sample to actually have higher morning cortisol values in comparison to other studies with young foster children because morning cortisol levels typically decrease across the first year of life (Ramsay & Lewis, 1994), but that was not the case. The intervention had no effect on morning cortisol levels.

The intervention did have an effect on stimulated cortisol pattern. In the group provided with attachment-focused intervention (PFR), one third of the children (7 of 21) demonstrated a shift from a flat cortisol pattern to an increase in cortisol over the course of the research visit. In the comparison group, only 1 of 25 children demonstrated an increase. Under the circumstances shared by the children in this sample, an increase in cortisol during the first half of a 90-min home visit by an unfamiliar person which included a brief departure from the home by the caregiver may indicate movement toward a more adaptive, functioning SRS and HPA system. This suggests that the implementation of an effective, relationship-focused intervention can help caregivers to nurture in ways that positively influence the physiological regulation of young children who have experienced the traumatic stresses associated with child welfare involvement. In the parent study, caregivers enrolled in the PFR condition had higher scores on postintervention observational ratings of caregiver sensitivity ($d = .42$), relative to caregivers in the comparison condition (Spieker et al., 2012), but this association was not apparent in the substudy. Thus, we have not measured a potential mechanism by which the intervention influenced cortisol pattern.

For nearly two thirds of the sample, cortisol levels did not change under those circumstances. While no SRS reactivity might suggest in typically developing children that they did not find the circumstances stressful, both the relationship histories and the unusually low cortisol levels present in this sample make this lack of responsiveness of more concern. Although the literature has shown that separation alone is not a reliable stressor for many toddlers and young children, a flat pattern of reactivity together with low morning cortisol levels suggest dysregulation in the form of hypocortisolism, or a downregulation of the stress response. Blunted cortisol reactivity such as this is thought to be an outcome of chronic stress, and has been detected in other studies with young children in foster care (Fisher et al., 2006). Five children in the study (3 in the intervention condition, 2 in the comparison condition) who demonstrated an increase in cortisol at the first research visit had a flat cortisol pattern in the second research visit—potentially a less adaptive response.

A small group of children in this study demonstrated a drop in cortisol over the course of the postintervention home visit. This drop possibly represents a small snapshot of the child's pattern of diurnal cortisol activity; we noted that the children in this category had higher initial cortisol levels on average (at S1 and S6) than did children in the other two groups. The children whose cortisol levels increased in response to the stressor also did not demonstrate a "typical" pattern, or an increase in cortisol, which would peak between 20 and 40 min and then gradually decline (Goldberg et al., 2003). Only 1 child at baseline and 2 at postintervention had such a pattern, and their cortisol level at 30 min barely exceeded the cutoff for the flat cortisol pattern. The children with the increasing pattern showed the expected increase, but because we did not collect saliva beyond 45 min, we were unable to observe the expected return to baseline.

TABLE 4. *Postintervention Classifications of Stimulated Cortisol Patterns by Intervention Group and Caregiver Type*

	PFR			EES		
	$n = 21$			$n = 25$		
T2	Decrease	Increase	Flat	Decrease	Increase	Flat
Foster	0	3	7	3	0	5
Birth	1	2	3	2	1	9
Kin	0	2	3	1	0	4
Totals	1	7	13	6	1	18

PFR = Promoting First Relationships; EES = Early Education Support.

A number of factors might have impacted which children benefitted from intervention in a way that was measurable in salivary cortisol. First, the frequency and severity of maltreatment that children in the sample may have experienced would certainly impact their responsiveness to stressors as well as their reaction to separation from a caregiver and their physiological coping mechanisms and responsiveness to intervention (Barnett, Manly, & Cicchetti, 1993). Unfortunately, were not able to review files to code for maltreatment frequency and severity and control for the level of trauma that children may have experienced. Second, 6 children in the experimental group and 12 in the comparison group were living with their birth parent at the time of intervention (Foster: PFR = 10, EES = 8; Kin: PFR = 5, EES = 5; see Table 4). While some data on the reasons for removal were available to us, the information did not make explicit the relationship between the reason for removal and the current caregiver, so we could not control for whether a child living in his or her birth home had been reunited with a previously abusive caregiver; again, this may have a strong impact on their cortisol patterns in response to separation as well as the potential effectiveness of an intervention focusing on the caregiver–child relationship.

Although it was not what we expected, in this sample we did not detect differences in either stress reactivity or response to intervention based on the type of caregiver the child was with. There were no statistically significant relationships between caregiver type (foster, birth, or kin) and cortisol level or reactivity to the experimental protocol at Visit 1, regardless of whether the cortisol was expressed in the original interval assay units, as the natural logarithm transform of the interval units, or as a ternary categorical measure summarizing the reactivity pattern. There also were no significant relationships between caregiver type and change from Visit 1 to Visit 2 in the level or reactivity in the pattern of the cortisol values, tested as interval, natural log transform, or categorical variables. We also did not find moderator or interactive effects of caregiver type on cortisol level or pattern when added to models, including the main intervention factor (PFR vs. EES). This was true for analyses using either continuous or categorical representations of the patterns of cortisol measures.

Limitations

Much of the literature on HPA activation in young children has focused on the connection between stimulated cortisol reactions and attachment strategies as measured by the laboratory-based Strange Situation whereas this study's procedures took place in the home. All subjects in this study were from a similar, high-risk background involving maltreatment and multiple removals and caregiver changes. Without a comparison group of children who have never been maltreated or separated, we do not know if a home-based research visit and caregiver separation constitute sufficient stressors for children this age, and this is an important limitation of our study. Other limitations are that we did not collect a 60-min saliva sample and were unable to collect diurnal cortisol samples in addition to the early morning sample and the stimulated samples. To reduce the intrusion on the already-stressed families in this sample, we felt that it was important not to add an additional researcher visit to collect morning cortisol; however, this introduced another potential source of error because we only have caregivers' reports as to when the child awoke, that the morning samples were collected within 30 min of waking, and that the child did not have anything to eat or drink. This data collection may have been difficult or inconvenient for some families, and their record of the timing may not be completely correct. Further exploration is required to better understand the caregiving correlates of the different patterns of cortisol reactivity, what they might mean longitudinally, and which children received the most benefit from the intervention and why.

A feature of the study design was that children were provided services with whichever provider they were currently living, so our sample includes foster, birth, and kin care providers. We found no significant impact of caregiver type on cortisol levels or reactivity. The circumstances of young children in foster care are not homogeneous, and previous maltreatment and loss may impact a child differently if he or she is in foster care, is living with relatives, or has been returned to a birth parent. We recommend that future studies focus on the physiology of relationship stress in these caregiver subgroups, and control for maltreatment history. Emerging models for examining cortisol reactivity such as the allostasis model (Blair et al., 2011; Skinner, Shirtcliff, Haggerty, Coe, & Catalano, 2011) and the adaptive calibration model (Del Guidice, Ellis, & Shirtcliff, 2011) also emphasize the role of individual sensitivity to environmental stressors and the ways in which cumulative stress impacts the functioning of the HPA axis. This holistic view of HPA axis reactivity may be especially salient in the case of young foster children, whose experiences both before and within the child welfare system can vary greatly.

In addition, the small sample size contributed to very large confidence intervals and a large odds ratio for the variables representing intervention group and dichotomous cortisol pattern at baseline, and limits the interpretability of the findings. Based on findings of the NICHD Early Child Care Research Network (1997), which found no significant relationship between attachment security and daycare in the first year, we also did not include data on the children's experiences of out-of-home care outside of their foster care placements.

Finally, families randomly assigned to the EES condition received a qualitatively different type of home visit which did not include any of the intervention strategies utilized by PFR; the intervention also was quantitatively different in that EES families received 3 home visits from their provider whereas the PFR assigned families received up to 10. All participants randomly assigned to EES completed all three home visits. Most participants completed the PFR intervention, but not all; 82.6% of participants randomly assigned to PFR received the full 10-visit intervention, 8.7% received at least 50% of the intervention visits, and 8.7% received less than 50%.

Our findings provide an additional dimension to a small group of studies that have investigated the impact of relationship-based interventions on the HPA axis regulation of infants in foster care. We did not find the impact of intervention on diurnal cortisol that has been reported in other studies (Fisher, Stoolmiller, Gunnar, & Burraston, 2007; Fisher, Van Ryzin, & Gunnar, 2011), as represented by cortisol level in the early morning. While Dozier et al. (2008) found an intervention effect on initial cortisol levels prior to the Strange Situation, our study is the first to demonstrate an effect on stimulated cortisol levels following a caregiver-separation-type stressor. The observed changes in HPA axis reactivity in the present study occurred in a randomized study of a sample of toddlers in state custody, a particularly vulnerable population, and were obtained in the home environment of the child regardless of whether their caregiver was currently foster, birth, or kin. Within that complicated context, there is a small, but clear, signal that an intervention which addresses the relationship between a child and his or her current caregiver has the potential to help regulate the stress reactivity of toddlers in foster care.

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