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Does HPA-Axis Dysregulation Account for the Effects of Income on Effortful Control and Adjustment in Preschool Children?

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Abstract

The effects of low income on children's adjustment might be accounted for by disruptions to hypothalamic–pituitary–adrenal (HPA)-axis activity and to the development of effortful control. Using longitudinal data and a community sample of preschool-age children ($N = 306$, 36–39 months) and their mothers, recruited to over-represent low-income families, we explored the associations among diurnal cortisol levels and effortful control, and we tested a model in which diurnal cortisol and effortful control account for the effects of family income on child adjustment. Continuous indicators of morning cortisol level and diurnal slope, as well as dichotomous indicators reflecting low morning levels and flat diurnal slope, were examined as predictors of rank-order changes in two dimensions of effortful control, executive control and delay ability. Low income was related to a flat diurnal cortisol slope, and above the effects of family income, a flat diurnal cortisol slope predicted lower social competence. Low morning cortisol level predicted smaller gains in executive control and higher total adjustment problems. Further, delay ability predicted lower adjustment problems above the effects of income and diurnal cortisol levels. The results suggest that HPA-axis dysregulation and effortful control contribute additively to children's adjustment.

Keywords

income; HPA axis; effortful control; social competence; adjustment problems; preschool

Effortful control, a core aspect of self-regulation, provides a critical foundation for children's social, emotional and behavioural adjustment (e.g., Blair & Razza, 2007). However, children growing up in low-income families tend to demonstrate lower effortful control, which might account for greater adjustment problems often observed in children in low-income families (Raver et al., 2011). Therefore, it is important to understand the factors that contribute to the development of effortful control. One such factor is the regulation of the neuroendocrine stress response system, as indicated by cortisol (Blair et al., 2011). The aims of this study were to test whether diurnal cortisol levels predicted relative changes in effortful control

across 9 months and whether diurnal cortisol levels and effortful control accounted for the relations of income to children's adjustment problems and social competence. This study sought to test a model in which the effects of low income on children's adjustment were partially accounted for by dysregulation of the HPA axis, which in turn would interfere with the development of effortful control.

Effortful control is defined as the ability to shift and focus attention and inhibit automatic responses and includes dimensions of executive attention and inhibition and the ability to delay gratification (Kochanska, Murray, & Harlan, 2000; Rothbart, Ahadi, & Evans, 2000). Measures of effortful control often combine the executive attention and inhibitory control dimensions with reward delay. However, evidence suggests that these aspects of effortful control may differ in their developmental course, predictors and relations with adjustment (Brock, Rimm-Kaufman, Nathanson, & Grimm, 2009; Carlson, 2005; King, Lengua, & Monahan, 2012; Li-Grining, 2007) and may stem from different brain regions. Executive attention and inhibitory control may reflect more directly activity in the prefrontal cortex, whereas the delay in reward contexts may reflect an additional motivational component related to activity in the mesolimbic system (Dixon, 2010). In addition, executive and delay components of effortful control might relate differently to cortisol, with previous evidence that executive but not delay tasks were related to cortisol (Davis, Bruce, & Gunnar, 2002). Therefore, in this study, we examined executive control and delay ability separately.

Effortful control predicts adjustment, including greater social-emotional competence (e.g., Eisenberg et al., 2003; Raver, Blackburn, Bancroft, & Torp, 1999) and lower externalizing (Eisenberg et al., 1995; Hughes & Ensor, 2009; Kochanska & Knaack, 2003; Lengua, 2003; NICHD Early Child Care Research Network, 2003; Rothbart, Ahadi, & Hershey, 1994) and internalizing problems (de Boo & Kolk, 2007; Eisenberg, Cumberland, et al., 2001; Lengua, 2003, 2006; Muris, van der Pennen, Sigmond, & Mayer, 2008). However, effortful control tends to be lower in children growing up in low-income households (e.g., Eisenberg, Gershoff, et al., 2001; Hughes, Ensor, Wilson, & Graham, 2010; Lengua, 2002; Li-Grining, 2007; Mezzacappa, 2004; Mistry, Benner, Biesanz, Clark, & Howes, 2010), potentially partially accounting for greater adjustment problems demonstrated by those children. In particular, the accumulation of stress or adversity associated with low income might divert the development of effortful control (Lengua, Honorado, & Bush, 2007). Thus, it is critical to identify stress-related factors that might account for the association between income and effortful control. One such factor might be the regulation of the neuroendocrine stress response system reflected in activity of the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA axis also has been linked with children's adjustment. High levels of cortisol or cortisol reactivity are related to lower social competence (Gunnar et al., 1997; Hart, Gunnar, & Cicchetti, 1995) and higher internalizing (Ashman, Dawson, Panagiotides, Yamada, & Wilkson, 2002) and externalizing problems (Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003), although low levels of cortisol are also related to externalizing (Alink et al., 2008). The HPA axis is a stress-sensitive system that responds to the environmental context. Activation of the HPA axis leads to a hormonal cascade that ends in the production of cortisol. The HPA axis follows a diurnal rhythm, with cortisol levels reaching their peak about 30 minutes after awakening and decreasing throughout the day to near zero at bedtime

(Kirschbaum et al., 1990). Stress, particularly severe or chronic adversity, can disrupt diurnal patterns or levels of cortisol (e.g., Miller, Chen, & Zhou, 2007). Variations in the typical diurnal pattern observed in children experiencing contextual stressors include children showing elevated levels later in the day (e.g., Gunnar et al., 1997) or flattened and lower levels across the day (Gunnar & Vazquez, 2001; Heim, Ehlert, & Hellhammer 2000; Tarullo & Gunnar, 2006). A meta-analysis suggests that acute stress is associated with elevations in cortisol, whereas chronic, pervasive stress is related to blunted levels (Miller et al., 2007). Further, low income is related to both low and high diurnal levels of cortisol, but most consistently to blunted diurnal cortisol (Dowd, Simanek, & Aiello, 2009). Thus, higher or lower levels of cortisol or blunted slopes may be indicative of a dysregulated stress response system.

Dysregulation of the HPA axis due to adversity might contribute to the lower levels of effortful control observed among children from low-income families. Neurobiological stress responses, particularly HPA-axis activity, are thought to affect activity in the prefrontal cortex (PFC), the area associated with effortful control (Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007). The mechanisms of this are not entirely clear, but the PFC is known to be rich in glucocorticoid receptors (Herman & Cullinan, 1997). Moreover, there are direct neural connections between the PFC and other areas of the brain involved in stress reactivity, including the amygdala and hippocampus. Thus, there may be both structural and functional mechanisms underlying HPA dysregulation and deficits in effortful control.

Given that both higher and lower levels of cortisol may indicate disruptions to the HPA axis, either might relate to effortful control, and in fact, there is evidence for both. Some evidence suggests that elevated cortisol interferes with inhibitory control processes (Braunstein-Bercovitz, Dimentman-Ashkenazi, & Lubow, 2001; Lupien, Gillin, & Hauger, 1999; Lyons, Lopez, Yang, & Schatzberg, 2000) and produces cognitive and affective impairments (McEwen, 1998). In a longitudinal study that utilized a low-income sample, higher home baseline levels of infant cortisol collected prior to an experimental manipulation were related to lower executive functioning when children were 3 years old (Blair et al., 2011). In toddlers, higher levels of cortisol were related to lower parental ratings of effortful control (Wataura, Donzella, Kertes, & Gunnar, 2004). Also, high levels of cortisol after a period of adaptation to preschool were related to lower attentional and inhibitory control (Gunnar et al., 1997). In contrast, another study found that higher home baseline cortisol levels were associated with better effortful control (Davis et al., 2002). In addition, one study found that a blunted cortisol pattern characterized by low cortisol levels throughout the day predicted smaller increases in effortful control across 6 months in preschool-age children (Zalewski et al., 2012). It is possible that inconsistent findings reflect the fact that significant disruptions to cortisol regulation, either high or low, interfere with the development of effortful control. Thus, associations might not be detected on a continuum, but rather at the extremes of cortisol levels. Thus, in addition to examining continuous cortisol indicators, we tested whether having markedly high or low morning cortisol or a flat diurnal slope was related to effortful control.

Alternatively, it is possible that methodological issues contribute to inconsistent patterns of association between cortisol and effortful control. First, differences across studies might be

related to the cortisol measures employed, including waking and bedtime samples (Bruce, Davis, & Gunnar, 2002; Zalewski et al., 2012), morning levels at school, daily output (Davis et al., 2002; Watamura et al., 2004) or baseline and reactivity to a challenge (Blair, Granger, & Razza, 2005; Blair et al., 2011). Second, the developmental period examined might result in different patterns of associations of cortisol to adversity and to other factors. For example, there is some evidence that young children may enter a stress hyporesponsive period in which low levels of cortisol are observed (Gunnar & Donzella, 2002), which might account for the lower cortisol levels in response to adversity seen in preschool children (Badanes, Watamura, & Hankin, 2011; Fernald, Burke, & Gunnar, 2008; Zalewski et al., 2012). Further, cortisol elevations in relation to adversity might be observed in infancy as children are responding to an adverse context. However, in the context of chronic adversity, cortisol levels might be blunted by the preschool period as children adapt to chronic experiences of adversity (Gunnar & Vazquez, 2001). Third, patterns of cortisol associations might differ if the study sample is homogenous on risk (e.g., low income or mid-income to upper-income sample) as opposed to representing the full range of risk for statistical reasons related to restricted range on either the predictor or outcome variables. A strength of this study is the use of a sample that represents the full range of income, allowing a robust test of the effects of income and providing adequate variability in other factors examined.

This study sought to test whether dysregulation of the neuroendocrine stress response system, as indicated by diurnal cortisol levels, was related to the development of effortful control and accounted for the effects of income on children's effortful control and adjustment. We hypothesized that dysregulation of the HPA axis might play a role in disrupting the development of effortful control, and in turn, both HPA-axis dysregulation and lower effortful control would account for the effects of low income on children's adjustment. Prior to addressing the central aim of this study, we explored the relations of diurnal cortisol levels (morning level and diurnal slope) to two aspects of effortful control, executive control and delay ability, testing whether associations could be detected using continuous versus categorical cortisol variables, indicating marked disruptions in HPA-axis activity (i.e., high vs low morning level and blunted diurnal slope) and testing whether disruptions in HPA-axis activity predicted relative changes in effortful control across time. From prior evidence, it was possible that high and low morning levels and a blunted diurnal pattern could be related to lower effortful control; yet, given inconsistent findings in the past, we approached this aim in an exploratory manner. This study was unique in utilizing longitudinal data to examine the relation of HPA-axis dysregulation to changes in effortful control and in testing a model in which HPA-axis dysregulation accounted for the relation of income to children's adjustment through its impact on developing effortful control.

METHOD

Participants

Study participants were 306 36-month-old children ($M = 37$, $SD = 0.84$ months) and their mothers who were recruited from various publicly and privately funded sources, including the university hospital birth registry, daycares, preschools, libraries, health clinics and charitable agencies and organizations serving low-income families in the urban and

suburban areas surrounding a Pacific Northwest university. Families were recruited for participation so that there was equal representation across income levels, with 29% of the sample at or near poverty ($N = 90$ at or below 150% of the federal poverty threshold), 27% lower income ($N = 83$ above 150% of the poverty threshold but below the local median income of \$58K), 25% middle to upper income ($N = 78$ above the median income to \$100K) and 18% affluent ($N = 54$ above \$100K). To participate, families required reasonable proficiency in English to comprehend the assessment procedures, and children diagnosed with a developmental disability were excluded. Participants included 50% girls; 81% of mothers were married or had long-time partners, 12% were never married, 7% were separated, divorced or widowed single heads of household. The racial and ethnic composition of the sample of children included 64% European American, 9% African American, 3% Asian American, 10% Latino or Hispanic, 2% Native or American Indian and 12% multiple racial and ethnic backgrounds. Three per cent of mothers had some high school attainment, 6% completed high school, 34% completed some college or technical/professional school, 30% were college graduates and 27% had postgraduate education.

Participants were assessed at two time points, and those missing data at either time point ($n = 47$) were compared with those missing no data ($n = 259$) to assess the extent of bias introduced by missing data. No participants were missing data on income. Complete data on cortisol, effortful control and adjustment were available for 88%, 95% and 94% of the participants, respectively. Levels of family income, cortisol, effortful control and parent report of adjustment were compared across participants missing any data and those missing no data. Participants missing data differed from those not missing data in that they had lower income (M missing = 7.15, M no missing = 9.03, $t[304] = 3.07$, $p < 0.01$) and lower T2 effortful control (M missing = 0.42, M no missing = 0.50, $t[304] = 1.99$, $p = 0.05$). However, the effect sizes of the associations of missingness to income ($r = -0.17$) and effortful control ($r = -0.12$) were modest and did not reach previously cited thresholds for introducing substantial bias (i.e., $r > 0.40$, Collins, Schafer, & Kam, 2001). Thus, little bias was introduced due to missing data.

Procedures

Families were assessed at two times separated by 9 months ($M = 9.40$, $SD = 0.66$) when children were 36–40 and 45–51 months. Assessments at both time points were nearly identical. Following the university's Social and Behavioural Sciences Institutional Review Board stipulations, both active parental consent and child assent were secured prior to data collection. Assessments included neuropsychological, physiological and questionnaire measures administered by trained experimenters. Children completed neuropsychological and behavioural measures of effortful control while mothers completed questionnaires in a separate room. Families were compensated \$70 for the first visit and \$90 for the second visit. Mothers provided permission to contact children's teachers if they were in a school or daycare setting to obtain teachers' ratings of children's adjustment in the classroom. Teachers completed forms when they had known the children for at least 1 month and were compensated \$10 for each form completed.

At the end of the session, mothers were trained in the collection of cortisol and were given a home collection kit and instructions to collect the saliva samples at home. Specifically, mothers were instructed to collect their children's saliva using two sorbettes per collection (Salimetrics, LLC, State College, PA) 30 minutes after the children woke in the morning prior to eating and 30 minutes prior to bedtime, for three consecutive days, and storing the sorbettes in pre-labelled swab storage tubes. Reminder and troubleshooting calls from staff were made on the first and third evenings to ensure proper collection, storage and mailing of samples. Neither mailing (Clements & Parker, 1998) nor thawing and refreezing (Garde & Hansen, 2005) saliva samples has been shown to influence cortisol concentrations or levels. Protocol violations were minimized by doing the following: (1) making troubleshooting and reminder calls to families on the first and last days of collection; (2) including detailed instructions, labelling and recording systems for families, in which any inconsistencies alerted our team to possible problems with sample collections; and (3) careful monitoring of timing and contents of returned samples so that any potential problems could be addressed with families as quickly as possible, including some requests to collect additional samples. Families were compensated \$30 for returning the saliva samples.

Measures

Descriptive statistics for all study measures are reported in Table 1.

Income—At T1, mothers reported on household income from all sources on a 12-point scale.

Cortisol—T1 saliva samples were sent to the university's Biobehavioral Behavioral and Nursing Systems laboratory for processing, where they were stored at -70°C . until extraction. For processing, all sample tubes were thawed to room temperature and centrifuged at 1000 rpm for 10 minutes in order to separate the saliva from the collection swab. The cleared eluent was then transferred to a 1.6 mL Eppendorf tube and stored at -70°C until testing for cortisol. Prior to assay, each sample was subjected to another centrifugation step of 5000 rpm for 5 minutes in order to separate out small particulates and residual mucin. In order to test for the presence of salivary cortisol, 25 μL of saliva from each sample was transferred into each of two wells, producing duplicate samples for each assay; sample values were then averaged. The concentration of cortisol in each sample was extrapolated from a standard curve generated in each test plate, and the results were averaged in order to give an adjusted result. Samples were assayed using the high-sensitivity cortisol salivary enzyme immunoassay kit provided by Salimetrics LLC (State College, PA). The sensitivity of this kit ranges from 0.005 to 2.5 $\mu\text{g}/\text{dL}$. All samples from the same subject for each set of saliva were included in the same assay batch to minimize inter-assay within-subject variability. Intra-assay reliabilities were obtained using the high and low cortisol controls provided by Salimetrics. The mean cortisol value (MCV) for the high concentration sample is 0.950 $\mu\text{g}/\text{dL}$; the MCV for the low concentration sample is 0.083 $\mu\text{g}/\text{dL}$. For the high cortisol concentration, the intra-assay CV was 6.3%; the low concentration, the intra-assay CV was 5.4%, all acceptable values.

Of 306 families, 35 (11%) did not return any samples; 257 families returned six samples; two families returned five samples; seven families returned four samples; and five families returned two samples. Therefore, 271 families had some cortisol data. Assay values over 2.0 $\mu\text{g/dL}$ (32 samples from 20 children) were deemed biologically implausible and were not used, consistent with methods used in other studies (Ashman et al., 2002). Only one case was fully discarded because all cortisol values were over 2.0 $\mu\text{g/dL}$. Samples collected 90 minutes after waking up or prior to bedtime were also discarded, resulting in an additional four children's data being eliminated above those eliminated for implausible values. Cortisol values were averaged across morning and evening samples, resulting in average cortisol values available for 270 children.

Assay results for the three mornings and evenings were averaged to produce average morning and average evening levels. The three morning cortisol values were correlated 0.27–0.43. The three evening values were correlated 0.23–0.32. The diurnal slope was computed by subtracting average evening from average morning values. The average morning score was 0.29 ($SD = 0.21$, range = 0.02–1.63), average evening score was 0.13 ($SD = 0.18$, range = 0.02–1.94), and average diurnal slope was 0.16 ($SD = 0.20$, range = –0.64–1.23). Average morning level was correlated with evening level (0.38) and with diurnal slope (0.37), indicating that these were correlated but not redundant indicators of diurnal levels. As is common with cortisol data, values were positively skewed, and log transformations were applied to average morning and average evening variables. By using the log-transformed morning and evening values, a diurnal pattern was recalculated. Analyses were conducted with log-transformed values. To explore whether associations with effortful control would be detected on a continuum or at the extremes of cortisol (e.g., Dozier et al., 2006), categorical variables for low morning and low diurnal slope were created at 1 SD and 1.5 SD s below the sample mean, and high morning values were calculated at 1 SD and 1.5 SD s above the mean.

Use of steroid medications, an inhaler, health factors and food intake can affect cortisol levels. Mothers completed a daily questionnaire regarding sampling times and children's health, medication use and eating times on sampling days. Questionnaires were reviewed to ensure compliance. During reminder calls, mothers were reminded to avoid sampling when their children were using steroid medications or were ill and were mailed additional materials if they accidentally sampled at those times.

Effortful control—Effortful control was assessed at both time points with identical measures of attention regulation, cognitive and behavioural inhibitory control, and delay of gratification. Measures were selected to be of varying difficulty for children across childhood so that identical measures could be used across time. Although some of the measures were normed for children older than those in this sample, there was still variability in performance even at these early ages. Scores from the range of measures were averaged so that there was variability in performance on the aggregate scores. Scores on all measures were the proportion correct out of the total possible so that scores were on a comparable scale over time. Measures included a combination of executive function subscales of the NEPSY, a developmental neuropsychological assessment battery (Korkman, Kirk, & Kemp, 1998), and tasks from Murray and Kochanska (2002). Given evidence that delay ability

might operate differently than the executive attention and inhibitory control aspects of effortful control, two separate effortful control variables were created: executive control and delay ability (Fisher, Tininenko, & Pears, 2007; Li-Grining, 2007).

Executive control was assessed using six tasks. The Inhibition and Auditory Attention subscales of the NEPSY were designed for use with children 5 years and older. However, the scales were administered to the children in this study to allow use of identical measures of effortful control longitudinally. The inhibition subtest assesses a child's ability to inhibit a dominant response in order to enact a novel response (T1 $M = 0.14$, $SD = 0.28$, $Range = 0.00-1.00$; T2 $M = 0.49$, $SD = 0.40$, $Range = 0.00-1.00$). The auditory attention subtest is a continuous performance test that assesses the ability to be vigilant and to maintain and shift selective auditory set (T1 $M = 0.09$, $SD = 0.24$, $Range = 0.00-0.89$; T2 $M = 0.26$, $SD = 0.34$, $Range = 0.00-0.98$).

Behavioural inhibitory control was assessed using the Bear-Dragon task (Kochanska, Murray, Jacques, Koenig, & Vandegeest, 1996), which requires the child to perform actions when a directive is given by a bear puppet, but not when given by a dragon puppet. Children's actions were scored as performing no movement, a wrong movement, a partial movement, or a complete movement, with scores ranging from 0 to 3 (T1 $M = 0.62$, $SD = 0.20$, $Range = 0.33-1.00$; T2 $M = 0.87$, $SD = 0.20$, $Range = 0.33-1.00$). Cognitive inhibitory control was assessed using the Day-Night task (Gerstadt, Hong, & Diamond, 1994), which requires the child to say 'day' when shown a picture of moon and stars and 'night' when shown a picture of the sun. Items were scored 1 for correctly providing the nondominant response or 0 for providing the dominant response (T1 $M = 0.44$, $SD = 0.33$, $Range = 0.00-1.00$; T2 $M = 0.62$, $SD = 0.30$, $Range = 0.00-1.00$).

The Dimensional Change Card Sort (DCCS; Zelazo, Muller, Frye, & Marcovitch, 2003) assesses cognitive inhibitory control, attention focusing, and set shifting. In this task, children were shown target cards that consisted of a silhouetted figure on a coloured background. Children were instructed to sort cards according to either the shape (six trials) or colour (six trials) properties on the target cards. If children correctly sorted >50% of the cards, they advanced to the next level in which the target cards integrated the sorting properties. Target cards consisted of a coloured figure on a white background, and children were again instructed to sort according to shape (six trials) and then colour (six trials). If they correctly sorted >50% of the cards, children advanced to the next level in which they were instructed to sort by one dimension (colour) if the card had a border on it and by the other dimension (shape) if the card lacked a border (12 trials) (T1 $M = 0.42$, $SD = 0.20$, $Range = 0.00-0.89$; T2 $M = 0.61$, $SD = 0.26$, $Range = 0.00-1.00$).

The Head, Toes, Knees, Shoulders (HTKS) task also integrates attention and inhibitory control (Ponitz et al., 2008). Children are asked to follow the instructions of the experimenter but to enact the opposite of what the experimenter directs (e.g., touch toes when asked to touch head). Behaviours were coded as 0 if the child touched the directed body part, 1 if the child self-corrected, and 2 if the child touched the opposite body part (T1 $M = 0.03$, $SD = 0.09$, $Range = 0.00-0.65$; T2 $M = 0.19$, $SD = 0.27$, $Range = 0.00-0.85$).

Twenty per cent of all executive control tasks were independently re-scored to assess inter-rater reliability. ICCs ranged from 0.72 to 0.98.

Children's ability to delay gratification was assessed using a gift delay task (Kochanska et al., 1996). In this task, children were told that they would receive a present but that the experimenter wanted to wrap it. The children were instructed to sit facing the opposite direction and to not peek while the experimenter noisily wrapped the gift. Children's peeking behaviour (frequency, degree, latency to peek, and latency to turn around) and difficulty with the delay (fidgeting, tensing, getting out of seat, grimacing, and talking) were rated. Latencies and behaviour scores were converted to proportions of total possible times/scores and averaged.

Confirmatory factor analyses were used to test the acceptability of a two-factor model of effortful control that specified executive control and delay factors. The executive control factor loaded on inhibition, auditory attention, bear–dragon, day–night, DCCS, and HTKS observed scores, and delay ability loaded on peeking frequency, latency to peek, latency to turn around, and difficulty with delay observed scores. Separate models were tested at each time point. At both T1 and T2, the models demonstrated acceptable fit to the data (T1 RMSEA = 0.04, CFI = 0.97, $\chi^2(42) = 64.95$, $p = 0.01$; T2 RMSEA = 0.03, CFI = 0.99, $\chi^2(42) = 50.27$, n. s.). All standardized loadings were significant (>0.36), and the correlations of the latent factors were 0.37 and 0.48 at times 1 and 2, respectively, supporting the examination of a two-factor model of effortful control. Consistent with previous research, an overall executive control score was computed as the mean of the proportion scores of the individual tasks. Executive control scores were considered missing if $>50\%$ of the component scores were missing. Internal consistency of the composite executive control measure was 0.67, and the inter-rater reliability was 0.83. An overall delay ability score was computed as the mean of the proportion scores for the individual delay indicators and was considered missing if $>50\%$ of the component scores were missing. Internal consistency was 0.77, and inter-rater reliability was 0.91.

Children's adjustment—Teachers reported on children's social competence and adjustment problems at T2 using the Social Skills Rating Scale (SSRS; Gresham & Elliot, 1990), a 30-item social competence scale, which assesses cooperation, assertiveness, and self-control ($\alpha = 0.91$), and the 13-item problem behaviour scale, including internalizing and externalizing problems ($\alpha = 0.87$). Teachers reported knowing the children in the study for an average of 11.63 months. ($SD = 11.44$, $Range = 1–48$).

RESULTS

Analytic Plan

Analyses were conducted to examine the relations of children's diurnal cortisol levels to effortful control, to test whether diurnal cortisol predicted changes in effortful control from T1 to T2, and to test whether diurnal cortisol and effortful control accounted for the effects of income on adjustment. Prior to testing the hypothesized associations, compliance with cortisol collection procedures was examined. To test hypothesized relations, first, correlations were examined using both continuous and categorical cortisol variables, with

categorical cortisol variables representing low and high morning levels and blunted diurnal slope, to determine whether associations could be detected on a continuum or at the extremes of cortisol. Based on these preliminary analyses, cortisol variables that were significantly related to effortful control were tested as predictors of relative changes in effortful control using multiple regression analyses. Second, correlations among the study variables were examined to assess the plausibility of the study hypotheses that HPA-axis dysregulation would account for the effects of income on effortful control and adjustment. Third, path analyses were conducted to test whether diurnal cortisol and effortful control accounted for the effects of income on children's adjustment. Multiple regression and path analyses were conducted using Mplus 6.0 (Muthen & Muthen, 2010) using Full Information Maximum Likelihood Estimation (FIMLE) which uses all available data to calculate parameter estimates. FIMLE has been found to be less biased and more efficient than other approaches to handling missing data (Arbuckle, 1996). Our examination of bias in missing data (above) suggested that the pattern of missing data introduced minimal bias and aligned with the assumptions of FIMLE. Therefore, analyses were based on an N of 306.

Cortisol Collection Protocol Compliance

Health, medication, and food ingestion were examined in relation to cortisol variables. One- to two-percent of children were reported to have an elevated temperature on one of the three days of saliva collection. Having an elevated temperature on day 3 was modestly related to higher morning cortisol levels ($r = 0.14$, $p = 0.02$). However, excluding those cases from the sample did not alter the pattern of the correlations of cortisol variables with other variables, and therefore these participants' samples were retained. Neither general inhaler use (3.5%) nor steroidal medication use (1.6%) was related to the cortisol variables. Eating within a half hour of morning saliva collection was reported in 10–15% of cases and was related to lower morning cortisol level ($r = -0.15$, $p = 0.01$). However, excluding data from the children who ate within a half hour of saliva collection from the sample did not alter the pattern of the correlations of cortisol variables with other variables, and these participants' samples were retained. Taking a nap during the day (67%) was not related to the cortisol variables. Average wake times and latencies to collection, that is, the difference between wake time and collection, were related to average morning levels, $r = -0.15$, $p < 0.05$ and $r = -0.12$, $p < 0.05$, respectively, and were included as covariates in all analyses. Bed time and latency were not correlated with cortisol variables.

Relations of Cortisol to Effortful Control

Correlations of cortisol levels and effortful control—To clarify the relation between HPA-axis functioning and effortful control we examined associations of T1 and T2 executive control and delay with T1 cortisol variables, including the continuous morning level and diurnal slope variables, as well as the categorical cortisol variables of low morning (0 = above the cut-offs, 1 = at or below the cut-offs of 1 *SD* and 1.5 *SD* below the sample mean), high morning (0 = below the cut-offs, 1 = at or above the cut-offs of 1 *SD* and 1.5 *SD* above the sample mean), and low diurnal slope (0 = above the cut-offs, 1 = at or below the cut-offs of 1 *SD* and 1.5 *SD* below the sample mean) (Table 2). Categorical indicators of markedly low morning levels were related to lower executive control. Specifically, morning levels that were 1 *SD* below the sample mean were related to lower T2 executive control,

and morning levels that were 1.5 *SD* below the mean were related to lower T1 and T2 executive control. Morning levels were unrelated to delay ability. Flatter diurnal slopes were related to lower delay ability at T1, and this association was significant when diurnal slope was measured continuously and at 1 *SD* below the mean. As a result, morning levels and diurnal slope were examined as predictors of relative changes in executive control and delay ability. Indicators of high morning cortisol were unrelated to effortful control and not analyzed further.

Cortisol as a predictor of changes in effortful control—To test whether cortisol predicted rank order changes in effortful control, multiple regression analyses using FIMLE estimates were conducted (Table 3). Controlling for child sex, family income, child wake time and latency to saliva collection, and covarying the effects of T1 effortful control, T1 cortisol variables were tested as predictors of T2 effortful control. Low morning levels at -1.5 *SD* below the mean predicted modestly smaller increases in executive control across 9 months. However the other indicators of morning level (continuous and -1 *SD*) were unrelated to changes in executive control. None of the cortisol variables predicted changes in delay ability.

Income, Cortisol and Effortful Control as Predictors of Adjustment

Correlations among income, cortisol, effortful control and adjustment—

Correlations among the variables are presented in Table 4 and were examined to assess the plausibility of the hypothesis that diurnal cortisol and effortful control might account for the effects of income on adjustment. Child sex was related to T1 delay ability and T2 executive control such that boys were lower in both. Therefore, sex was included as a covariate in subsequent analyses. Higher income was related to a lower likelihood of demonstrating the flat diurnal slope, and it was related to higher levels of executive control and delay ability. Income was also related to lower total problems and higher social competence. Low morning cortisol was correlated with lower executive control at T1 and T2, and it was related to higher total problems and lower social competence. Executive control was related to higher social competence. Thus, it was plausible that low morning cortisol and executive control could account for the relation of income to children's adjustment. Having a flat diurnal slope was related to lower delay ability at T1 and lower social competence at T2. Also, delay ability was related to lower total problems and higher social competence. Thus, it was plausible that flat diurnal slope and delay ability could account for the relation of income to adjustment.

Income, cortisol and effortful control as predictors of adjustment—Path analyses were conducted to test the proposed model in which HPA-axis dysregulation predicts smaller increases in effortful control, and both HPA-axis dysregulation and effortful control account for the effects of income on adjustment (Figure 1). Because low morning cortisol (1.5 *SD* below the mean) was related to lower executive control, higher total problems, and lower social competence, and because flat diurnal slope (1 *SD* below the mean) was related to lower social competence, these cortisol variables were included in the model. The model tested whether income predicted low morning cortisol and flat diurnal slope, which in turn, were tested as predictors of effortful control and adjustment. In

addition, these analyses tested whether diurnal cortisol levels and effortful control accounted for the effects of income on total problems and social competence by examining the indirect effects of income on adjustment through cortisol and effortful control. Child sex, wake time and latency to saliva collection were covaried.

The model provided a reasonable fit to the observed data ($CFI = 0.90$, $RMSEA = 0.07$). T1 income was related to lower T1 executive control and delay ability, and predicted a greater likelihood of demonstrating the flat diurnal cortisol slope. Above the effects of T1 executive control, lower income predicted modestly lower levels of T2 executive control. In addition, low morning cortisol predicted modestly lower levels of T2 executive control. Only T1 delay ability was significantly related to T2 delay ability. Higher T2 total problems were predicted by lower income, lower delay ability and low morning cortisol levels, with all effects being modest in magnitude. Lower T2 social competence was predicted by lower income and low diurnal cortisol slope, again with modest effects. Tests of the indirect effects of income on adjustment through diurnal cortisol and effortful control indicated a nonsignificant indirect effect of income on total problems ($\beta = -.02$, $SE = 0.03$, *n.s.*). There was a significant indirect effect of income on social competence ($\beta = 0.05$, $SE = 0.02$, $p = 0.01$) largely accounted for by two trends toward effects of income through flat diurnal slope ($\beta = 0.02$, $SE = 0.01$, $p = 0.10$) and of income on social competence through executive control ($\beta = 0.02$, $SE = 0.01$, $p = 0.10$).

DISCUSSION

This study examined the hypothesis that dysregulation of the HPA-axis and low effortful control might account for the effects of low income on children's adjustment problems and social competence. The study employed a large community sample that represented the full range of income and examined the relations of preschoolers' diurnal cortisol to changes in their effortful control across 9 months, addressing whether diurnal HPA axis function might play a role in the development of effortful control. There was partial support for the proposed associations. Low morning cortisol predicted smaller relative increases in effortful control, and both diurnal cortisol and effortful control predicted children's adjustment above the effects of income, suggesting that diurnal cortisol and effortful control represent relevant factors in children's adjustment. However, neither cortisol levels nor effortful control accounted for the effects of income, but rather contributed independently to children's adjustment.

A preliminary step was to test whether relations of diurnal cortisol to effortful control would be detected on a continuum, or whether disrupted levels, high or low, would be related to effortful control. These were exploratory analyses given the lack of consistent findings and methods across previous studies. We found that morning cortisol was related to lower executive control, but only at markedly low levels of morning cortisol ($-1.5 SD$). This suggests that disruptions to diurnal cortisol levels may play a role in the development of effortful control, but perhaps not when there are modest levels of dysregulation. Indeed, markedly low morning levels predicted smaller increases in executive control across 9 months. A flat diurnal slope was related to lower delay ability concurrently, and this association was detected across the continuum of diurnal slope. However, diurnal slope did

not predict changes in delay ability, suggesting the possibility that the association is accounted for by a third common variable, such as income.

Notably, high levels of morning cortisol were unrelated to effortful control in this study. Rather, low morning cortisol and a flat diurnal slope were related to lower effortful control, consistent with the findings of two previous studies with preschool children (Davis et al., 2002; Zalewski et al., 2012), but in contrast to other studies (e.g., Blair et al., 2011; Watamura et al., 2004), albeit the measures of cortisol and effortful control and sample characteristics differed somewhat across studies. This may suggest that markedly low levels of cortisol are a particularly detrimental patterning of HPA-axis functioning that may interfere with the development of executive control in the preschool period when effortful control is demonstrating precipitous growth. The specific mechanisms that may be involved in these processes are not clear. It may be that low cortisol levels reflect an overall downregulation of the HPA axis that is adaptive to conditions of chronic stress and that chronic stress also interferes with the development of regions of the prefrontal cortex involved in executive control. Alternatively, low cortisol may be preceded by high levels of cortisol, which may produce changes in the development of the PFC. More longitudinal research is needed to better explicate these processes in typical and high-adversity samples. As discussed above, different findings across studies might be accounted for by several factors including the cortisol indicator utilized, the developmental period, the risk level of the sample, and perhaps other factors as well. At this point, there are not a sufficient number of studies that vary on each of these factors to draw any conclusions about how these factors influence the relations between the HPA axis and executive processes or to provide guidance on the specific cortisol variables to include in studies. Future research on cortisol can contribute to clarifying these associations by including multiple cortisol indicators and specifying sample characteristics that might impact associations.

It is also noteworthy that diurnal cortisol predicted changes only in executive control, not delay. This finding is similar to the results of a previous study in which cortisol predicted executive but not delay aspects of effortful control (Davis et al., 2002). It is not surprising that there are effects of cortisol on executive functions considering the neurobiological connections between the HPA axis and prefrontal cortex (Blair et al., 2011). However, the differentiation of effects on delay ability, which likely also includes a motivational component, requires further investigation. It is possible that reward motivation is more characterological than executive control, thus, less amenable to being shaped by experience.

The central aim of this study was to test whether HPA-axis dysregulation and effortful control accounted for the association of low income with children's adjustment. There was mixed support for this hypothesis. Lower income was related to a higher likelihood of demonstrating a flat diurnal slope. Lower income was also related to lower executive control, delay ability and social competence, and higher adjustment problems. Thus, it was plausible that diurnal cortisol and effortful control might account for the relation of income to children's adjustment. However, the results of path analyses did not support the model. Above the effects of income, low morning cortisol level and flat diurnal slope predicted children's adjustment problems and social competence, respectively. Further, delay ability also predicted lower adjustment problems. However, including diurnal cortisol and effortful

control in the model did not result in decreases in the association between income and adjustment, and there was little evidence of an indirect effect of income on adjustment through cortisol and effortful control.

The indirect effect of income on adjustment problems was not significant, and there were trends towards indirect effects of income on social competence through flat diurnal slope and executive control. Further, the fact that low morning cortisol predicted higher total problems and flat diurnal slope predicted lower social competence above the effects of income and effortful control suggests that these indicators of HPA-axis functioning are playing a role in children's adjustment through other pathways not examined here. In addition, diurnal cortisol and delay ability operated independently in predicting adjustment, whereas the relation of executive control to adjustment appeared to be accounted for by income and diurnal cortisol levels. These findings were surprising in part because the initial analyses demonstrated that markedly low morning levels predicted less growth in executive control, pointing to a possible pathway of the effects of cortisol levels on children's adjustment. It is possible that lower executive control and lower morning cortisol are both indications that children's self-regulation is being taxed by the low-income environment and, thus, are overlapping factors to some extent. Nonetheless, disruptions to these self-regulatory systems appear to have independent and additive implications for children's adjustment problems and social competence. Taken together, the findings of this study point to unique effects of income, diurnal cortisol and effortful control, suggesting that each of these factors contributes to adjustment problems and, together, they additively increase children's risk for developing adjustment problems.

Some limitations of this study might also account for the mixed support for the hypothesized relations. The 9-month span across assessments might not be sufficient to capture the hypothesized associations. Future studies should examine developmental changes in effortful control and children's adjustment as they relate to time-varying cortisol levels. In addition, a broader array of cortisol variables, including cortisol reactivity to challenge, could further clarify the role of HPA activity in the development of effortful control. However, only diurnal cortisol levels were assessed in this study. Also, obtaining multiple samples within a day would provide a more accurate characterization of the diurnal pattern. Nonetheless, the fact that we obtained two samples each day across 3 days provides a stable estimate of the average daily decline in cortisol level. A significant challenge in obtaining cortisol data in community samples is the potential for protocol violations, including samples collected at times or under conditions other than specified. An optimal approach to addressing this is to utilize technology that records the times families open the collection tubes (Mems Caps) and objective measures of children's sleep/wake times. However, these technologies were not employed. Rather, we took a number of measures to reduce the likelihood of protocol violations, including careful labeling and documentation, close monitoring of samples, and calls to families on collection days. Despite these measures, it is possible that families did not accurately record their sample collection times or conditions, and bias might have been introduced if these protocol violations were more common among lower-income families. It is also possible that the observed associations would be different in a high-risk sample. Whereas only the dichotomous indicators of markedly low morning

cortisol and flat diurnal slope were related to children's effortful control and adjustment in this sample, in a high-risk sample, continuous indicators might capture this association.

In summary, low morning cortisol and flat diurnal slope appear to reflect dysregulation of the neuroendocrine stress response system in the preschool period, both predicting smaller increases in effortful control and relating to children's adjustment. These effects were above the effects of income but did not account for the effects of income, suggesting that these are additional risk factors to consider in explaining children's adjustment. This study's use of multiple indicators of cortisol and effortful control points to the possibility of specificity in associations of cortisol to executive control and delay ability and to adjustment problems and social competence. Elucidating the potential neurobiological pathways by which low income confers risk for adjustment problems in young children is an important task of developmental scientists, providing clarification about the potential mechanisms of the effects of adversity and informing intervention efforts. Major disruptions to HPA-axis functioning, rather than modest to moderate dysregulation, relate to the development of executive control and have implications for adjustment. This is useful information in considering populations of children who might benefit from preventive interventions, indicating that they should be targeted at children who experience pervasive, chronic adversity given the compounding of risk associated with concomitant disruptions to their self-regulation.

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Figure 1.

Standardized path coefficients for the effects of family income and cortisol levels on executive control, delay ability, total problems and social competence. (Only significant paths are depicted for ease of presentation. Child sex, morning wake time and latency to saliva collection after wake time were included as covariates but are not depicted here. Low morning cortisol is coded 0 = above the cut-off and 1 = at or below the cut-off of 1.5 *SD* below the sample mean. Flat cortisol slope is coded 0 = above the cut-off and 1 = at or below the cut-off of 1 *SD* below the sample mean.)

Table 1

Mean, standard deviation and range for study variables

	<i>M</i>	<i>SD</i>	Min–Max
Morning cortisol level (ln)	–0.63	0.27	–1.72–0.21
Diurnal slope (ln)	0.46	0.36	–0.58–1.24
T1 executive control	0.29	0.15	0.00–0.77
T2 executive control	0.49	0.20	0.00–0.91
T1 delay ability	0.62	0.25	0.09–1.00
T2 delay ability	0.76	0.23	0.08–1.00
T2 total problems	10.78	6.30	1.00–35.00
T2 social competence	47.17	7.86	24.00–68.00

Table 2

Correlations of executive control and delay with morning level and diurnal slope of cortisol measured continuously at 1 and 1.5 *SD* cut-offs

	T1 executive control	T2 executive control	T1 delay ability	T2 delay ability
Morning level: continuous	0.00	0.06	0.07	0.04
Low morning level: -1 <i>SD</i>	-0.09	-0.13*	-0.08	-0.05
Low morning level: -1.5 <i>SD</i>	-0.12*	-0.16*	-0.05	-0.00
High morning level: +1 <i>SD</i>	-0.01	-0.01	0.07	0.10
High morning level: +1.5 <i>SD</i>	-0.07	-0.04	0.02	0.04
Diurnal Slope: Continuous	-0.00	0.01	0.21*	0.07
Low diurnal slope: -1 <i>SD</i>	-0.03	-0.01	-0.22*	-0.09
Low diurnal slope: -1.5 <i>SD</i>	0.03	0.06	-0.12*	0.04

Values for low morning cortisol and low diurnal slope are dichotomous indicators with 0 = above the cut-off and 1 = at or below the cut-off of either -1 or -1.5 *SD* below the sample mean. Values for high morning cortisol are dichotomous indicators with 0 = below the cut-off and 1 = at or above the cut-off of either +1 or +1.5 *SD* above the sample mean.

*
 $p < 0.05$.

Table 3

Standardized regression coefficients for the effects of cortisol morning levels and diurnal slopes on rank order changes in executive control and delay ability across 9 months

	<u>T2 executive control</u>		<u>T2 delay ability</u>	
	β	<i>SE</i>	β	<i>SE</i>
Step 1: covariates				
Child sex	-0.03	0.02	-0.04	0.06
Family income	0.12*	0.03	0.05	0.06
Wake time	-0.06	0.05	0.09	0.06
Latency to saliva collection	-0.10*	0.05	-0.07	0.06
T1 executive control	0.45**	0.07	—	—
T1 delay ability	—	—	0.49**	0.05
Step 2: diurnal cortisol (tested individually)				
Morning level: continuous	0.02	0.05	-0.01	0.06
Low morning level: -1 <i>SD</i>	-0.06	0.05	0.01	0.06
Low morning level: -1.5 <i>SD</i>	-0.10*	0.05	0.02	0.06
Diurnal slope: continuous	0.01	0.05	-0.01	0.06
Low diurnal slope: -1 <i>SD</i>	0.02	0.05	0.00	0.06
Low diurnal slope: -1.5 <i>SD</i>	0.05	0.05	0.07	0.06

Sex is coded 1 = girls and 2 = boys.

*
 $p < 0.05$,

**
 $p < 0.01$.

Table 4
Correlations among income, diurnal cortisol levels, effortful control and adjustment

	Sex	Income	Low morning cortisol	Flat cortisol slope	T1 executive control	T1 delay ability	T2 executive control	T2 delay ability	T2 total problems
Child sex	—								
Family income	-0.06	—							
Low morning cortisol	-0.09	-0.08	—						
Flat cortisol slope	0.01	-0.11*	0.17*	—					
T1 executive control	-0.09	0.19*	0.00	-0.00	—				
T1 delay ability	-0.14*	0.24*	0.07	-0.21*	0.26*	—			
T2 executive control	-0.13*	0.25*	-0.18*	-0.02	0.50*	0.31*	—		
T2 delay ability	-0.11	0.15*	-0.01	-0.10	0.27*	0.50*	0.37*	—	
T2 total problems	0.07	-0.22*	0.13*	0.00	-0.06	-0.15*	-0.09	-0.14*	—
T2 social competence	-0.06	0.25*	-0.12*	-0.17*	0.14*	0.05	0.18*	0.13*	-0.23*

Sex is coded 1 = girls and 2 = boys. Low morning cortisol is coded 0 = above the cut-off, 1 = at or below the cut-off of 1.5 SD below the sample mean. Flat cortisol slope is coded 0 = above the cut-off, 1 = at or below the cut-off of 1 SD below the sample mean.

* $p < 0.05$,

** $p < 0.01$.