



# Early adversity, hypocortisolism, and behavior problems at school entry: A study of internationally adopted children



Kalsea J. Koss<sup>a,\*</sup>, Shanna B. Mliner<sup>b</sup>, Bonny Donzella<sup>b</sup>, Megan R. Gunnar<sup>b</sup>

<sup>a</sup> Department of Psychology and Human Development, Vanderbilt University, 230 Appleton Place, Nashville, TN 37203, United States

<sup>b</sup> Institute of Child Development, University of Minnesota, 51 E. River Road, Minneapolis, MN 55455, United States

## ARTICLE INFO

### Article history:

Received 14 August 2015

Received in revised form

14 December 2015

Accepted 14 December 2015

### Keywords:

Hypocortisolism

Hypothalamic–pituitary–adrenal axis

Orphanage

Early adversity

Problem behavior

Adoption

## ABSTRACT

The hypothalamic–pituitary–adrenal (HPA) axis is influenced by early life adversity; however, less is known about the potential for recovery following marked improvements in care. The present study examined longitudinal changes in children's cortisol reactivity in the laboratory (4 assessments over 2 years) after adoption. Post-institutionalized ( $N=65$ ) and post-foster care children ( $N=49$ ) demonstrated blunted reactivity relative to non-adopted peers ( $N=53$ ). Furthermore, post-institutionalized children exhibited no evidence of expected adaptation to repeated sessions in the 2 years following adoption. As evidenced by blunted cortisol reactivity, flatter diurnal slope, and lower home morning cortisol, we found support for hypocortisolism among children experiencing adverse early care. Hypocortisolism served as a mediator between adversity and teacher-reported attention and externalizing problems during kindergarten. Early adversity appears to contribute to the down-regulation of the HPA axis under both basal and stress conditions.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Early adversity appears to affect the development of hypothalamic–pituitary–adrenal (HPA) axis reactivity in both animals and humans leading to reduced resilience and heightened risk for mood and health disorders (Danese and McEwen, 2012). However, the literature is mixed, with hyper-, hypo-, and no changes in HPA axis function being obtained in different studies (Strüber et al., 2014). There are a number of possible reasons for these mixed results, but one source of inconsistent findings can be attributed to whether basal or stress reactivity is the outcome measure. Initial research focused on stress reactivity (Liu et al., 1997). However, once researchers began to study ambulatory cortisol patterns (Hellhammer et al., 2004) and vulnerable children (e.g., Carlson et al., 1995) the focus shifted to basal diurnal activity. With this shift came greater concern with low or blunted patterns of hormone production (Heim et al., 2000).

Many but not all studies of children exposed to early adverse care have reported a lower morning and flatter daytime cortisol rhythm (for review see Strüber et al., 2014). While this altered pattern has at times been described as hypocortisolism, this conclusion

is premature. Hypocortisolism involves not only lower basal levels and a flatter diurnal pattern, but also blunted stress responses (Heim et al., 2000). We are aware of only two studies, both with adults, that examined associations between the hypocortisol patterns in diurnal activity (i.e., low morning and a flatter diurnal slope) and cortisol stress responses, with mixed results (Kidd et al., 2014; van Eck et al., 1996). Examining whether hypocortisolism in cortisol reactivity and home diurnal patterns co-occur or are divergent patterns was one of the primary goals of the present study. To achieve this, we sought to create an index of hypocortisolism reflecting indices of both home diurnal and laboratory stress reactivity that capture the down-regulation of the HPA axis in the context of early adversity.

We examined children adopted internationally with a focus on those adopted from institutional or orphanage care because these children experience a marked improvement in care at the time of adoption and provide a good model of early adversity (van Ijzendoorn and Juffer, 2005). There have been only two studies of cortisol reactivity among post-institutionalized (PI) youth, both of which used the Trier Social Stress Test (Gunnar et al., 2009; McLaughlin et al., 2015). One found no evidence of a blunted stress response among 10- to 12-year-olds (Gunnar et al., 2009), while the other found blunted responding but only if the youth were removed from institutional care after two years of age (McLaughlin et al., 2015). The heterogeneity in findings may suggest that the

\* Corresponding author.

E-mail address: [kalsea.j.koss@vanderbilt.edu](mailto:kalsea.j.koss@vanderbilt.edu) (K.J. Koss).

HPA stress response recovers in some but not all children. If so, we have little knowledge of how responses to stress change in the immediate years after adoption.

As an extension of our previous work in this sample on diurnal HPA activity following international adoption, we examined changes in the children's cortisol reactivity to laboratory challenges over the first two years post adoption from institutional care settings. Previously, comparable to other studies of early adversity (Strüber et al., 2014), we found that these children exhibited a flatter diurnal slope that was associated with less supportive socioemotional care prior to adoption (Koss et al., 2014). Notably, however, we found that children who had been adopted at earlier ages from international foster care settings also exhibited a similarly blunted diurnal cortisol rhythm. Thus the effects were not due specifically to institutional care but were common more broadly to orphaned and abandoned children. Examining cortisol reactivity to stressors, its potential recovery following adoption, and association with institutional versus other types of pre-adoption conditions was a second goal of this study.

Finally, both hyper- and hypo-cortisolism are described as the result of chronic or frequent stressors and are expected to mediate impairments in health and behavior (McEwen, 1998; Strüber et al., 2014). Furthermore, associations between hyper- and hypo-cortisolism and behavior problems may differ depending on the type of behavioral problems evidenced. Although findings remain somewhat mixed, elevated cortisol is often associated with internalizing problems whereas blunted or low cortisol may be associated with externalizing problems (for a review see Gunnar and Vazquez, 2006). Children experiencing early adversity in the form of institutional care exhibit a number of behavioral and emotional problems (Juffer et al., 2004). We previously reported that blunted diurnal cortisol predicted parent-reported broadband behavior problems prior to kindergarten entry (Koss et al., 2014). In the present study we examined whether the combination of diurnal home and laboratory cortisol reactivity patterns, indicative of hypocortisol, served as a mediator between early adversity and the type of behavior and emotional problems as reported by parents and teachers during kindergarten (e.g., internalizing, externalizing, ADHD problems).

The goals of the present study were three-fold. Building on our previous investigation of diurnal cortisol, we sought to examine differences in children's cortisol reactivity to a laboratory setting among children with varying degrees of early life experiences. This included investigations of the impact of early life adversity on cortisol reactivity as well as examinations of longitudinal change, indicative of recovery of the HPA axis, in children's cortisol reactivity following adoption. Second, we examined the extent to which indices of diurnal cortisol and stress reactivity together reflect hypocortisolism in the context of chronic stress. Lastly, we examined whether this hypocortisolism may serve as a mediating mechanism for distinct types of behavioral problems.

## 2. Method

### 2.1. Participants

Participants included 167 children taking part in a larger longitudinal study of the transition to family care following international adoption. Sixty-five children (38 female, 27 male) were adopted from orphanages or institutions (post-institutionalized; PI), 49 children (19 female, 30 male) were adopted from international foster care (post-foster care; PFC) and 53 non-adopted (NA) same-aged children (27 female, 26 male) who were born and raised in similar types of families (education/income) that adopt internationally-born children. PI and PFC participants were recruited through adoption agencies, an international adoption medical clinic, and

enrollment on the International Adoption Registry maintained by our research group. NA children were recruited through a University department-maintained participant pool recruited through letters mailed at birth and on-line advertising. PI children met the following criteria: their last care type prior to adoption was an institutional setting ( $M$  age at adoption = 24.66 months,  $SD = 5.04$ ,  $M = 75.9\%$  of pre-adoptive life in institution,  $SD = 29.4$ ), began study participation within 3 months of their adoption, and were 18–36 months at recruitment ( $T1$   $M$  age = 26.36 months,  $SD = 5.05$ ). PFC children met the following criteria: were adopted from international foster care ( $M$  age at adoption = 9.66 months,  $SD = 1.47$ ), spent most of their pre-adoption lives in foster care and less than 50% in institutional settings ( $M = 87.3\%$  of pre-adoptive life in foster care,  $SD = 12.2$ ;  $M = 10.0\%$  of pre-adoptive life in institutional care,  $SD = 12.3$ ), and were 18–36 months at recruitment ( $T1$   $M$  age = 32.48 months,  $SD = 5.27$ ). NA children met the following criteria: were 18–36 months at recruitment ( $T1$   $M$  age = 27.80 months,  $SD = 5.77$ ) and were reared in their families of origin. Exclusions included facial indices of fetal alcohol exposure using the FAS Facial Photographic Analysis software (7 PI, 2 PFC; Astley and Clarren, 2000) and congenital and endocrine disorders (2 PI). NA children were included as a typically developing comparison group; NA children were excluded if they experienced early adversity or had been diagnosed with neurodevelopmental disorders (autism 1 NA, maltreatment 1 NA).

Children participated in four laboratory sessions roughly 2, 8, 16, and 24 months after adoption timed from the PI children's entry into the US (PI time since arrival  $M = 1.70$  months,  $SD = .78$ ). At the first laboratory session, children were between the ages of 18 and 36 months ( $M$  age = 28.61 months,  $SD = 5.90$ ). Behavior problems were assessed during kindergarten ( $M = 5.98$  years,  $SD = .29$ ) by both parents and teachers. There were 64 Asian children (26 PI, 37 PFC, 1 NA), 58 Caucasian (10 PI, 48 NA), 23 Black/African (23 PI), 11 American Indian/Alaskan Native (2 PI, 9 PFC), and 11 multiracial or other racial backgrounds (4 PI, 3 PFC, 4 NA). Additionally, 17 children were Hispanic/Latino (2 PI, 12 PFC, 3 NA). For additional pre-adoptive participant and family demographics of this sample see Koss et al. (2014). All procedures were approved by the University's institutional review board.

### 2.2. Measures and procedures

#### 2.2.1. Laboratory salivary cortisol

Saliva was collected using procedures described in detail previously (Koss et al., 2014). Samples were stored at  $-20^{\circ}\text{C}$  prior to assaying in duplicate using a time-resolved fluorescence immunoassay (DELFA). Intra-assay and inter-assay coefficients of variation were 6.7% and 8.8% or less, respectively. Each laboratory session was approximately 2 h and consisted of a number of challenging tasks, including brief separations, exposure to novel and arousing stimuli, interactions with strangers, transitions between tasks and rooms, and electrophysiological assessments (see Supplemental materials for a detailed description of tasks and timing of cortisol sampling). Three saliva samples were collected throughout the laboratory session at each of the four assessments (12 total laboratory samples). The samples were not collected in response to any one task but rather reflect children's reactivity to the demands of the laboratory session as a whole. The majority of laboratory sessions occurred in the mid-morning and early afternoon hours (Sample 1  $M$  time range: 10:46 am–11:04 am;  $SDs$  92–115 min). Analyses controlled for individual differences in testing and waking times by including calculated time-since-waking (time-since-waking  $M$  range: 225–267 min,  $SDs$  105–182 min). Cortisol values more than 4  $SDs$  of the mean were Winsorized (4–7 values at each session). Data for children with fevers at the laboratory session were deleted from the data ( $n = 2$ ). Parent-reported

medications were coded for known effects on the HPA axis and these weights were used as covariates in the analyses (Granger et al., 2009).

### 2.2.2. Diurnal cortisol

These procedures are described in full in Koss et al. (2014). Briefly, parents collected wakeup, midday, and bedtime samples on three days after each of the laboratory sessions. Factor scores for children's diurnal cortisol (intercept/morning cortisol and slope/diurnal change) were extracted from between-level portion of the multilevel structural equation linear growth model (1 estimate of the person-level intercept and the 1 estimate of the person-level slope). Nine percent of children (153/167) in the present analysis were missing home diurnal data, but this did not differ by group, most demographics, or study variables (8/65 PI, 3/49 PFC, 3/53 NA). However, girls (11/84) were more likely to be missing diurnal cortisol compared to boys (3/83;  $\chi^2(1)=4.89$ ,  $p<.05$ ).

### 2.2.3. Child adjustment problems

Parents and teachers completed the MacArthur Health and Behavior Questionnaire (HBQ; Essex et al., 2002) during kindergarten. We examined the behavior problems scales: internalizing, externalizing, and ADHD symptoms (parent-version 75 items; teacher-version 60 items). Each item was rated on a three-point Likert scale with excellent internal reliability in the present study ( $\alpha$  range = .84–.91 across reporters and scales). At kindergarten, we had HBQ data on 128 participants (45 PI, 41 PFC, 42 NA; 124 both reporters, 3 teacher only, 1 parent only). The parent-reported assessment was completed by the primary caregiver ( $N=115$  mothers,  $N=10$  fathers). All teacher assessments were collected a minimum of two months after the start of kindergarten ( $M=6.89$  months,  $SD=5.84$ ). There were no differences in those with and without kindergarten behavior problem data with regard to cortisol data and early life history variables.

## 2.3. Data analysis plan

Analyses were conducted in four parts. First, descriptive statistics were examined and ANOVAs were conducted to examine group differences in children's kindergarten behavior problems. Second, latent growth models were fit examining cortisol reactivity in the laboratory using multilevel structural equation modeling (MSEM) in MPLUS (Muthén and Muthén, 1998–2012). Next, regression analyses were conducted to examine whether home diurnal hypoactivity predicted laboratory hypoactivity. To do so, latent intercept and slope factors (e.g., cortisol activity) were extracted from the between-level portion of the growth model reported here and the ones reported in our previous work for the home diurnal cortisol analysis; these estimates represent the person-level portion of the models. This resulted in four estimates of HPA activity for each individual: 1 estimate of the diurnal cortisol slope, 1 estimate of the diurnal cortisol intercept, 1 estimate of the laboratory reactivity slope, and 1 estimate of the laboratory reactivity intercept. Following significant association between basal and laboratory hypocortisolism, principal component analysis was conducted to create a hypocortisolism factor using home and laboratory cortisol. Lastly, the HPA axis factor scores were examined as mediators between early adversity and children's adjustment problems during kindergarten.

## 3. Results

### 3.1. Descriptive statistics and group behavioral differences

Cortisol values were highly correlated within collection period and modestly correlated across collection periods (see correlation and descriptive statistics table in Supplemental materials). Symptoms of internalizing, externalizing, and ADHD at kindergarten were moderately correlated within and across reporters. All cortisol values were highly skewed (skewness range 1.95–4.75) and were transformed using the natural log transformation.

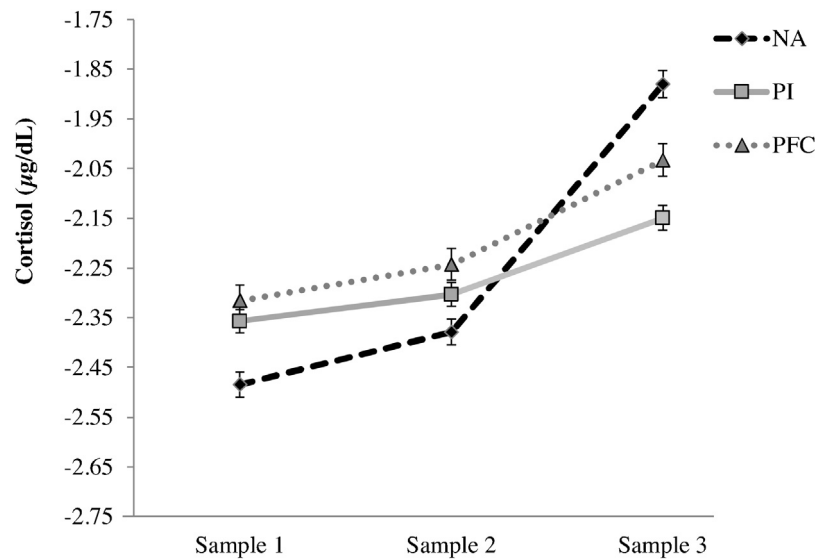
There were no significant group differences in teacher- or parent-reported internalizing problems ( $F(2,123)=.48$ , ns;  $F(2,122)=.95$ , ns, respectively) or parent-reported externalizing problems ( $F(2,122)=2.12$ , ns). There were group differences in teacher- and parent-reported ADHD symptoms ( $F(2,124)=4.49$ ,  $p<.05$ ;  $F(2,122)=3.36$ ,  $p<.05$ , respectively) and teacher-reported externalizing problems ( $F(2,122)=5.03$ ,  $p<.01$ ). Fisher's LSD post-hoc analyses ( $ps<.05$ ) showed that, compared to NA children, PI and PFC children had higher rates of teacher-reported ADHD symptoms, while only PIs had higher rates of teacher-reported externalizing behaviors and parent-reported ADHD symptoms (see supplemental materials for group-specific behavioral problem means).

### 3.2. Laboratory cortisol reactivity

Latent growth models were fit examining cortisol reactivity in the laboratory using multilevel structural equation modeling (MSEM). This approach accounts for nested data; cortisol samples were nested within time and individuals. Group differences and change over time in cortisol reactivity were examined. The group predictors were dummy-coded with NA children set as the comparison group. Given the non-linear nature of reactivity data, the latent basis approach was used. This allows for fitting different shapes to the data by freeing time scores on the latent slope parameter (McArdle and Epstein, 1987); the time scores for the latent slope factor were constrained to 0 for the first sample, 1 for the second sample, and the third sample was free to be estimated. Missing data were estimated using maximum likelihood estimation with robust standard errors using a numerical integration algorithm. Child sex was examined as a potential time-invariant covariate of the between-subjects latent cortisol intercept and slope. Child sex was not a significant covariate and thus was not included in the analyses.

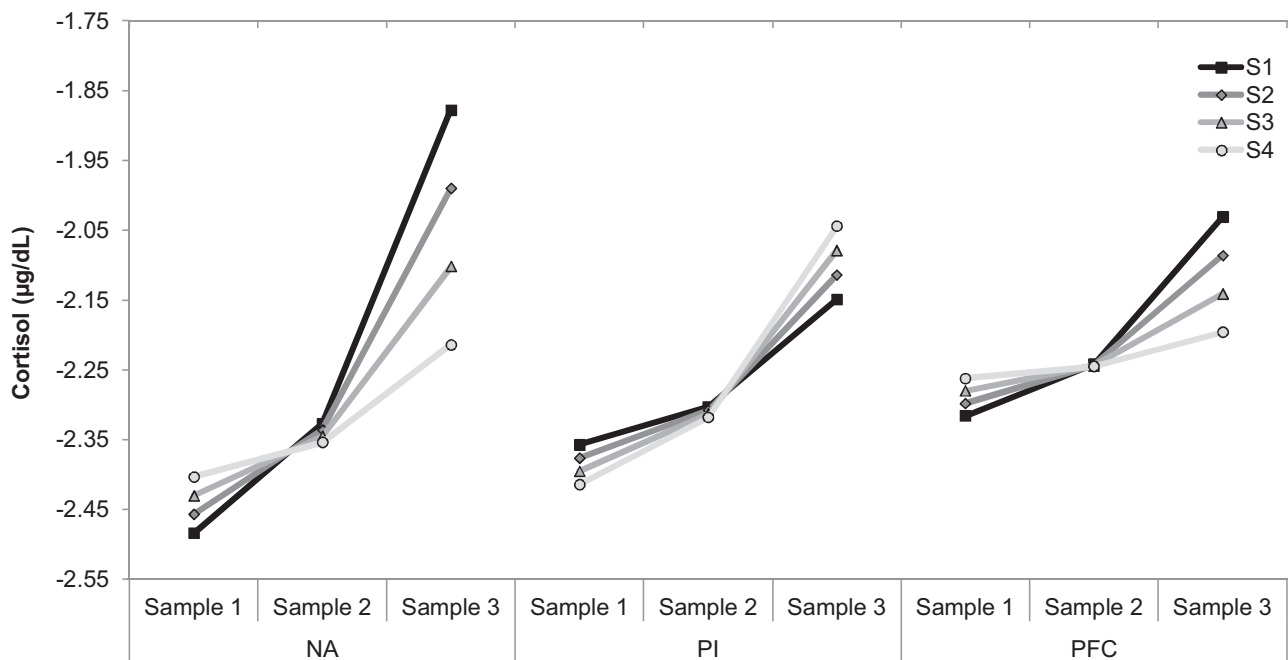
Results for the MSEM linear growth analyses are depicted in Table 1. Overall, children demonstrated increasing cortisol levels over each laboratory session. Group was modeled at the between-level and was a significant predictor of cortisol reactivity (e.g., latent slope) but not the latent intercept. Both PI and PFC children had reduced cortisol reactivity in comparison to NA children (see Fig. 1). Re-parameterization of the dummy-coded group variable demonstrated no difference between PI and PFC children at the person-level (intercept:  $\beta=.05$ ,  $SE=.13$ , ns; slope:  $\beta=.02$ ,  $SE=.03$ , ns; PI as comparison group). Group accounted for a large portion of the variance in cortisol reactivity at the between-level (intercept  $R^2=.06$ , slope  $R^2=.65$ ).

Longitudinal change was modeled at the within-level portion of the model. It was expected that cortisol reactivity would adapt/reduce over time as the children became familiar with similar procedures at each testing session. There was an effect of collection period and the group X collection period interaction suggesting group differences in changes in reactivity across the four sessions. Indicative of adaption, there was a significant negative effect of collection period on cortisol reactivity consistent with reduced reactivity at subsequent sessions. Additionally, there was a significant collection period X group interaction on the slope



**Fig. 1.** Cortisol reactivity in response to laboratory procedures in non-adopted (NA), post-institutionalized (PI), and post-foster care (PFC) children.

Note: The figure depicts the between-level of the MSEM results reflecting an aggregate of cortisol reactivity across the longitudinal study at the person-level for longitudinal estimates at the collection period-level see Fig. 2.



**Fig. 2.** Predicted change over time in children's cortisol reactivity among non-adopted (NA), post-institutionalized (PI), and post-foster care (PFC) children. Graph depicts the estimated collection period by group interaction on children's cortisol reactivity. Change across sessions only statistically significant for the NA group.

for the PI group indicating a difference in the PI-NA comparison. Fig. 2 depicts the predicted longitudinal change in cortisol reactivity for each group across the four collection periods. NA children demonstrated the expected adaptation whereas the PI children had no significant change across the two-year period (and this non-significant change was in the opposite direction of the NA and PFC children). To probe the collection period X group difference interaction on cortisol reactivity, the comparison group in the dummy-coding was changed. Using the PFC children as the comparison group, there was no change across collection periods ( $\beta = -.02$ ,  $SE = .02$ , ns), there was no effect of the NA-PFC group comparison ( $\beta = .01$ ,  $SE = .06$ , ns), and a trend for the PI-PFC comparison ( $\beta = .03$ ,

$SE = .02$ ,  $p = .088$ ) on cortisol reactivity. These results suggest that the PFC children have a pattern of longitudinal change that falls in between the longitudinal patterns represented by the NA and PI groups. Probing this collection period X group interaction suggests that there are no differences between the NA-PFC comparison and there are only trend-level differences in the PI-PFC comparison. These findings along with the graphical representation suggest that the PFC group shows evidence of adaptation/habituation to subsequent experiences in the laboratory setting (similar to the NA children); however, this change is to a lesser degree thus finding only trend-level evidence when compared to the PI youth. Lastly, time-since-waking was included as a time-varying covariate and



**Table 1**  
Multilevel latent growth model results examining children's cortisol reactivity.

	B (SE)	R <sup>2</sup>
<b>Within-level</b>		
Collection period → cortisol intercept	.03 (.04)	
Collection period → cortisol slope	-.04 (.02)*	
Collection period × PI group → cortisol intercept	-.04 (.06)	
Collection period × PI group → cortisol slope	.05 (.02)*	
Collection period × PFC group → cortisol intercept	.04 (.04)	
Collection period × PFC group → cortisol slope	-.01 (.01)	
Medication use → cortisol intercept	.02 (.02)	
Medication use → cortisol slope	-.01 (.01)	
TSW sample 1 → cortisol sample 1	-.06 (.01)***	
TSW sample 2 → cortisol sample 2	-.09 (.01)***	
TSW sample 3 → cortisol sample 3	-.06 (.02)***	
Cortisol intercept residual variance	.29 (.03)***	
Cortisol slope residual variance	.02 (.01)*	
Cortisol intercept-slope covariance	-.04 (.01)***	
Within-subject cortisol intercept R <sup>2</sup>		.01
Within-subject cortisol slope R <sup>2</sup>		.08
<b>Between-level</b>		
Cortisol intercept mean	-2.48 (.11)***	
Cortisol intercept variance	.08 (.02)***	
Cortisol slope mean	.16 (.05)***	
Cortisol slope variance	.001 (.001)	
Cortisol sample 3 time score	3.88 (.66)***	
Cortisol intercept-slope covariance	-.001 (.003)	
PI group → cortisol intercept	.13 (.13)	
PI group → cortisol slope	-.11 (.04)*	
PFC group → cortisol intercept	.17 (.14)	
PFC group → cortisol slope	-.08 (.04)*	
Between-subject cortisol intercept R <sup>2</sup>		.06
Between-subject cortisol slope R <sup>2</sup>		.65

Note: The within-level reflects intra-individual change in children's cortisol reactivity across the four sessions. The between-level reflects inter-individual differences between children. The slope factor captures cortisol reactivity to laboratory procedures. Unstandardized parameter estimates and standard errors (SE) are presented. Bold values are significant. N = 167 children, 618 observations. TSW = time-since-waking. \*\*\* $p < .001$ , \*\* $p < .01$ , \* $p < .05$ .

medication use was included as a time-invariant covariate within each collection period. There was no effect of medications. Predictors and covariates at the within-level accounted for a small portion of the variance in the latent intercept and slope (intercept  $R^2 = .01$ , slope  $R^2 = .08$ ).

### 3.3. Relation between home and laboratory cortisol

Because of problems with high collinearity between the home diurnal intercept (morning cortisol) and slope (diurnal change), separate linear regression analyses were conducted examining these measures predicting the laboratory slope (cortisol reactivity). Child sex, age at the start of the study, age at family entry, and laboratory intercept were included as covariates in the first step. Both lower home morning cortisol ( $\beta = .24$ ,  $p < .001$ ) and blunted diurnal change ( $\beta = -.35$ ,  $p < .001$ ) significantly predicted a more blunted cortisol response to laboratory challenge.

#### 3.3.1. Hypocortisolism factor

Given the relations between home and laboratory cortisol, we entered each of these cortisol measures from the home (Koss et al., 2014) and laboratory latent growth curve analyses into a principle component analysis with varimax rotation to obtain a hypocortisolism index. Two factors with eigenvalues greater than one emerged accounting 87.61% of the total variance. The diurnal intercept (higher levels associated with greater morning cortisol), diurnal slope (lower levels associated with steeper change across the day), and laboratory slope (higher levels associated with greater cortisol reactivity) loaded strongly on the first factor and accounted for 55.01% of the variance (factor loadings from the rotated solution

.82, -.97, and .75, respectively). This factor was then reversed-scored to reflect lower morning, flatter daytime slope, and more blunted laboratory response and labeled as hypocortisolism. The second factor was only comprised of the intercept of the laboratory session (e.g., greater anticipatory response; factor loading .92) and accounted for 32.54% of the variance. The two distinct factors of HPA axis activity were extracted and factor scores were used as predictors of children's behavior.

#### 3.3.2. Racial/ethnic differences among all cortisol variables

There were no race and ethnic differences within the PI and within the PFC groups among any of the cortisol variables (see Supplemental materials for complete analysis).

### 3.4. Hypocortisolism and children's behavior problems

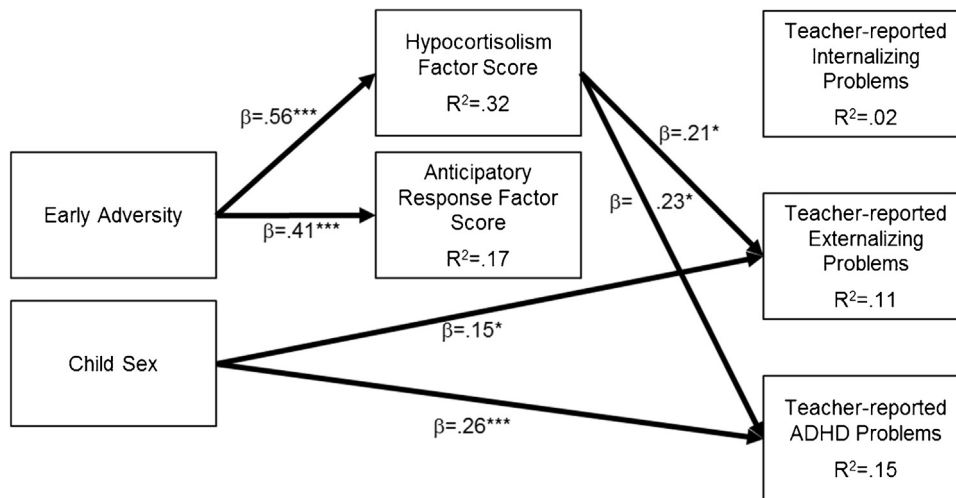
Mediation analyses were conducted in the SEM framework examining the hypocortisolism factor as a mediator between age at family entry and teacher- and parent-report of internalizing, externalizing, and ADHD symptoms during kindergarten. To yield a more continuous measure of adversity, age at the entry into the family was used as an index of early adversity rather than group (a categorical measure). This measure overlapped entirely with group while still allowing for graded differentiation between earlier and later adopted PFC children and earlier and later adopted PI children. Use of this variable as a measure of early life adversity was used to account for the fact that the PFC group was similar to both the PI and NA children when considering different indices of HPA axis activity. Child sex was included as a covariate in the model due to sex differences in adjustment problems. Child age at the start of the longitudinal study and child age at kindergarten were included as covariates of hypocortisolism and child outcomes, respectively. Separate SEM models were fit for teacher- and parent-reported outcomes. Error variances were allowed to correlate among shared measurement scales.

#### 3.4.1. Teacher-reported outcomes

The model with teacher-reported outcomes provided adequate fit to the data and accounted for varying amounts of variance in HPA activity and problem behavior (see Fig. 3;  $\chi^2(10) = 18.60$ ,  $p < .05$ ; CFI = .95; RMSEA = .08; SRMR = .05). Early adversity was associated with both factors of HPA functioning identified in the previous analyses; a longer duration of early life adversity was associated with greater degree of hypocortisolism ( $\beta = .56$ ,  $p < .001$ ) and a higher laboratory intercept ( $\beta = .41$ ,  $p < .001$ ). Only the hypocortisolism factor in turn was associated with higher rates of externalizing problems ( $\beta = .21$ ,  $p < .05$ ) and ADHD symptoms ( $\beta = .23$ ,  $p < .05$ ). The indirect effects of early adversity on externalizing problems and ADHD symptoms through the hypocortisolism factor were significant providing support for the notion that hypocortisolism serves as a mediator for both child outcomes (externalizing  $\beta = .12$ ,  $p < .05$ , ADHD  $\beta = .13$ ,  $p = .05$ ). Using the proportion of the maximum (Preacher and Kelley, 2011) as an effect size measure suggests a medium effect size for both outcomes (externalizing  $K^2 = .10$ ; ADHD  $K^2 = .11$ ). See Fig. 3 for significant covariate effects.

#### 3.4.2. Parent-reported outcomes

The model with parent-reported outcomes also provided adequate fit to the data ( $\chi^2(10) = 20.44$ ,  $p < .05$ ; CFI = .94; RMSEA = .08; SRMR = .05; internalizing  $R^2 = .03$ ; externalizing  $R^2 = .10$ ; ADHD  $R^2 = .12$ ). However, neither of the HPA axis factors were related to any of the parent-reported outcomes and thus mediation was not examined (hypocortisolism factor: internalizing  $\beta = .14$ , ns; externalizing  $\beta = .12$ , ns; ADHD  $\beta = .05$ , ns; anticipatory response



**Fig. 3.** Hypocortisolism as a mediator of early adversity and teacher-reported problems.

Note: Standardized estimates provided. Only significant pathways depicted (correlations and non-significant effects omitted). \* $p < .05$ , \*\*\* $p < .001$ . The hypocortisolism factor score reflects reduced morning diurnal cortisol, shallower diurnal slopes, and blunted cortisol reactivity in the laboratory. The anticipatory response factor score reflects the initial laboratory cortisol sample.

factor: internalizing  $\beta = .05$ , ns; externalizing  $\beta = -.03$ , ns; ADHD  $\beta = -.06$ , ns).

#### 4. Discussion

The results revealed that children adopted during their second and third years from international institutional care show blunted cortisol stress responses. Furthermore, unlike children born and raised in well-educated and highly resourced birth families, the PI children did not exhibit an adaptation of the HPA response to the laboratory events across the testing sessions, even though each session was highly similar to the previous session. Children adopted earlier from international foster care, who presumably experienced less and shorter durations of adversity, also showed an overall blunted cortisol stress response; however, they did exhibit some degree of adaptation over time similar to the direction exhibited by the NA children. We found good evidence for hypocortisolism in that blunted stress reactivity was related to a low morning levels and flatter diurnal slope at home. Finally, the hypocortisolism index that included early morning cortisol (low), diurnal slope (flatter), and laboratory stress response (blunted) mediated between early adversity and teacher-reported ADHD and externalizing problems, both of which differed by group.

To our knowledge, this is the first study to track changes in young children's cortisol stress reactivity during the transition to family care following international adoption. The first two years post-adoption are a time of rapid recovery of physical, social, and cognitive health (van Ijzendoorn and Juffer, 2005). Despite this, we saw little evidence of recovery of HPA axis stress responding. Notably, we observed a significant cortisol response to the laboratory session in all groups, but the responses of the PI and PFC children were blunted. The PI children were blunted in their response at the first session and did not significantly change in this over the two years following adoption. The PI children were characterized by non-significant change that was in the opposite direction than the two comparison groups further suggesting a lack of adaptation to repeated testing. In contrast, the NA children, and to a modest extent the PFC children, showed adaptation longitudinally. The degree of PFC children's longitudinal change was to a lesser extent when compared to NA children; the PFC group's already blunted cortisol reactivity found at the start of the study may have limited the degree of adaptation observed. Because we

saw evidence for blunting of the cortisol stress response in the PFC group, as in our results for the diurnal cortisol rhythm (Koss et al., 2014), we cannot attribute the blunting of the response solely to the experience of institutional care. A multitude of adverse conditions experienced by orphaned and abandoned children around the world from conception to adoption could be contributing to shaping this pattern of hypocortisolism.

Our results challenge the argument that there is a sensitive period prior to two years when the HPA axis will recover its responsiveness as argued for the children fostered out of institutional care in the Bucharest Early Intervention Study (McLaughlin et al., 2015). Because the PFC children who were all adopted before or roughly at a year and the PI children who were all adopted over 15 months of age both showed a similar blunting effect, adoption prior to two years may not be the key to recovery. Of course, we were only examining the children during the first two years post adoption. It may take until the children reach late childhood or adolescence to observe greater recovery among earlier adopted children.

The second major finding was clear evidence that hypoactivity in home diurnal and laboratory stress reactivity are related and, thus, a more general characterization of early adversity as associated with hypocortisolism is justified, at least among internationally adopted children. This is important for several reasons. First, if we had merely found a less marked response to the laboratory challenges one interpretation could always be stress inoculation. That is, because adopted youth were more familiar with harsh and threatening conditions they were better able to cope with the laboratory challenges (Lyons and Parker, 2007). However, better coping in the laboratory should be related, if anything, to a healthier and more robust diurnal cortisol pattern and this was not the case in the present study. The second reason it was important to find that home and laboratory hypocortisol responding were associated is that it further raises questions about why in research with murine models of early life stress hypercortisolism is typically noted (Meaney and Szyf, 2005), while in primate (Capitanio et al., 2006) and human studies, hypocortisolism appears to be the more typical outcome. This is a major challenge to models linking early adversity to later emotional and physical health problems via hyper-activity of the HPA axis. Of course, it poses less of a problem to models arguing that it is extra-hypothalamic CRH and not cortisol that is the active agent of early life stress effects (Baram et al., 2001) or dual pathway

models arguing for differential pathways and outcomes leading to hypo- versus hyper-cortisolism (Strüber et al., 2014).

Finally, the results provide evidence that hypocortisolism mediates between early adverse care and the type of behavior problems frequently noted for children adopted from conditions of neglect and disrupted care. We did not find any evidence that early adversity in the form of institutional or international foster care are associated with internalizing problems at this early age. Given this, it is not surprising that we saw no evidence of mediation for internalizing problems. However, consistent with previous studies, we did see elevated levels of attention problems and, according to teacher-report, externalizing problems (Juffer et al., 2004). Furthermore, for teacher-report, we did find clear evidence that hypocortisolism mediated between early adversity and both ADHD and externalizing symptoms.

Hypocortisolism has been associated with externalizing behavior problems, particularly during the elementary school years, even among low risk children (Alink et al., 2008). Thus, to find that hypocortisolism served as a mediator of externalizing problems is consistent with previous work and might suggest that it is part of the neurobiological pathway to some aspects of externalizing behavior. ADHD symptoms are very commonly reported among children who have experienced deprivation early in life, particularly those reared in poor quality institutional settings (Humphreys and Zeanah, 2015). Nonetheless, there is little evidence that hypocortisolism is a part of the pathophysiology of ADHD. Nor is it the case that hypocortisolism typically is associated with ADHD except in the presence of comorbid conduct problems (e.g., Fairchild, 2012). This does not mean that chronic stress during the first years of life is uninvolved in the pathophysiology of attention problems and more generally in executive function deficits (Arnsten, 2009). Indeed, there is good evidence that reductions in the quality of care received by young mammals impairs the development of frontal brain regions (Bock et al., 2005) and that methylphenidate fosters recovery of function following stress, consistent with its effects on children diagnosed with ADHD (Zehle et al., 2007). It is possible that early adversity induced hyperactivity of the HPA axis both stimulates a down-regulation of the HPA axis and damages the development of neural systems that support attention regulation.

There are a number of limitations in this study. First, because we examined cortisol responses to multiple events in the laboratory as opposed to one defined stressor, we cannot be certain that any one event was more important in producing the overall pattern of rising cortisol across the laboratory sessions. Thus children's cortisol reactivity in the present study likely reflects the demands of the laboratory session as a whole. Additionally, this design and the timing of the samples preclude the ability to assess recovery of the HPA axis following the conclusion of a specific stressor. Second, we have relatively little objective data on the actual care the children received prior to adoption and we could not, obviously, randomly assign children to being orphaned or abandoned or to the type and quality of care they would receive. The fact that our findings are consistent with those of the Bucharest study where they were able to achieve random assignment for removal from institutional care gives some confidence in the findings. We cannot rule out genetic differences between the adopted and non-adopted children as an explanation of the findings; however, because the adopted children came from so many races and countries, it seems unlikely that a simple genetic explanation can account for these findings. Epigenetic differences, nonetheless, seem likely and intriguing as a possible explanation of these findings. Third, it is a bit disconcerting that we obtained mediation effects with teacher- but not parent-report, when previously we had these effects using parent-report (Koss et al., 2014). However, the instruments used in the two analyses were different and our previous parent-report findings assessed different broadband

behavioral difficulties. In addition, entering formal schooling may have precipitated a change in child functioning that the parents have not yet incorporated into their views of their child's problem behaviors. These differences may also reflect differences in children's behavior across settings (e.g., home versus school settings) as parent and teacher reports were only modestly correlated. Furthermore, it should be pointed out that parent-reported outcomes reflect the views of the primary caregiver and in the case of the present study mostly represent maternal reports. Assessments completed by mothers versus fathers may differentially impact the findings and future research should include assessments by both parents. Finally, our sample size was comparable to that in other studies of PI children (e.g., McLaughlin et al., 2015), but nonetheless was relatively small. Thus, as always with small samples, caution is warranted in interpretation of the results.

Nevertheless, the findings argue that the type of early adversity experienced by children adopted internationally as infants and toddlers is clearly associated with hypocortisolism. The down-regulation of the HPA axis at the adrenal level is present both in basal and stress activity. It is present not only shortly after adoption, but at least for the first several years. While it may improve for some children, we do not yet really know for whom this will be the case. Finally, it appears to mediate statistically between early adversity and problems in behavior regulation reflected in attention/hyperactivity, aggression, and conduct issues. What we need to understand now is whether this hypocortisolism is part of the pathophysiology of these problems or merely an index or marker of the degree and duration of early adversity which, through some other mechanism(s), produces the epigenetic and neural changes leading to behavior regulatory problems. Furthermore, we need additional longitudinal research to know whether there will be recovery of HPA axis function with time or development.

### Conflict of interest

All authors report no conflicts of interest.

### Contributors

Megan Gunnar designed the study with input from Shanna Mliner and Bonny Donzella who executed data collection and preparation. Kalsea Koss provided statistical data analysis. Megan Gunnar and Kalsea Koss provided data interpretation and manuscript writing. All authors have contributed to and approved the final manuscript.

### Role of the funding source

Study sponsors provided financial support but did not have a role in the data collection, analysis, or interpretation. The decision to submit the manuscript for publication was the sole decision of the authors.

### Acknowledgments

The authors would like to thank the other members of the Minnesota International Adoption Project team for their efforts in collecting these data, including: Bao Moua, Kristin Frenn, and Meg Bale. We would also like to thank the parents and children without whom this study would not have been possible. This work was supported by grants R01 MH080905 and P50 MH078105 from the National Institute of Mental Health awarded to Megan Gunnar. Support was also provided to Kalsea Koss by National Institute of Mental Health training grants (T32 MH015755 and T32 MH018921) during the preparation of this article.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2015.12.018>.

## References

- Alink, L.R.A., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Mesman, J., Juffer, F., Koot, H.M., 2008. Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev. Psychobiol.* 50, 427–450.
- Arnsten, A.F., 2009. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat. Rev. Neurosci.* 10, 410–422.
- Astley, S., Clarren, S., 2000. Diagnosing the full spectrum of fetal alcohol exposed individuals: introducing the 4-digit diagnostic code. *Alcohol Alcohol.* 35, 400–410.
- Baram, T.Z., Yi, S., Avishai-Eliner, S., Schultz, L., 2001. Developmental neurobiology of the stress response: multilevel regulation of corticotropin-releasing hormone function. *Mol. Psychiatr.* 6, 647–656.
- Bock, J., Gruss, M., Becker, S., Braun, K., 2005. Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlations with developmental time windows. *Cereb. Cortex* 15, 802–808.
- Capitanio, J.P., Mendoza, S.P., Mason, W.A., Maninger, N., 2006. Rearing environment and hypothalamic-pituitary-adrenal regulation in young rhesus monkeys (*Macaca mulatta*). *Dev. Psychobiol.* 46, 318–330.
- Carlson, M., Dragomir, C., Earls, F., Farrell, M., Macovei, O., Nystrom, P., Sparling, J., 1995. Effects of social deprivation on cortisol regulation in institutionalized Romania n infants. *Soc. Neurosci. Abstr.* 21, 524.
- Danese, A., McEwen, B.S., 2012. Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol. Behav.* 106, 29–39.
- Essex, M.J., Boyce, T., Goldstein, L.H., Armstrong, J.M., Kraemer, H.C., Kupfer, D., 2002. The confluence of mental, physical, social and academic difficulties in middle childhood. II: developing the MacArthur health and behavior questionnaire. *J. Am. Acad. Child Psychiatry* 41, 588–603.
- Fairchild, G., 2012. Hypothalamic-pituitary-adrenocortical axis function in attention-deficit hyperactivity disorder. *Curr. Top. Behav. Neurosci.* 9, 93–111.
- Granger, D.A., Hibel, L.C., Fortunato, C.K., Kapelewski, C.H., 2009. Medication effects on salivary cortisol: tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology* 34, 1437–1448.
- Gunnar, M.R., Frenn, K., Wewerka, S., Van Ryzin, M.J., 2009. Moderate versus severe early life stress: associations with stress reactivity and regulation in 10- to 12-year old children. *Psychoneuroendocrinology* 34, 62–75.
- Gunnar, M.R., Vazquez, D., 2006. Stress neurobiology and developmental psychopathology. In: Cicchetti, D., Cohen, D. (Eds.), *Developmental Psychopathology: Developmental Neuroscience*, vol. 2. Wiley, New York, pp. 533–577.
- Heim, C., Ehler, U., Hellhammer, D.K., 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 25, 1–35.
- Hellhammer, J., Schlotz, W., Stone, A.A., Pirke, K.M., Hellhammer, D., 2004. Allostatic load, perceived stress, and health: a prospective study in two age groups. *Ann. N. Y. Acad. Sci.* 1032, 8–13.
- Humphreys, K.L., Zeanah, C.H., 2015. Deviations from the expectable environment in early childhood and emerging psychopathology. *Neuropsychopharmacology* 40, 154–170.
- Juffer, F., Stams, G.J.M., van IJzendoorn, M.H., 2004. Adopted children's problem behavior is significantly related to their ego resiliency, ego control, and socioeconomic status. *J. Child Psychol. Psychiatry* 45, 697–706.
- Kidd, T., Carvalho, L.A., Steptoe, A., 2014. The relationship between cortisol responses to laboratory stress and cortisol profiles in daily life. *Biol. Psychol.* 99, 34–40.
- Koss, K.J., Hostinar, C.E., Donzella, B., Gunnar, M.R., 2014. Social deprivation and the HPA axis in early development. *Psychoneuroendocrinology* 50, 1–13.
- Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Meaney, M.J., 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 227, 1659–1662.
- Lyons, D.M., Parker, K.J., 2007. Stress inoculation-induced indications of resilience in monkeys. *J. Trauma Stress* 20, 423–433.
- McArdle, J.J., Epstein, D., 1987. Latent growth curves within developmental structural equation models. *Child Dev.* 58, 110–133.
- McEwen, B., 1998. Stress, adaptation, and disease: allostasis and allostatic load. *Ann. N. Y. Acad. Sci.* 840, 33–44.
- McLaughlin, K.A., Sheridan, M.A., Tibu, F., Fox, N.A., Zeanah, C.H., Nelson, C.A., 2015. Causal effects of the early caregiving environment on development of stress response systems in children. *Proc. Natl. Acad. Sci.* 112, 5637–5642.
- Meaney, M.J., Szyf, M., 2005. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin. Neurosci.* 7, 103–123.
- Muthén, L.K., Muthén, B.O., 1998–2012. *Mplus User's Guide*, 7th edition. Muthén & Muthén, Los Angeles, CA.
- Preacher, K.J., Kelley, K., 2011. Effect sizes for mediation models: quantitative strategies for communicating indirect effects. *Psychol. Methods* 16, 93–115.
- Strüber, N., Strüber, D., Roth, G., 2014. Impact of early adversity on glucocorticoid regulation and later mental disorders. *Neurosci. Biobehav. R* 38, 17–37.
- van Eck, M., Nicolson, N.A., Berkhof, H., Sulon, J., 1996. Individual differences in cortisol responses to a laboratory speech task and their relationships to responses to stressful daily events. *Biol. Psychol.* 43, 69–84.
- van IJzendoorn, M., Juffer, F., 2005. Adoption is a successful natural intervention enhancing children's IQ and school performance. *Curr. Dir. Psychol. Sci.* 14, 326–330.
- Zehle, S., Bock, J., Grzegorz, J., Gruss, M., Braun, K., 2007. Methylphenidate treatment recovers stress-induced elevated dendritic spine densities in rodent dorsal anterior cingulate cortex. *Dev. Neurobiol.* 27, 1891–1900.