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Individual differences in the activity of the hypothalamic pituitary adrenal axis: Relations to age and cumulative risk in early childhood

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ABSTRACT

This study examined individual differences in the function of the hypothalamic-pituitary-adrenal (HPA) axis with regard to age and cumulative risk during challenging laboratory tasks administered at 6, 12, 24, and 36 months. Saliva samples were collected from a majority-minority sample of $N = 185$ children (57% African American, 50% female) prior to and following these tasks and later assayed for cortisol. Cumulative distal risk was indexed via a composite of maternal marital status, maternal education, income-to-needs ratio, the number of children in the household, and maternal age at childbirth. Probing of hierarchical models in which cortisol levels and age were nested within child revealed significant differences in cortisol as a function of both age and cumulative risk, such that children exposed to high levels of risk exhibited higher levels of cortisol both within and across age. These results highlight the sensitivity of the HPA axis to environmental context at the level of the individual, even as that sensitivity is manifest against the background of species-typical biological development.

1. Introduction

Decades of research link altered hypothalamic-pituitary-adrenal (HPA)-axis activity to adverse caregiving environments, but less is known about whether more distal forms of adversity are reliably associated with individual differences in HPA-axis activity. Studies that have addressed this question have typically examined HPA-axis activity at a single age (or a single cross-age composite), and thus few studies have followed the association between adversity and individual differences in HPA-axis activity through early childhood. Moreover, distal adversity has most commonly been indexed by poverty and measured in majority-Caucasian samples, and thus the operationalization of environmental adversity has been a narrow one that often excludes the effects of cumulative risk and minority status. This study seeks to address these gaps in the literature by examining the relation between exposure to cumulative distal risk and the activity of the HPA axis in a diverse sample across early childhood.

1.1. The activity of the HPA axis in early childhood: relations to age

The HPA axis is one of the principal systems that mediate the physiological response to challenge. When activated in response to novel events, unfamiliar circumstances, or distressing conditions, the HPA axis initiates a signaling cascade resulting in the release of cortisol into the bloodstream (Chrousos and Gold, 1992). Levels of cortisol in blood and saliva are highly correlated (Kirschbaum and Hellhammer, 1989), and therefore assaying saliva offers a minimally-invasive method for studying the HPA axis in young children.

Studies indicate that although HPA-axis activity is responsive to challenge in infancy (e.g., Gunnar, 1992; Lewis and Thomas, 1990), over the course of early childhood the threshold for response changes, such that by preschool most children do not exhibit reactivity to laboratory-based challenge tasks (Gunnar et al., 2009; Lupien et al., 2009). There is evidence that this reduction in HPA reactivity across early childhood is coupled with decreases in HPA-axis activity under various conditions at rest: Watamura et al. (2004) reported significantly lower cortisol levels at 30 months than at 12 months, while Ursache and

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colleagues reported a similar finding for baseline (i.e., pre-challenge) levels of cortisol from 7 to 24 months (Ursache et al., 2014; note that here and throughout "baseline" refers to cortisol levels prior to the onset of a challenging task). Though there is considerable debate regarding the meaning of this apparent shift (e.g., Hostinar et al., 2014), some have speculated (Gunnar and Quevedo, 2007) that it could be analogous to the stress hyporesponsive period (SHRP) described in rodents (see Nelson, 2015 for a review). Animal models suggest that the SHRP serves to protect the anabolic process of brain development in early life from the catabolic effects of glucocorticoids (Levine, 1999).

1.2. The HPA axis and environmental adversity

Although Hostinar et al. (2014) proposed that in humans the developmental transition towards lower levels of HPA-axis activity is instantiated by social support, there is evidence that this shift may be undermined by exposure to environmental adversity. Poverty has been associated with increased diurnal levels of cortisol among infants (Saridjan et al., 2010), elevated levels of baseline cortisol among toddlers (Blair et al., 2011), higher levels of cortisol throughout laboratory procedures among preschoolers (Blair et al., 2005), and higher overnight levels of cortisol among school-aged children (Evans and English, 2002). In the largest prospective study to date, longer exposure to poverty was associated with higher levels of cortisol at 48 months of age (Blair et al., 2013).

Defining environmental adversity exclusively in terms of poverty (e.g., household income below a certain threshold) overlooks other risk factors that, though correlated with poverty, co-occur with poverty at varying frequencies at the individual level of analysis (Sameroff et al., 1993). Studies demonstrate that it is the accumulation of risk factors, rather than the presence of any specific factor, that is most strongly associated with individual differences in development (cf., Appleyard et al., 2005; Masten and Wright, 1998; Trentacosta et al., 2008). It is therefore surprising that few studies have examined HPA-axis activity in early childhood as a function of cumulative risk. Blair and colleagues linked exposure to high levels of cumulative risk with higher aggregate levels of cortisol during a laboratory procedure at 15 months (Blair et al., 2008), whereas Zalewski et al. (Zalewski et al., 2016) found that exposure to higher levels of cumulative risk were associated with 'blunted' or 'flattened' diurnal patterns of HPA axis activity at 36 months of age.

1.3. Current study

The current study employs a prospective longitudinal design to examine the function of the HPA axis prior to and following a series of developmentally-appropriate, challenging laboratory tasks adapted from a commonly-used protocol (Lab-TAB; Goldsmith and Rothbart, 1999). Environmental adversity was operationalized as cumulative distal risk, rather than poverty, and the study sample was majority minority. This allowed us to examine whether the accretion of distal risk factors might reliably be associated with HPA-axis function, and whether the effects of risk were significant after controlling for the effects of race to the extent permitted by our analyses. Based on prior research, we hypothesized that levels of cortisol prior to and following challenging tasks would decrease as a function of age (with the caveat that even at younger ages we may not observe a significant increase in cortisol in response to challenge, given that our tasks do not involve nociceptive stimuli). However, we also hypothesized that children exposed to higher levels of cumulative risk would exhibit higher cortisol levels at each age.

The latter hypothesis was based on both diathesis-stress (and in particular, the concept of allostatic load; McEwen, 1998) and biological sensitivity to context theories (BSC; Boyce and Ellis, 2005). As Hostinar and Gunnar (2013) note, both of these theoretical perspectives would predict that exposure to greater adversity would be associated with higher levels of activity in the physiological systems that mediate the stress response, including the HPA axis. These theories differ, however, in two important ways: first, according to diathesis-stress theory and the concept of allostatic load, higher levels of cortisol are an unfortunate consequence of exposure to adversity that impose a drag (or load) on optimal physiological function, whereas according to BSC, elevated cortisol levels are an adaptive response that prepares the individual for the challenges likely to be encountered in an adverse environment. Second, BSC asserts that elevated levels of physiological activity would also be observed in the context of highly-supportive environments, a prediction that is not tested here given that low levels of risk do not indicate high support. In short, the current study was not designed to evaluate competing accounts for the etiology of altered HPA-axis activity, but rather to test a broader hypothesis about that activity informed by both of these theoretical perspectives.

2. Methods

2.1. Participants

Participants were full-term, healthy infants recruited at 3 months of age by the Durham Child Health and Development Study (DCHDS) via fliers and postings at birth and parenting classes or through phone contact via birth records. The DCHDS included $N = 206$ children (48.5% female), 85 of whom were European American (41.3%) and 121 of whom were African American (58.7%). The final sample for analysis ($N = 185$, 50.3% female) consisted of 80 European-American children (43.2%) and 105 African American children (56.8%). There were 21 children without cortisol data who were excluded from the analysis. These children did not differ from the analysis sample by race ($p = 0.259$), gender ($p = 0.338$), or cumulative distal risk ($p = 0.105$). Note that these participants did not necessarily leave the study, but rather did not provide cortisol data at any age. For further information about attrition from the study between 6 and 36 months, see Holochwost et al. (2016).

2.2. Procedures

At 6, 12, 24, and 36 months children participated in one or more challenging tasks designed to elicit a physiological stress response. The nature of these tasks changed across assessments to be developmentally appropriate. At 6 months, children and their parents participated in the still-face procedure (SFP; Tronick et al., 1978), during which mothers look at their child for 90 s without facial movement or vocalization, and then in the arms restraint task (Goldsmith and Rothbart, 1999), wherein mothers gently hold their child's arms down for 2 min while maintaining a neutral expression. Children participated in the strange situation procedure at 12 months (SSP; Ainsworth et al., 1978), which features two episodes of maternal separation. At 24 months children participated in the barrier task (Goldsmith and Rothbart, 1999), in which an attractive toy is taken from the child and placed behind a transparent barrier for 2 min. Finally, at 36 months children participated in the gift-wrap task (Kochanska et al., 2000), wherein the child attempts to refrain from opening a wrapped present while left alone for 2 min. At each of the four laboratory visits, three saliva samples were obtained from the child. The first sample was obtained a few minutes after the child's arrival at the lab. The mean time of initial sample was 12:34 p.m. ($SD = 2.74$ h) at 6 months, 12:44 p.m. at 12 months ($SD = 2.96$), 12:26 p.m. at 24 months ($SD = 2.96$) and 12:17 p.m. at 36 months ($SD = 2.76$). The other two samples were taken 15 min and 30 min after the conclusion of the challenge tasks following a strict protocol in which researchers used a timer to cue the collection of the post-challenge samples.

2.3. Measures

2.3.1. Salivary cortisol

Prior to the collection of saliva samples, mothers were asked if their child had been sick or was administered prescription medication in the last 24 h (these samples were excluded from analyses, after Granger et al., 2012). All saliva samples were assayed in duplicate using a commercially-available immunoassay designed specifically for measuring salivary cortisol (Salimetrics, Carlsbad, CA). The test volume was 25 uL; the range of sensitivity was 0.007–3.0 ug/dL and average intra- and inter-assay coefficients of variation were less than 15 and 10%, respectively. The mean of each duplicate assay was used in analyses. Cortisol concentrations greater than 3 standard deviations above the mean were excluded from analysis, and prior to analysis all cortisol values were subject to natural logarithmic transformations to correct for positive skew (see Granger et al., 2012; for a review).

2.3.2. Cumulative distal risk

During the 6-month laboratory visit, children's primary caregivers completed a series of self-report measures about their households from which data regarding five common risk factors were taken (Burchinal et al., 2008): marital status, maternal education, pre-tax household income, the number of children (age ≤ 18 years) in the household, and maternal age at childbirth. An income-to-needs ratio (INR; McLoyd, 1998) was calculated for each household by dividing pre-tax income by the federal poverty level (Health and Human Services Poverty Guidelines, 2004) for the number of people that income supported (i.e., the number of people in the household). Responses on measures of each risk factor were coded as evidencing risk (1) or not (0) using thresholds taken from the literature; thus the 28.6% of the sample indicating single marital status were designated at risk, as was the 16.0% of the sample that reported their highest level of education as less than high school (Burchinal et al., 2008; Trentacosta et al., 2008). An income-to-needs ratio of less than 1.3 (accounting for 27.2% of the sample) was considered evidence of risk (Lanza et al., 2011; Rhoades et al., 2011), as was the presence of three or more children in the household (24.3% of the sample; Popp et al., 2008) and a maternal age of 19 or less at the time of the first child's birth (27.8% of mothers; Lanza et al., 2011). Cumulative risk scores were calculated as the proportion of dichotomized risk factors present relative to the number of factors for which data were provided at 6 months (after Mistry et al., 2010), yielding a mean risk score of 0.270 (SD = 0.306).

2.4. Data analysis

Data analyses were conducted in the following steps: first, preliminary analyses were performed to examine levels of cortisol prior to and following challenge tasks at each age, associations between levels of cortisol and potential covariates (i.e., child gender and race), patterns of missing data, and to investigate the issue of measurement invariance within the limitations imposed by our study design. Then four sets of preliminary models were tested, in which cortisol values for each portion of the laboratory visit (baseline, post–15 min, and post–30 min) within each age (6, 12, 24, or 36 months) were modeled. The first model in each set included parameter estimates for the fixed linear effect of trial and the random intercept. Two subsequent models in each set added a fixed quadratic effect for trial and then a random slope. A fifth and final set of preliminary models followed these same procedures, but estimated cortisol levels at baseline across age.

Based on the results of these preliminary models an overall unconditional model was estimated using the following equation, in which the k^{th} portion of the laboratory visit (baseline, post–15 min, or post–30 min) was nested within the j^{th} age (6, 12, 24, or 36 months) of the i^{th} child:

$$\text{cortisol}_{ijk} = \text{intercept} + \text{time}_{ij} + \text{time}^2_{ij} + \text{age}_{ij} + \text{trial}_{jk} + \text{trial}^2_{jk} + (\text{age}_{ij}\ \text{X}\ \text{trial}_{jk}) + (\text{age}_{ij}\ \text{X}\ \text{trial}^2_{jk}) + \text{error}_{ijk}$$

Trial and *trial*^{2} captured the linear and quadratic components of cortisol's course during each lab visit. The inclusion of both these terms was based on the results of our preliminary models, which indicated that there was a significant linear effect for trial at each age and a significant quadratic effect for trial at three of four ages. *Trial* was coded so that 0 corresponded to the baseline cortisol measurement. *Time* refers to the time of day cortisol was collected; including *time* and its square controlled for diurnal variation in cortisol production (after Mills-Koonce et al., 2011). *Age* was entered as a categorical variable for the purposes of estimating both fixed and random effects, given that our fifth and final preliminary model indicated there was a significant non-linear component to baseline cortisol levels across age, a finding that is consistent with previous research conducted with this sample (Hill-Soderlund et al., 2015). Age was coded such that at 6 months = 0, and therefore all analyses involving age used 6 mos. as the reference category. Based on the results of the preliminary models the overall unconditional model included estimates of the random intercept for child and age, but not for the random slope.

The final conditional model added gender and race as covariates and risk as a focal predictor, and was estimated using the following equation:

$$\begin{aligned}\text{cortisol}_{ijk} = {} & \text{intercept} + \text{gender}_i + \text{race}_i + \text{risk}_i + \text{time}_{ij} + \text{time}^2_{ij} \\ & + \text{age}_{ij} + \text{trial}_{jk} + \text{trial}^2_{jk} + (\text{risk}_i\ \text{X}\ \text{age}_{ij}) + (\text{risk}_i\ \text{X}\ \text{trial}_{jk}) \\ & + (\text{risk}_i\ \text{X}\ \text{trial}^2_{jk}) + (\text{age}_{ij}\ \text{X}\ \text{trial}_{jk}) + (\text{age}_{ij}\ \text{X}\ \text{trial}^2_{jk}) \\ & + (\text{risk}_i\ \text{X}\ \text{age}_{ij}\ \text{X}\ \text{trial}_{jk}) + (\text{risk}_i\ \text{X}\ \text{age}_{ij}\ \text{X}\ \text{trial}^2_{jk}) + \text{error}_{ijk}\end{aligned}$$

Note that while this model estimates main effects of gender and race, interaction terms including these covariates were excluded. The model included random intercepts for child and age, but not for the random slope, and all models were estimated using full information maximum likelihood under the assumption that data were missing at random (Allison, 2009; see preliminary analyses below) using SAS 9.3.

Examining the main effects of age allowed us to test our first hypothesis – that levels of cortisol prior to and following challenging tasks would decrease as a function of age – whereas examining the interaction effects among *risk*, *age*, and both *trial* and *trial*^{2} allowed us to test our second hypothesis: that the effects of age on cortisol would be moderated by cumulative risk. To aid in the interpretation of these interactions two additional steps were taken: first, model-implied estimates for cortisol values were calculated for children exposed to low (25th percentile = a cumulative risk score of 0) and high (75th percentile = a risk score of 0.40) levels of risk at each age for each portion of the laboratory visit, which provided information about the associations of risk with cortisol at different points in the visit at each age. Second, interactions were probed following the guidelines established by Aiken and West (1991) and using the online utilities developed by Preacher et al. (2006) to characterize trajectories of HPA-axis activity at each age among children exposed to low and high levels of risk.

3. Results

3.1. Preliminary analyses

Table 1 presents descriptive statistics for and bivariate correlations among gender, race, cumulative risk, and cortisol for each portion of the procedure at each age. Four points are of note: first, mean levels of cortisol exhibited an overall pattern of decline as a function of increasing child age. Second, cumulative risk was positively correlated with multiple cortisol measurements at 6, 12, and 24 months. Third, both male gender and African American race were correlated with higher cortisol levels at certain measurements. Fourth, some cortisol data were missing for each measurement, though over eighty percent of children (83.2%) had cortisol data for at least half of the samples and nearly sixty percent (59.5%) had data for 9 of 12 samples. The degree of

Table 1
Pearson correlations among and descriptives for demographics, cumulative risk, and salivary cortisol.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1. Gender (male)	–														
2. Race (AA)	0.12	–													
3. Cumulative Risk	0.09	0.39**	–												
4. Cort (6 m, basal)	0.02	0.03	0.02	–											
5. Cort (6 m, +15 min)	0.02	0.24*	0.23*	0.40**	–										
6. Cort (6 m, +30 min)	−0.08	0.28**	0.20*	0.34**	0.73**	–									
7. Cort (12 m, basal)	−0.21*	0.12	0.23*	0.32**	0.42**	0.46**	–								
8. Cort (12 m, +15 min)	−0.13	0.18	0.22*	0.17	0.08	0.08	0.60**	–							
9. Cort (12 m, +30 min)	−0.07	0.20*	0.25*	−0.02	0.11	0.20	0.44**	0.76**	–						
10. Cort (24 m, basal)	0.09	0.19*	0.28**	0.06	0.16	0.24*	0.19	0.27*	0.13	–					
11. Cort (24 m, +15 min)	0.02	0.21*	0.20*	0.07	0.10	0.13	0.28*	0.34**	0.30**	0.56**	–				
12. Cort (24 m, +30 min)	−0.14	0.13	0.09	−0.06	0.26*	0.24	0.32**	0.25*	0.19	0.39**	0.69**	–			
13. Cort (36 m, basal)	−0.17*	0.25**	0.05	−0.08	−0.07	0.05	0.12	0.23*	0.26*	0.19*	0.16	0.18	–		
14. Cort (36 m, +15 min)	−0.04	0.17*	0.06	−0.02	0.02	0.06	0.17	0.18	0.22*	0.09	0.30**	0.22*	0.66**	–	
15. Cort (36 m, +30 min)	0.05	0.11	−0.01	0.09	0.14	0.15	0.02	0.10	0.15	0.08	0.23**	0.25**	0.43**	0.70**	–
N	185	185	185	85	111	101	92	114	104	132	140	130	141	148	146
M	0.50	0.57	0.27	−1.79	−1.86	−1.88	−1.60	−1.80	−1.93	−1.96	−2.35	−2.38	−2.02	−2.41	−2.50
SD	0.50	0.50	0.31	0.72	0.74	0.74	0.72	0.73	0.66	0.58	0.57	0.56	0.54	0.53	0.50
SE	0.04	0.04	0.02	0.08	0.07	0.07	0.07	0.07	0.06	0.05	0.05	0.05	0.05	0.04	0.04

Note: For pairwise correlations N = [85,185]. AA = African American; Cort = salivary cortisol (natural logarithm). ** $p < 0.05$; * $p < 0.01$.

missingness across all samples and missingness for any individual sample were regressed on gender, race, and cumulative risk, revealing that AA children were more likely than their EA counterparts to be missing the post–30 min sample at 12 months. Missingness of cortisol data was therefore classified as MAR. A series of confirmatory factor analyses indicated that cortisol samples collected at each point in the procedure exhibited a similar relation to a single latent variable at each age (see online supplementary material).

Fig. 1 presents mean cortisol levels for each portion of the procedure at each age. As suggested by the means and standard errors, there was no significant difference in cortisol for measures taken at baseline and 15 min after the challenge task ($p = 0.072$) or baseline and 30 min after the challenge task at 6 months ($p = 0.066$). However, significant decreases in cortisol were observed for baseline versus +30 min at 12 months ($p = 0.006$), and for both baseline versus +15 min and baseline versus +30 min at 24 ($p < 0.001$) and 36 months ($p < 0.001$).

The preliminary models described above indicated that there were significant fixed effects for *trial* at each age, a significant effect for $trial^2$ at 6, 24, and 36 months, and a significant effect for age^2 for baseline

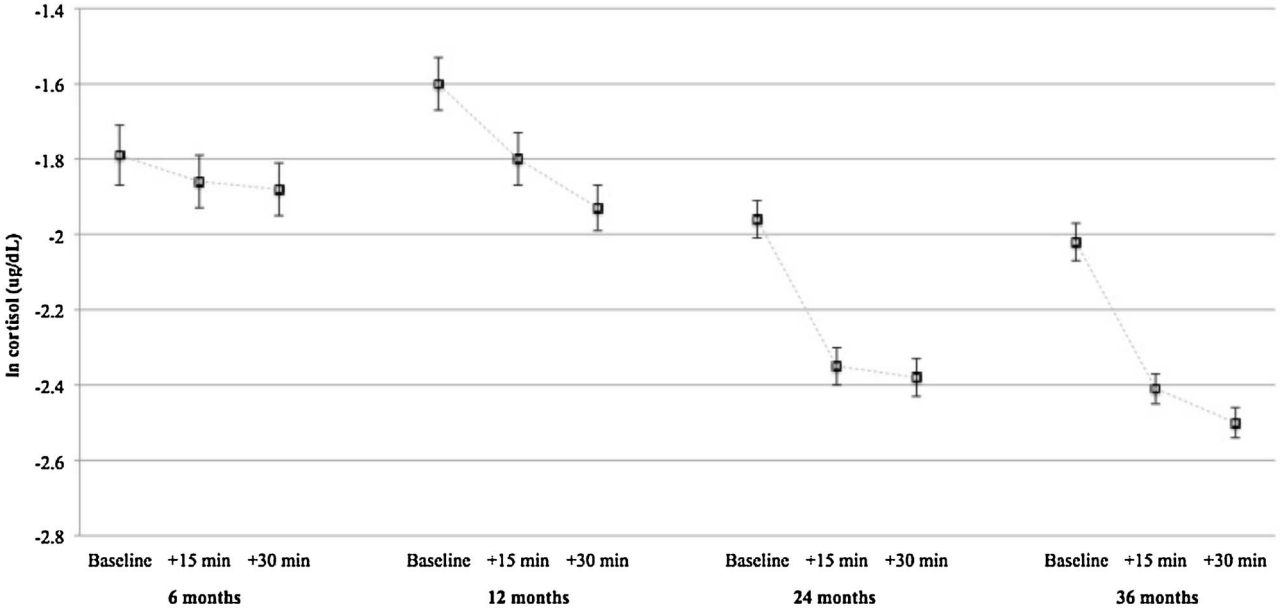

Fig. 1. Observed cortisol concentrations for each portion of the procedure at 6, 12, 24, and 36 months. Error bars represent two standard errors about the mean.

Table 2
Parameter estimates for unconditional mixed model of cortisol and age.

Fixed Effects	B	*p*
Intercept	−1.23	0.021
Time	−0.040	0.624
Time^2	0.0001	0.966
Age (6 vs 12 months)	0.106	0.202
Age (6 vs 24 months)	−0.195	0.013
Age (6 vs 36 months)	−0.277	< 0.001
Trial	−0.160	0.130
Trial^2	0.040	0.415
Age X Trial (6 vs 12 months)	0.022	0.883
Age X Trial (6 vs 24 months)	−0.438	0.002
Age X Trial (6 vs 36 months)	−0.396	0.004
Age X Trial ^{2} (6 vs 12 months)	−0.037	0.590
Age X Trial ^{2} (6 vs 24 months)	0.146	0.027
Age X Trial ^{2} (6 vs 36 months)	0.115	0.074
Random Effects	Est.	*p*
Intercept for Child	0.067	.001 < 0.001
Intercept for Age	0.151	< 0.001
Residual	0.164	< 0.001
-2 Log-Likelihood	2642.2	

values of cortisol across age. These models also indicated that when the variance in cortisol at each age was partitioned the random intercept accounted for a substantial portion of the variance in cortisol at each age, while the random effect for the slope did not (see supplementary materials). Based on these results, the unconditional model presented in Table 2 included a quadratic term for *trial*, as well as random intercepts for child and age. As a result, while baseline values of cortisol at each age were allowed to vary at random by child, the linear and curvilinear rates of change in cortisol from baseline were constrained to be the same across children.

In the unconditional model parameter estimates for *age* were significant for the 6 versus 24 ($B = -0.195$, $p = 0.013$) and 6 versus 36-month contrasts ($B = -0.277$, $p < 0.001$), indicating that baseline levels of cortisol were lower at these ages than at 6 months. Significant interactions between *age* and *trial* for these same contrasts suggest a relation between age and the linear course of cortisol during the procedure (6 vs. 24 months: $B = -0.438$, $p = 0.002$; 6 vs. 36 months: $B = -0.396$, $p = 0.004$), whereas the significant interaction between *age* and *trial*^{2} for the 6 versus 24 month contrast indicates an association between age and the curvilinear course of cortisol as well ($B = 0.146$, $p = 0.027$). Note that the terms *trial* and *trial*^{2} represent the instantaneous rate of change when *trial* or *trial*^{2} equals zero, and, given that zero corresponds to baseline, the *trial* and *trial*^{2} terms can be thought of as the instantaneous rate of change in cortisol from baseline.

3.2. Results of the conditional model

3.2.1. Effects of age

Our first hypothesis was that levels of cortisol both at baseline and following the challenge tasks (i.e., at the post-15 and 30 min measurements) would decrease as a function of age. The parameter estimates for the final conditional model, presented in Table 3, contribute preliminary support for this hypothesis: the estimates of *age* for the 6 versus 24

Table 3
Parameter estimates for conditional mixed model of cortisol, age, and cumulative risk.

Fixed Effects	B	*p*
Intercept	−1.01	0.061
Gender	−0.076	0.160
Race	0.214	< 0.001
Risk	−0.240	0.275
Time	−0.080	0.336
Time^2	0.001	0.643
Age (6 vs 12 months)	−0.022	0.838
Age (6 vs 24 months)	−0.266	0.008
Age (6 vs 36 months)	−0.339	< 0.001
Trial	−0.358	0.007
Trial^2	0.108	0.085
Risk X Age (6 vs 12 months)	0.529	0.066
Risk X Age (6 vs 24 months)	0.343	0.197
Risk X Age (6 vs 36 months)	0.261	0.315
Risk X Trial	0.911	0.015
Risk X Trial^2	−0.315	0.074
Age X Trial (6 vs 12 months)	0.261	0.173
Age X Trial (6 vs 24 months)	−0.240	0.190
Age X Trial (6 vs 36 months)	−0.323	0.067
Age X Trial ^{2} (6 vs 12 months)	−0.133	0.137
Age X Trial ^{2} (6 vs 24 months)	0.087	0.315
Age X Trial ^{2} (6 vs 36 months)	0.120	0.151
Risk X Age X Trial (6 vs 12 months)	−1.10	0.031
Risk X Age X Trial (6 vs 24 months)	−0.955	0.047
Risk X Age X Trial (6 vs 36 months)	−0.475	0.310
Risk X Age X Trial^2 (6 vs 12 months)	0.431	0.073
Risk X Age X Trial ^{2} (6 vs 24 months)	0.305	0.180
Risk X Age X Trial ^{2} (6 vs 36 months)	0.060	0.786
Random Effects	Est.	*p*
Intercept for Child	0.052	< 0.001
Intercept for Age	0.150	< 0.001
Residual	0.162	< 0.001
-2 Log-Likelihood	2166.2	

Table 4
Model-implied estimated cortisol concentrations.

Age (mos.)	Risk	Cortisol concentration (ln (ug/dL)) Basal			15-min post-challenge			30-min post-challenge		
		Est.	*SE*	95% CI	Est.	*SE*	95% CI	Est.	*SE*	95% CI
6	Low	−1.77	0.081	[−1.85, −1.70]	−2.02	0.072	[−2.09, −1.95]	−2.10	0.070	[− −2.17, −2.03]
	High	−1.87	0.112	[−1.98, −1.76]	−1.82	0.103	[−1.92, −1.72]	−1.88	0.102	[−1.98, −1.78]
12	Low	−1.81	0.082	[−1.89, −1.73]	−1.95	0.071	[−2.02, −1.88]	−2.09	0.072	[−2.16, −2.02]
	High	−1.58	0.102	[−1.68, −1.48]	−1.74	0.103	[−1.84, −1.64]	−1.87	0.103	[−1.97, −1.77]
24	Low	−2.20	0.072	[−2.27, −2.13]	−2.44	0.071	[−2.51, −2.37]	−2.51	0.073	[−2.58, −2.44]
	High	−1.73	0.095	[−1.83, −1.64]	−0.2-.24	0.097	[−2.34, −2.14]	−2.46	0.097	[−2.56, −2.36]
36	Low	−2.11	0.060	[−2.17 −2.05]	−2.56	0.058	[−2.62, −2.50]	−2.56	0.058	[−2.62, −2.50]
	High	−2.10	0.082	[−2.18, −2.02]	−2.48	0.079	[−2.56, −2.40]	−2.61	0.077	[−2.69, −2.53]

($B = -0.266$, $p = 0.008$) and 6 versus 36-month ($B = -0.339$, $p < 0.001$) contrasts were significant, indicating that baseline levels of cortisol across the three measurements were lower at 24 and 36 months than at 6 months among children exposed to low levels of risk (i.e., for whom risk = 0). These results were consistent with those reported for the unconditional model in Table 2. However, the unconditional and conditional models diverge in that whereas the interactions between *age* and both *trial* and *trial*^{2} were significant for the unconditional model, they were not significant in the conditional model, which added the variables *risk*, *gender,* and *race*. Rather, in the conditional model significant interactions were observed among *age*, *risk*, and *trial*.

3.2.2. Effects of risk

These significant three-way interactions tentatively support our second hypothesis: that the relation of age and cortisol is moderated by cumulative risk. Significant interactions among *age*, *risk*, and *trial* for both the 6- versus 12- ($B = -0.955$, $p = 0.047$) and 6- versus 24-month contrasts ($B = -1.10$, $p = 0.031$) were observed. However, no higher-order interaction including *age* and *risk* was significant for the 6 versus 36-month contrast, allowing us to conclude that the relation of age and cortisol at 36 months was not moderated by risk. Note that these results were consistent with those for an alternative version of the model that excluded the interactions among *age*, *risk*, and *trial*^{2} (see online Supplementary materials).

To aid in the interpretation of these interactions model-implied estimates of cortisol concentrations were calculated for children exposed to low and high levels of risk at each portion of the procedure for every age. These estimates are presented in Table 4 and Fig. 2. Note that an alternative version of the conditional model that included a random effect for slope was also estimated (see Supplementary materials). Given that both the parameter and model-implied estimates for this alternative model were very similar to those presented in Table 4 and Fig. 2, we report the results for these more parsimonious models. Following the estimates depicted in Fig. 2 from left to right revealed the following pattern of results:

Children exposed to low and high levels of risk exhibited similar baseline cortisol values at 6 months, but children exposed to high levels of risk exhibited significantly higher levels of cortisol at both 15 min (low risk = −2.02 (0.072), high risk = −1.82 (0.103)) and 30 min post-challenge (low risk = −2.10 (0.070), high risk = −1.88 (0.102)). At 12 months children exposed to high levels of risk displayed higher cortisol at each point in the procedure: baseline (low risk = −1.81 (0.082), high risk = −1.58 (0.102)), 15 min (low risk = −1.95 (0.071), high risk = −1.74 (0.103)), and 30 min post-challenge (low risk = −2.09 (0.072), high risk = −1.87 (0.103)). At 24 months children exposed to high levels of risk exhibited higher levels of cortisol at both baseline (low risk = −2.20 (0.072), high risk = −1.73 (0.095)) and 15 min post-challenge (low risk = −2.44 (0.071), high risk = −2.24 (0.097)). Consistent with the *ns* three-way interaction among *age*, *risk*, and *trial* for the 6 versus 36-month contrast, there were

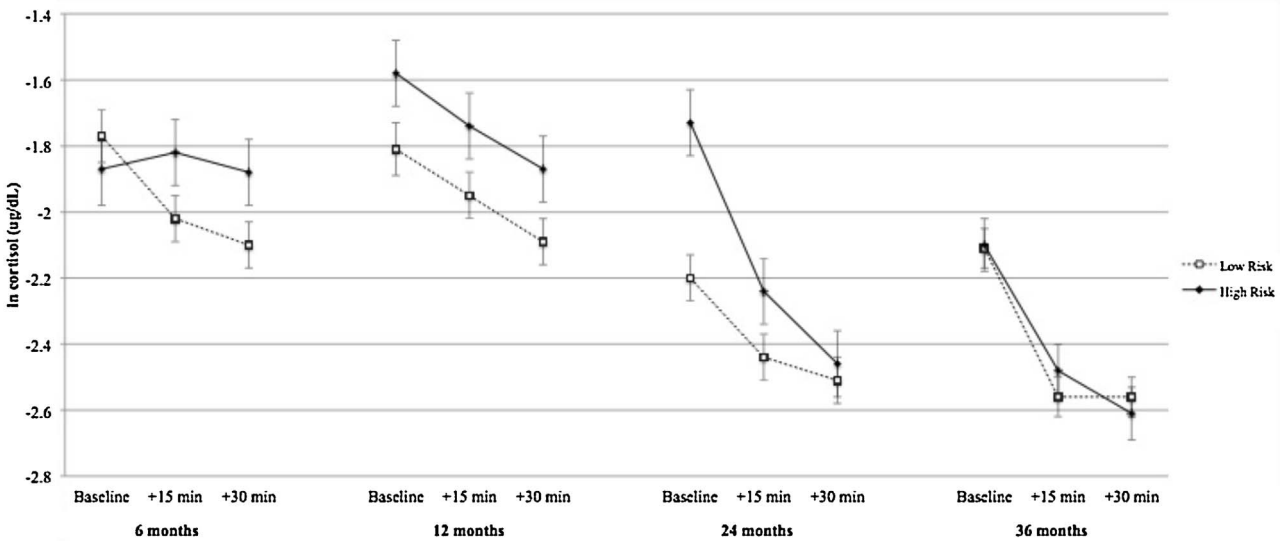

Fig. 2. Model-implied cortisol concentrations for each portion of the procedure at 6, 12, 24, and 36 months for children at low (25th percentile) and high (75th percentile) levels of cumulative risk. Error bars represent two standard errors about the mean.

no significant differences in cortisol as a function of risk for 36 months.

The model-implied estimates presented in Table 4 and Fig. 2 also revealed differences in baseline cortisol *across age of assessment* as a function of risk. Children exposed to high levels of cumulative risk exhibited significantly higher levels of baseline cortisol at 12 months (−1.58 (0.102)) than at 6 months (−1.87 (0.112)), and their baseline levels at 24 months (−1.73 (0.095)) were comparable to those observed at 12 months. In contrast, children exposed to low levels of risk exhibited similar levels of baseline cortisol at 6 (−1.77 (0.081)) and 12 (−1.81 (0.082)) months and significantly lower baseline cortisol levels at 24 months (−2.20 (0.072)) than at 12 months.

Finally, the significant interactions among *age*, *risk*, and *trial* for the 6- versus 12- and 6- versus 24-month contrasts were probed following the procedures outlined in the data analysis section above. These analyses revealed differences in cortisol activity as a function of risk *within age of assessment* at 6 months, but not at 12 or 24 months. At six months the simple slope for cortisol for children exposed to low levels of risk was negative and significant, indicating that these children exhibited a linear decline in cortisol from baseline ($B = -0.358$, $p = 0.008$). This is consistent with the point estimates presented in Table 4 and Fig. 2, which indicate that cortisol levels among low-risk children were significantly lower at 15 (−2.02 (0.072)) and 30 min (−2.10 (0.070)) post-challenge than at baseline (−1.77 (0.081)). However, children exposed to high levels of risk did not exhibit a comparable decline ($B = 0.143$, $p = 0.377$), and there was no significant difference in cortisol levels at baseline, 15 min, and 30 min post-challenge. Although at 12 months children exposed to low ($B = -0.197$, $p = 0.282$) and high levels of risk ($B = -0.201$, $p = 0.162$) did not exhibit a significant linear change in cortisol from baseline, both groups of children did exhibit significant linear declines in cortisol from baseline at 24 months (low risk: $B = -0.598$, $p < 0.001$; high risk: $B = -0.622$, $p < 0.001$), and again point estimates indicated that cortisol values were lower at 15- (low risk = −2.44 (0.071), high risk = −2.24 (0.097)) and 30-min (low risk = −2.51 (0.058), high risk = −2.46 (0.097)) post-challenge than at baseline.

4. Discussion

In this study we examined the associations of age and cumulative distal risk with the function of the HPA axis in early childhood. We hypothesized that although advancing age would be associated with lower levels of cortisol, the relation of age with cortisol would be moderated by cumulative risk. Specifically, we hypothesized that higher levels of risk would be associated with higher levels of cortisol both prior to and following challenging tasks. These hypotheses were largely supported, though our findings revealed a number of nuances regarding the associations of age and risk with levels of cortisol in young children.

Our initial inspection of the descriptives suggested that cortisol decreased as a function of age, and the results of our unconditional model supported this: baseline levels of cortisol were lower at 24 and 36 months than at 6 months, and there was a negative relation between age and the linear component of cortisol's trajectory from baseline over the course of the procedure at these ages. It is of note that in our sample levels of cortisol were comparable at 6 and 12 months. This may suggest that the most substantial reductions in HPA-axis activity during early childhood occur between 12 and 24 months. Though more research is clearly required on this point, this assertion is consistent with both the non-linear effects for age in our preliminary models (see Supplementary material, Model 5B) and a recent literature review (Gunnar et al., 2009).

Cumulative risk was not associated with differences in cortisol prior to or following challenging tasks at 36 months, but it was associated with such differences at 6, 12, and 24 months. At 6 months, children exposed to high levels of cumulative risk exhibited higher cortisol 15 and 30 min after the challenge task than children exposed to low levels of risk. Moreover, unlike their peers children exposed to high levels of risk did not exhibit a significant, linear decrease in cortisol from baseline, as indicated by the non-significant simple slope and a comparison of baseline and subsequent cortisol estimates. At 12 months, children exposed to high levels of risk exhibited higher cortisol at each point in the procedure, and though the simple slopes and point estimates indicated that children exposed to both low and high levels of risk exhibited decreases in levels of cortisol activity at 24 months, children exposed to high levels of risk displayed higher levels of cortisol at baseline and 15 min after the challenge task.

Among children exposed to low levels of cumulative risk, baseline levels of cortisol were lower at 24 months than at 6 months. This finding is consistent with results reported by both Watamura et al. (2004) and Ursache et al. (2014). The sample in Watamura's study was middle- to upper-middle class in its composition (Watamura, personal communication), which may in part explain the consistencies between her results and those observed among children in our sample exposed to low levels of risk. In our sample children exposed to high levels of cumulative risk exhibited higher baseline levels of cortisol at 12 months than at 6 months, a finding that builds upon previous research demonstrating an association between higher baseline cortisol levels and lower income in a cross-sectional study of young children (Blair et al., 2005).

Though the findings regarding baseline levels of cortisol can be interpreted without concern about the confounding of age and the specific challenge task employed, this is not the case when comparing post-challenge levels of cortisol across ages. Bearing this confound in mind, our results may be interpreted as follows: assume, as Gunnar and Cheatham (2003) suggest, that decreasing levels of HPA-axis activity over the course of early childhood evolved to protect the developing brain from exposure to high levels of a catabolic glucocorticoid. However, both BSC and diathesis-stress theory assert that this pattern of decreasing HPA-axis activity may be altered under conditions of high environmental risk. According to BSC theory, in an environment of high risk it may be more adaptive for the child to maintain higher overall levels of HPA-axis activity, providing increased metabolic resources to prepare for and face the challenges that a high-risk environment is disproportionately likely to present, challenges that are far more severe than those encountered in a laboratory setting. In contrast, according to diathesis-stress theory and the concept of allostatic load higher levels of HPA-axis activity are a maladaptive consequence of exposure to risk. In either case, in our results we may be witnessing adaptations of biological systems at the level of the individual as they unfold against the background of species-typical, genetically-encoded tendencies towards lower levels of HPA-axis activity with advancing age. Our results suggest that these tendencies are strong: by 24 months even children exposed to high levels of risk exhibit decreasing HPA-axis activity across the procedure. However, the fact that at multiple points in the procedure at 6, 12, and 24 months these children exhibit higher levels of activity than their peers speaks to the countervailing strength of environmental adversity and its capacity to prompt endophenotypic re-calibration.

This same account may also partially explain the finding that there was a main effect for race, indicating a positive association between African American race and baseline cortisol levels. As reported in a previous study by Blair et al. (2011), this finding was observed after controlling for measured aspects of risk, raising the possibility that African American race is serving as a proxy for unmeasured aspects of risk that may, like measured aspects, to some extent counteract the age-associated tendency towards lower levels of HPA-axis activity. These unmeasured aspects of risk may include intergenerational effects on the physiological response to stress (Blair et al., 2011) and discrimination on the basis of race in daily life (Sanders-Phillips et al., 2009). However, future research with large, diverse samples that allow modeling interactions among race, age, and cortisol at multiple points in time

(i.e., prior to and following challenge) will be required to further our understanding of the extent to which the relations of race and risk with HPA-axis activity are dissociable.

4.1. Limitations and future directions

The findings presented above are subject to three specific limitations. First, we have interpreted both the *absence of a significant increase* in cortisol from baseline and a *significant decrease* in cortisol as evidence for the hyporesponsivity of the HPA axis. The breadth of this interpretation is deliberate, as the current literature offers scant basis for asserting whether the SHRP manifests as the absence of an HPA-axis response to challenge or a negative response. Hostinar et al. (2014) argue that beginning in infancy the presence or assistance of conspecifics creates a permissive context for the dampening of HPA-axis activity (see also Lupien et al., 2009). However, the empirical evidence does not as of yet indicate whether this dampening is characterized by the lack of an HPA-axis response to challenge or an active suppression of HPA-axis activity.

Second, as is the case in most studies that employ salivary cortisol to index HPA-axis activity, three saliva samples were taken over the course of the procedure at each age. The fact that no significant increase in cortisol from baseline following challenge was observed at any age raises the possibility that peak levels of cortisol may have been reached between the samples taken at 15 and 30 min post-challenge, though this is unlikely given the protracted course of cortisol's response. It is more likely that the challenge tasks used at each age were not experienced by children as equally challenging. To overcome this limitation our science must establish measurement invariance across challenging tasks. Our study was not designed to accomplish this, and therefore we can offer only very preliminary evidence that weak factorial invariance may apply to our tasks. Future studies including multiple cortisol samples at each point in the procedure are required to address this question.

Finally, our study is subject to the limitations that apply to any prospective longitudinal study. Like other studies of its type (e.g., Blair et al., 2011), it allows us to investigate the association between risk experienced on one point in time and subsequent development, but it cannot speak to the contemporaneous association between risk and HPA-axis activity. Although we did not observe differences in cortisol levels as a function of risk at 36 months, we cannot conclude that there is no association between cumulative risk and HPA-axis activity at this age. Rather, it is possible that the countervailing influence of a biological predisposition towards lower levels of HPA-axis activity obscures this relation, that the prospective effects of risk measured at 6 months have begun to wane at 36 months, or that children progressively habituate to the tasks. Adding repeated measures of cumulative risk at each age (which were not available here) as a time-varying focal predictor would allow for an examination of both the longitudinal and contemporaneous associations between cumulative risk and HPA-axis activity, whereas including pre- and neo-natal measures of risk would provide further insight into when these associations become embedded. Some scholars (e.g., Pluess and Belsky, 2011) have argued that though the systems that mediate the physiological response to stress remain open to environmental input after birth, much of the calibration of these systems occurs *in utero*, and indeed there is evidence that the activity of the HPA axis early in life systematically varies as a function of the pre-natal environment (Seckl and Holmes, 2007). The fact that in the current study differences as a function of risk were already observed at 6 months suggests that embedding may occur earlier in development, and perhaps prenatally.

One contribution of this study is the demonstration that a cumulative index of distal risk factors was associated with HPA-axis function at certain ages in a diverse sample. This raises two questions for future research: could this finding be replicated in other samples featuring different racial or ethnic composition, by expanding, for example, the methods employed by Luecken and colleagues in their work with Mexican-American families (Luecken et al., 2013)? And would a similar finding result from studies conducted with samples of other nations or cultures, and if so, how might cultural context account for observed differences (Super and Harkness, 1986)?

One of the advantages of employing risk indices that include only distal risk factors is that data on these factors are relatively easy to collect, making it possible to conduct studies of risk and cortisol in samples that are sufficiently large and diverse to address these questions. Another advantage of focusing on distal risk is that with some recalibration measures of these factors can be used across cultures. For example, although the precise value of the income-to-needs ratio that is indicative of risk might vary by culture, in nearly every culture there is some ratio value associated with a level of material deprivation that constitutes a risk to optimal child development.

Making the bold assumption that this research could produce reliable evidence for a human analog to the SHRP across races and cultures, it would prompt questions regarding how contextual factors such as distal risk influence the magnitude and chronicity of this phenomenon. For example, previous research has demonstrated that higher levels of cumulative distal risk constrain positive parenting behaviors and promote negative ones (Holochwost et al., 2016; Popp et al., 2008). If it is the case that the emergence and consolidation of reduced HPA-axis activity in early childhood is contingent, in part, on the permissive context provided by parents and caregivers (Hostinar et al., 2014) then the accretion of distal sources of risk could influence HPA-axis activity by undermining that context. Adding measures of parenting to future studies would not only allow for an examination of whether negative parenting behaviors mediated the effects of distal risk on HPA-axis activity, but would also allow additional hypotheses unique to BSC theory to be tested. As noted in the introduction, low levels of distal risk do not imply high levels of social support. Positive parenting behaviors, however, do indicate this support. According to BSC theory, environments featuring high levels of adversity (as indexed by risk) *or* high levels of support (as indexed by positive parenting behaviors) should both be associated with higher levels of HPA-axis activity. One clear direction for future research would therefore be to examine whether these divergent environmental contexts lead to similar patterns of HPA-axis function, as BSC theory suggests.

4.2. Conclusion

The results reported here contribute to our understanding of how age and distal risk may jointly influence HPA-axis function in early childhood. They await replication in large-scale studies featuring diverse samples of participants from different cultures. These studies could also contribute evidence for a human analog to the SHRP and insight regarding its nature (i.e., active or passive suppression of HPA-axis activity), while addressing how parenting contributes to the instantiation of both biological preparedness for and sensitivity to developmental context. The results from these studies would add to an emergent literature that is moving beyond a demonstration of risk's capacity to get 'under the skin' to examine precisely how risk exerts its pernicious effects on development.

Conflict of interest

In the interest of full disclosure, Douglas A. Granger is founder and Chief Scientific and Strategy Advisor at Salimetrics LLC and SalivaBio LLC (Carlsbad, CA) and these relationships are managed by the policies on conflict of interest at the Johns Hopkins University School of Medicine and Office of Research Integrity and Adherence at Arizona State University. Granger and the Institute for Interdisciplinary Salivary

Bioscience Research moved from Arizona State University to the University of California Irvine in 2016 where similar policies will be established. No other authors have conflicts of interest to disclose.

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Contributors

Steven J. Holochwost designed the study, analyzed all data, and drafted the manuscript. Jean-Louis Gariépy contributed to the design of the study and revised it for intellectual content. W. Roger Mills-Koonce and Cathi B. Propper oversaw the acquisition of the data and revised the manuscript for intellectual content. Jacek Kolacz and Douglas A. Granger advised on the interpretation of data analysis and revised the manuscript for intellectual content.

All authors have approved the article in its present form.

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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.2017.03.023.

References

Aiken, L.S., West, S.G., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage Publications, Inc, Thousand Oaks, CA US.

Ainsworth, M.D.S., Blehar, M.C., Waters, E., Wall, S., 1978. Patterns of Attachment. Erlbaum, Hillsdale, NJ.

Allison, P.D., 2009. Missing data. In: Millsap, R.E., Maydeu-Olivares, A. (Eds.), The Sage Handbook of Quantitative Methods in Psychology. Sage Publications Ltd, Thousand Oaks, CA, pp. 72–89.

Appleyard, K., Egeland, B., van Dulmen, M.H.M., Sroufe, L.A., 2005. When more is not better: the role of cumulative risk in child behavior outcomes. J. Child Psychol. Psychiatry 46, 235–245. http://dx.doi.org/10.1111/j. 1469-7610.2004.00351.x.

Blair, C., Granger, D., Razza, R., 2005. Cortisol reactivity is positively related to executive function in preschool children attending head start. Child Dev. 76, 554–567. http://dx.doi.org/10.1111/j.1467-8624.2005.00863.x.

Blair, C., Granger, D.A., Kivlighan, K.T., Mills-Koonce, R., Willoughby, M., Greenberg, M.T., Hibel, L.C., Fortunato, C.K., 2008. Maternal and child contributions to cortisol response to emotional arousal in young children from low-income, rural communities. Dev. Psychol. 44, 1095–1109. http://dx.doi.org/10.1037/0012-1649.44.4.1095.

Blair, C., Granger, D.A., Willoughby, M., Mills-Koonce, R., Cox, M., Greenberg, M.T., Kivlighan, K.T., Fortunato, C.K., 2011. Salivary cortisol mediates effects of poverty and parenting on executive functions in early childhood. Child Dev. 82, 1970–1984. http://dx.doi.org/10.1111/j.1467-8624.2011.01643.x.

Blair, C., Berry, D., Mills-Koonce, R., Granger, D., the Investigators, F.L.P., 2013. Cumulative effects of early poverty on cortisol in young children: Moderation by autonomic nervous system activity. Psychoneuroendocrinology 38, 2666–2675. http://dx.doi.org/10.1016/j.psyneuen.2013.06.025.

Boyce, W.T., Ellis, B.J., 2005. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Dev. Psychopathol. 17, 271–301. http://dx.doi.org/10.1017/S0954579405050145.

Burchinal, M., Vernon-Feagans, L., Cox, M., 2008. Cumulative social risk, parenting, and infant development in rural low-income communities. Parent.: Sci. Pract. 8, 41–69. http://dx.doi.org/10.1080/15295190701830672.

Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. J. Am. Med. Assoc. 267, 1244–1252.

Evans, G.W., English, K., 2002. The environment of poverty: multiple stressor exposure, psychophysiological stress: and socioemotional adjustment. Child Dev. 73, 1238–1248.

Goldsmith, H.H., Rothbart, M.K., 1999. The Laboratory Temperament Assessment Manual: Locomotor Version. Department of Psychology, University of Wisconsin.

Granger, D.A., Fortunato, C.K., Beltzer, E.K., Virag, M., Bright, M.A., Out, D., 2012. Salivary bioscience and research on adolescence: an integrated perspective. J. Adolesc. 35, 1081–1095. http://dx.doi.org/10.1016/j.adolescence.2012.01.005.

Gunnar, M.R., Cheatham, C.L., 2003. Brain and behavior interfaces: stress and the developing brain. Infant Mental Health J. 24, 195–211. http://dx.doi.org/10.1002/imhj.10052.

Gunnar, M., Quevedo, K., 2007. The Neurobiology of stress and development. Annu. Rev. Psychol. 58, 145–173. http://dx.doi.org/10.1146/annurev.psych.58.110405.085605.

Gunnar, M.R., 1992. Reactivity of the hypothalamic-pituitary-adrenocorticoid system to stressors in normal infants and children. Pediatrics 90, 491–497.

Gunnar, M.R., Talge, N.M., Herrera, A., 2009. Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. Psychoneuroendocrinology 34, 953–967. http://dx.doi.org/10.1016/j.psyneuen.2009.02.010.

Health and Human Services Poverty Guidelines, 69 Fed. Reg. 7336 (February 13, 2004).

Hill-Soderlund, A.L., Holochwost, S.J., Willoughby, M.T., Granger, D.A., Gariépy, J., Mills-Koonce, W.R., Cox, M.J., 2015. The developmental course of salivary alpha-amylase and cortisol from 12 to 36 months: Relations with early poverty and later behavior problems. Psychoneuroendocrinology 52, 311–323. http://dx.doi.org/10.1016/j.psyneuen.2014.08.011.

Holochwost, S.J., Gariépy, J., Propper, C.B., Gardner-Neblett, N., Volpe, V., Neblett, E., Mills-Koonce, W.R., 2016. Sociodemographic risk, parenting, and executive functions in early childhood: The role of ethnicity. Early Childhood Res. Q. 36, 537–549. http://dx.doi.org/10.1016/j.ecresq.2016.02.001.

Hostinar, C.E., Gunnar, M.R., 2013. The Developmental effects of early life stress: an overview of current theoretical frameworks. Curr. Dir. Psychol. Sci. 22, 400–406. http://dx.doi.org/10.1177/0963721413488889.

Hostinar, C.E., Sullivan, R.M., Gunnar, M.R., 2014. Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: a review of animal models and human studies across development. Psychol. Bull. 140, 256–282. http://dx.doi.org/10.1037/a0032671.

Kirschbaum, C., Hellhammer, D.H., 1989. Salivary cortisol in psychobiological research: an overview. Neuropsychobiology 22, 150–169. http://dx.doi.org/10.1159/000118611.

Kochanska, G., Murray, K.T., Harlan, E.T., 2000. Effortful control in early childhood: continuity and change, antecedents, and implications for social development. Dev. Psychol. 36, 220–232. http://dx.doi.org/10.1037/0012-1649.36.2.220.

Lanza, S.T., Rhoades, B.L., Greenberg, M.T., Cox, M., 2011. Modeling multiple risks during infancy to predict quality of the caregiving environment: contributions of a person-centered approach. Infant Behav. Dev. 34, 390–406. http://dx.doi.org/10.1016/j.infbeh.2011.02.002.

Levine, S., 1999. Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. Physiol. Behav. 73, 255–260. http://dx.doi.org/10.1016/S0031-9384(01)00496-6.

Lewis, M., Thomas, D., 1990. Cortisol release in infants in response to inoculation. Child Dev. 61, 50–59. http://dx.doi.org/10.2307/1131046.

Luecken, L.J., Lin, B., Coburn, S.S., MacKinnon, D.P., Gonzales, N.A., Crnic, K.A., 2013. Prenatal stress, partner support, and infant cortisol reactivity in low-income Mexican American families. Psychoneuroendocrinology 38, 3092–3101. http://dx.doi.org/10.1016/j.psyneuen.2013.09.006.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445. http://dx.doi.org/10.1038/nrn2639.

Masten, A.S., Wright, M.O., 1998. Cumulative risk and protection models in child maltreatment. J. Aggress. Maltreat. Trauma 2, 7–30. http://dx.doi.org/10.1300/j146v02n01_02.

McEwen, B.S., 1998. Protective and damaging effects of stress mediators. New Engl. J. Med. 338, 171–179.

McLoyd, V.C., 1998. Socioeconomic disadvantage and child development. Am. Psychol. 53, 185–204. http://dx.doi.org/10.1037/0003-066X.53.2.185.

Mills-Koonce, W.R., Garrett-Peters, P., Barnett, M., Granger, D.A., Blair, C., Cox, M.J., 2011. Father contributions to cortisol responses in infancy and toddlerhood. Dev. Psychol. 47, 388–395. http://dx.doi.org/10.1037/a0021066.

Mistry, R., Benner, A.D., Biesanz, J.C., Clark, S.L., Howes, C., 2010. Family and social risk, and parental investments during the early childhood years as predictors of low-income children's school readiness outcomes. Early Chidhood Res. Q. 25, 432–449. http://dx.doi.org/10.1016/j.ecresq.2010.01.002.

Nelson, R.J., 2015. Introduction to Behavioral Endocrinology. Sinauer Associates, Sunderland, MA.

Popp, T.K., Spinrad, T.L., Smith, C.L., 2008. The relation of cumulative demographic risk to mothers' responsivity and control: examining the role of toddler temperament. Infancy 13, 496–518. http://dx.doi.org/10.1080/15250000802329446.

Preacher, K.J., Curran, P.J., Bauer, D.J., 2006. Computational tools for probing interaction effects in multiple linear regression, multilevel modeling: and latent curve analysis. J. Educ. Behav. Stat. 31, 437–448.

Rhoades, B.L., Greenberg, M.T., Lanza, S.T., Blair, C., 2011. Demographic and familial predictors of early executive function development: contribution of a person-centered perspective. J. Exp. Child Psychol. 108, 638–662. http://dx.doi.org/10.1016/j.jecp.2010.08.004.

Sameroff, A.J., Seifer, R., Baldwin, A., Baldwin, C., 1993. Stability of intelligence from preschool to adolescence: the influence of social and family risk factors. Child Dev. 64, 80–97. http://dx.doi.org/10.1111/j.1467-8624.1993.tb02896.x.

Sanders-Phillips, K., Settles-Reaves, B., Walker, D., Brownlow, J., 2009. Social inequality and racial discrimination: risk factors for health disparities in children of color. Pediatrics 124, S176–S186. http://dx.doi.org/10.1542/peds.2009-1100E.

Saridjan, N.S., Huizink, A.C., Koetsier, J.A., Jaddoe, V.W., Mackenbach, J.P., Hofman, A., Kirshbaum, C., Verhulst, F.C., Tiemeier, H., 2010. Do social disadvantage and early family adversity affect the diurnal cortisol rhythm in infants? The Generation R. Study. Hormones Behav. 57, 247–254. http://dx.doi.org/10.1016/j.yhbeh.2009.12.001.

Super, C.M., Harkness, S., 1986. The developmental niche: a conceptualization at the interface of child and culture. Int. J. Behav. Dev. 9, 545–569.

Trentacosta, C.J., Hyde, L.W., Shaw, D.S., Dishion, T.J., Gardner, F., Wilson, M., 2008. The relations among cumulative risk, parenting: and behavior problems during early childhood. J. Child Psychol. Psychiatry 49, 1211–1219.

Tronick, E.Z., Als, H., Adamson, L., Wise, S., Brazelton, T.B., 1978. The infant's response to entrapment between contradictory messages in face-to-face interaction. Am. Acad. Child Psychiatry 17, 1–13.

Ursache, A., Blair, C., Granger, D.A., Stifter, C., Voegtline, K., Investigators, F.L.P., 2014. Behavioral reactivity to emotion challenge is associated with cortisol reactivity and regulation at 7, 15, and 24 months of age. Dev. Psychobiol. 56, 474–488. http://dx.doi.org/10.1002/dev.21113.

Watamura, S.E., Donzella, B., Kertes, D.A., Gunnar, M.R., 2004. Developmental changes in baseline cortisol activity in early childhood: relations with napping and effortful control. Dev. Psychobiol. 45, 125–133. http://dx.doi.org/10.1002/dev.20026.

Zalewski, M., Lengua, L.J., Thompson, S.F., Kiff, C.J., 2016. Income, cumulative risk, and longitudinal profiles of hypothalamic–pituitary–adrenal axis activity in preschool-age children. Dev. Psychopathol. 28, 341–353. http://dx.doi.org/10.1017/S0954579415000474.