Understanding the Relation of Low Income to HPA-Axis Functioning in Preschool Children: Cumulative Family Risk and Parenting As Pathways to Disruptions in Cortisol

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Abstract  This study examined the relation of low income and poverty to cortisol levels, and tested potential pathways from low income to disruptions in cortisol through cumulative family risk and parenting. The sample of 306 mothers and their preschool children included 29 % families at or near poverty, 27 % families below the median income, and the remaining families at middle and upper income. Lower income was related to lower morning cortisol levels, and cumulative risk predicted a flatter diurnal slope, with a significant indirect effect through maternal negativity, suggesting that parenting practices might mediate an allostatic effect on stress physiology.

Keywords  Low income · Cumulative risk · Parenting practices · Cortisol · Children

Introduction

Accumulating evidence demonstrates that poverty, low income, and associated demographic risk factors are detrimental to children’s stress physiology [1]. Allostatic load, or the physiological costs associated with adaptation, is a term used to describe how particular environmental stressors impart negative consequences on physical and mental health [2]. With 21 % of children living in poverty and 42 % living in low income in the United States [3], it is a social imperative to understand at what level of low income do deleterious
effects on children’s developing stress physiology meaningfully emerge. Beyond documenting relations between low income and stress physiology, examination of the specific risk and parenting factors that account for the relations between income and disrupted stress physiology are needed [4].

The hypothalamic–pituitary–adrenal (HPA) axis is a stress sensitive system that responds to environmental context and produces the hormone cortisol [5]. From an allostatic load perspective, stress may lead to high levels of HPA-axis responding, with children and adolescents producing higher levels of cortisol. As detailed by Gunnar and Vazquez [6], the allostatic load perspective also holds that the HPA-axis may actually down-regulate after chronic periods of elevated stress responding. This process is also sometimes referred to as ‘hypocortisolism’ [6–8]. In contrast to an allostatic load theory, the observation of hypocortisol responding could also reflect a habituation to chronic stress [9], in that individuals adapt to their environment and learn to cope with the constant challenges. To date, neither theory explaining the observation of attenuated cortisol responding has been ruled out. Empirical evidence demonstrates that lower income is related to both higher and lower levels of cortisol in children and adolescents [4]. Such differences seen in the alteration of cortisol levels has led some in the field to posit that either form of an altered HPA-axis—either higher or lower levels of cortisol—to be indicative of an inflexible stress response system [10, 11]. Although both higher and lower levels of cortisol may indicate an altered HPA-axis system, and both relate to lower income, there remain unexamined aspects of how the continuum of income relates to cortisol levels. For instance, studies examining the effects of low income on cortisol have used either federal poverty cut off levels or examined the continuum of income.

Additionally, studies vary in the indicator of cortisol utilized. For example, some studies assess cortisol level using waking and bedtime samples [12, 13], while others have assessed morning levels of cortisol at school or determine daily output [1, 14]. In addition, many studies examine cortisol reactivity to a laboratory challenge or stressor, which also produces distinct patterns of findings [15]. Such variation in sampling of income levels and cortisol measure obtained (e.g. reactivity level, diurnal level, daily output) across studies may contribute to inconsistencies seen in the pattern of relations with cortisol.

Poverty and Lower Income Relate to Higher Cortisol Levels

Many studies examining relations of cortisol and income demonstrate an inverse relation, such that lower income is related to higher levels of cortisol in children. Overnight urinary cortisol levels were significantly higher for 9-year old children in poverty compared to children from a middle-class background [16]. Using the same sample but in a longitudinal design, greater time spent in poverty was related to greater cortisol output, when children were tested at age 13 [17]. Comparing low-, middle-, and high-income families with 6–10 year olds, cortisol was found to be higher for children from low-income families compared to high-income families when cortisol was measured at school during the morning. However, by high school, the associations between income and cortisol level had disappeared [1]. Studies in which the sample included a narrow range of income also have found that lower income is related to higher cortisol. For example, in a study that included only children in poverty, that is 5-year old children attending a Head Start program, a lower income-to-needs ratio was associated with higher levels of cortisol during a lab protocol measuring executive functioning [18]. Finally, in a more nuanced study of poverty and families’ perception of poverty, a significant interaction revealed that for families in poverty reporting low perceived economic sufficiency, infants had higher basal cortisol
levels at age 7 months and demonstrated steeper declines between 7 and 48 months than did children in families in poverty reporting higher perceived sufficiency [10]. Taken together, these studies clearly suggest that poverty and low income are associated with higher levels of cortisol.

Poverty and Lower Income Relate to Lower Cortisol Levels

Conversely, several studies have demonstrated a positive relation between income and cortisol levels such that children living in lower income families exhibit blunted or reduced hormone levels. Notably, all of these studies involved samples of preschool-age children, suggesting that developmental period may be an important consideration in understanding the effect of income on stress physiology. For example, preschoolers of depressed mothers living in impoverished urban Mexico exhibited lower baseline cortisol reactivity levels collected at the home [19]. Similarly, in a sample of US preschool children, living in poverty predicted a blunted diurnal pattern, as indicated by lower morning levels that remained low through the day [12]. Finally, another study found that financial strain was related to blunted basal cortisol levels (sample collected at 10 a.m. and 4 p.m.) and attenuated stress reactivity in preschool children [15].

Given inconsistencies in the pattern of associations between income and cortisol levels, the first aim of this study was to clarify this relation by examining the continuum of income, as well as different poverty cutoffs, and examining their relations to continuous and categorical indicators of cortisol levels, borrowing from an approach used in a previous study. Using a categorical indicator of cortisol level, prior research has shown that different risk factors were differentially associated with low and high levels [11]. To date, no studies examining cortisol and income have employed both a continuous and categorical approach to testing how various aspects of the income continuum relate to high and low levels of cortisol. Therefore, we addressed the first aim of this study by examining a continuous indicator of income, as well as categorical indicators of poverty, in relation to continuous and categorical indicators of cortisol levels. The effects of poverty were explored at 100, 150 and 200 % of poverty to determine whether there is a threshold effect for poverty. In addition, several indicators of daily cortisol levels were examined, including morning level, evening level and diurnal slope. These were examined as categorical variables, with 1 SD cutoffs, to assess whether the impact of risk occurs at the ends of the distribution. The findings from these analyses will hopefully guide future research in selection of cortisol variables and sampling.

Risk Factors that Mediate the Relation Between Cortisol and Income

Children growing up in poverty and lower income families encounter significantly more risk factors than children from higher economic backgrounds [20]. Exposure to risk may be an important mechanism through which income negatively impacts children’s cortisol [4]. Individual risk factors such as maternal depression [20–22], maternal stress [23], single parent status [12], family transitions [10] have been shown to relate to cortisol in children. However, few if any prior studies specifically examined these risk factors as potential mediators of the relation between income and altered cortisol. Interestingly, the only study that tested risk as a potential mediator of the effects of income on HPA-axis functioning used a cumulative risk model [17]. In this longitudinal study, a sum of physical risk factors including crowding in the home, noise, substandard housing and social risk factors including family turmoil, child’s separation from their parents, and exposure to violence was used to create a cumulative risk index. However, cumulative risk did not mediate the
effects of income. Considering allostatic load models of the effects of contextual risk on the HPA-axis functioning, we anticipate that the accumulation of family risk, rather than the experience of any one risk factor alone, would be related to disruptions in the HPA-axis. That is, the presence of multiple risk factors, particularly when exposure to multiple risk is chronic, is expected to overburden the stress-response system and result in disruptions to HPA-axis functioning. Based on our prior findings, we predicted that cumulative risk would account for the relation between income and lower morning cortisol levels and a blunted diurnal pattern [12].

Parenting, Cumulative Risk, and Cortisol

Parenting behaviors may alter cortisol levels, potentially representing a proximal link in the overall pathway by which low income and cumulative risk eventually disrupts children’s cortisol levels. Research has examined the relation of parenting to HPA-axis functioning both in high-risk samples, such as low maternal education and high maternal stress, and low-risk samples. In general, parenting that is lower in sensitivity, higher in intrusiveness and harshness is associated with lower morning cortisol levels [24], flatter diurnal slope [25], greater reactivity [26], and slower cortisol recovery after a stressor [27]. Studies typically examine one or two parenting variables at a time, with more emphasis on affective (i.e. sensitivity, harshness) parenting behaviors compared to control-related parenting behaviors (i.e. limit setting). This study sought to examine several parenting behaviors simultaneously to see which specific parenting behaviors, if any, accounted for the relations of income and cumulative family risk to disrupted cortisol patterns.

Parenting has been posited to mediate the relation between risk and child cortisol levels. Specifically, two studies have examined environmental risk and parenting as mediators of the relation between poverty and disruptions to children’s cortisol. In a sample of preschool-age children representing the full range of income, there was a trend toward an association between maternal negative affect and children’s diurnal cortisol pattern, with negative affect trending toward mediating the relation between poverty status and a low, flat diurnal pattern [12]. Another study found that positive parenting, but not negative parenting, explained a small but significant portion of higher basal cortisol levels in young children, over and above environmental risk factors [10]. These two studies point to the possibility that parenting may mediate the relations of poverty and cumulative risk to disruptions in children’s cortisol levels.

This Study

This study sought to clarify the relations of low income and poverty to cortisol levels, and to examine potential pathways from low income to disruptions in cortisol through cumulative family risk and parenting. The first aim of the study was to test the relation between low income and regulation of the HPA system. To do this we examined income both continuously and at the poverty cutoffs of 100, 150 and 200% of poverty. The second aim of the study was to examine the effects of income-related cumulative risk or adversity on the regulation of the HPA system, testing whether cumulative risk accounted for the effects of low income. Specifically, we expected that the accumulation of risk, not necessarily any particular family risk factor, would be related to disruptions in the HPA-axis system. The third aim was to test parenting behaviors as a mediator of the effects of low income and cumulative risk on the HPA system.
Method

Participants

Study participants were 306 mothers and their 36 month-old children (M = 37, SD = .84 mos.) who were recruited from various public- and privately-funded sources, including daycares, preschools, libraries, health clinics, and charitable agencies and organizations serving low income families (e.g., county-sponsored “play and learn” groups for mothers and children, food banks, Catholic Community Services). Families at these sites received information forms about the study and could indicate their interest in participating in the study on the information forms returned through their organization or mailed directly to the research project in postage paid envelopes. Recruitment sites received an honorarium of $100.00 when 90 % or more of their families returned the forms, regardless of the number of families indicating interest in participating. If a site returned 75 or 50 % of the forms, the site received $75.00 or $50.00, respectively.

Families were recruited for participation so that there was equal representation across income levels. The sample was roughly evenly distributed across income levels, with 29 % of the sample at or near poverty (N = 90 at or below 150 % of the federal poverty threshold), 27 % low income (N = 83 below the local median income of $58 K), 25 % middle income (N = 78 above the median income to $100 K), and 18 % upper income (N = 54 above $100 K). 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. 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 or other. Mothers’ educational distribution included 3 % mothers with some high school attainment, 6 % completed high school, 34 % with some college, technical school or professional school, 30 % college graduates, and 27 % with post-graduate education. 81 % of mothers were married or had long time partners, 12 % were never married, 7 % were separated, divorced or widowed and were the single heads-of-household.

Procedures

Families were assessed in research offices on the university campus. At the beginning of the assessment, following the guidelines stipulated by the Social and Behavioral Sciences Institutional Review Board at the University of Washington, both active parental consent and child assent were secured prior to data collection. Assessments included neuropsychological, task performance, physiological, and questionnaire measures administered by a team of trained experimenters. Children completed neuropsychological and behavioral measures of effortful control, while mothers completed questionnaire measures in a separate room from which they were able to observe their children. Then mothers joined children to engage in a series of parent–child interaction tasks. Families were compensated $70 for this first visit.

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 child’s saliva 30 min after the child woke in the morning and 30 min prior to bedtime, for three consecutive days. Mothers were to place a sorbetto (Salimetrics, LLC State College, PA, USA) under the child’s tongue for
1 min and then place the sorbettes into a prelabeled swab storage tube. Mothers repeated this process with another sorbette to ensure adequate saliva volume. A staff member called families on the first night to ensure proper collection and answer questions. A reminder call was placed on the third evening to prompt mother’s to return the packets via the mail. Mailing saliva has been shown not to influence saliva collection [28] and this method has been successfully used in childhood samples [29].

Measures

*Income and Demographics*

Demographic questions completed by the parent included child’s race and ethnicity and parent education. Parents reported on household income from all sources. Poverty status was determined using the 2009/2010 HHS Poverty Guidelines, in place at the start of the study, which is an income-to-needs ratio based on the number of people in the home. Fifteen percent (n = 47) of the families’ income fell at or below 100 % of the poverty threshold, an additional 14 % (n = 44) were at or below 150 % of the poverty threshold, and another 10 % (n = 30) were at or below 200 % of the poverty threshold.

*Cumulative Family Risk*

Cumulative risk included 8 factors: low maternal education, single parent status, divorce, adolescent parent, maternal depression, negative life events, residential instability, and household density. The correlations among the risk factors ranged from .02 to .50 in magnitude indicating that they were related but not redundant. A total family adversity score was the sum of all of the component factors. Dichotomous risk factors were scored 0 = not present, 1 = present. Continuous risk factor scores were converted into proportions of the total possible score so that they ranged from 0 to 1, and thus, were weighted equally with the dichotomous variables without loss of their continuous scale. The average cumulative risk score was 1.01 (SD = .83; range 0–4.6).

Mothers reported on their educational attainment. Risk was indicated by mothers’ not graduating from high school, with 3 % of mothers meeting this criterion. Mothers reported on their marital status, and families were identified as single parent families if the mother indicated she was never married, currently widowed, separated or divorced, or living for less than 1 year with a live-in partner, with 19 % mothers meeting these criteria. Family structure transitions were indicated by mothers reporting being divorced in the child’s lifetime and occurred in 3 % of the families. Mothers reported their age at the time of the study child’s birth, and 3 % of the mothers were considered an adolescent parent given that they were 19 years or younger when the child was born. Residential instability was indicated by the family changing households 3 or more times in the previous 3 years, encompassing the majority of the child’s lifetime, and 10 % of the children experienced 3 or more moves. Household density was calculated as the number of individuals living in the family home divided by the number of total rooms (kitchen, dining, living, family, bed and bathrooms) in the family home. The average ratio was .52, indicating that on average, there were twice as many rooms in the house as there were individuals living in the home. The score was converted to a proportion of the highest score in the sample.
Negative Life Events

Negative life events were assessed with parent report on the General Life Events Schedule for Children [30], previously shown to have significant associations with child adjustment [31]. The 29 events include a range of moderate to major negative events including changing schools, death of a family member or friend, parental arrest, loss friends or pets. Parents reported the occurrence of events within the previous 9 months, and total scores were the number of events that occurred. The average number of negative life events was 5.3 (SD = 4.0; range 0–26). The total score was converted into a proportion of the possible 29 events.

Maternal Depression

Mothers reported on their own depressive symptoms over the previous month using the 20-item Center for Epidemiological Studies–Depression Scale (CES-D) [32], a widely used self-report scale designed to measure depressive symptoms in the general population. Participants indicate whether each symptom was present on a scale of 0 (rarely or never) to 3 (most of the time), and the items were summed for a total score, with higher scores indicating higher levels of depression. Internal consistency of .89 has been reported and was .88 in this study. The average score in this sample was 10.02 (SD = 8.39, range 0–46.67), with a clinical cut-off of 16. The total score was converted into a proportion of the total possible score of 60.

Parent–Child Interaction

Parenting was assessed from interactions between mothers and their children. Mothers and children engaged in a series of activity segments (7 min restricted play, 7 min free play, 7 min instructional activity, 3 min clean-up) [33] totaling approximately 25 min (including instructions between tasks). In restricted play, the mother was instructed to allow the child to play with all toys in the room except those in a specified place, a freely accessible shelf of highly desirable toys. This increased the opportunity to observe parental control strategies and consistency of rule enforcement. This was followed by a period of free play (the mother and child were informed that they can now play with the previously restricted toys) facilitating observation of maternal involvement, positive affect and responsiveness to child’s cues. The next segment involved mothers helping children to build a Lego figure that was challenging for the child, increasing the likelihood of observing maternal scaffolding, which includes respect for autonomy, guidance, and low intrusiveness. Finally, a clean up segment increased the opportunity to observe control strategies and consistency.

Parental warmth, negativity, limit setting, scaffolding, and responsiveness were coded for each 1-min epoch for all segments, and then averaged across epochs and across segments. Parenting behaviors were coded from videotapes of the mother–child interactions by advanced undergraduates using a coding system that was adapted from three existing, well-established coding systems: the system for coding interactions and family functioning (SCIFF) [34], the Parenting Style Ratings Manual [35], and the parental warmth and control scale—revised [36] and used previously by this research team [37]. All codes were rated on 5-point Likert scales with 1 indicating the lowest level of behavior and 5 indicating the highest level of behavior on that scale. Mothers were rated on their displays of positive and negative emotion as well as the quantity and quality of their engagement with their children during the tasks. Positive Affect captures the frequency and level of
behavioral and verbal expressions of happiness, comfort and connection in the interaction, and warmth toward the child. Interactiveness assesses the quantity of verbal and non-verbal engagement with the child. Positive affect and interactiveness were combined into a measure of warmth. “Negativity” assesses the overall negative tone or level of tension expressed by the mother and included verbal and non-verbal expressions of irritation or frustration with the child that were critical, rejecting or invalidating. Limit Setting involves ratings of mothers’ clarity, consistency, and follow-through of their directives when child behavior during tasks required it. Necessary limit setting includes protecting child’s safety, protecting property, and parent efforts to modulate child affect or behavior. Scaffolding was a combination of guidance and structuring of the interaction, encouragement of child autonomy, and low levels of negative or intrusive control. In effect, this reflected the parent’s ability to intervene in some way when the child needed it and disengage when the child was functioning independently again in a way that helps the child regulate his/her emotional state. Maternal Responsiveness to children’s expressions of negative affect was also rated and indicated the mother’s sensitivity to cues of the child. Inter-rater reliability was assessed by independent recoding of 20 % of the interactions and was indicated by the intra-class correlation (ICC). The ICCs for parenting dimensions aggregated across the four tasks for warmth, negativity, scaffolding, limit setting and responsiveness were .80, .75, .81, .73, and .67, respectively.

Cortisol Assay

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 1,000 rpm for 10 min in order to separate the saliva from the collection swab. The cleared eluant was then transferred to a 1.6 ml Eppendorf tube and stored at minus 70 °C until testing for cortisol. Prior to assay, each sample was subjected to another centrifugation step of 5,000 rpm for 5 min 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, USA). The sensitivity of this kit ranges from .005 to 2.5 μg/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 = .950 μg/dl; the MCV for the low concentration sample = .083 μg/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.

Cortisol processing

Of 306 families, 33 (11 %) did not return any samples. One family returned 2 of the 6 six samples requested; 1 family returned 3, 4 returned 4, and 2 families returned 5 samples of the 6 requested. As a result, some cortisol data were available from 265 families. Assay results that were over 2.0 μg/dl (29 samples from 20 children) were deemed biologically
implausible and the values were not used, consistent with methods used in other studies [20]. Values in samples that had been collected 90 min after wake up or prior to bedtime were also discarded. Only one case was fully discarded because all cortisol values were over 2.0 μg/dl.

Use of steroid medications, an inhaler, health, food intake and napping have been shown to affect cortisol levels. Mothers completed a daily questionnaire regarding sampling times and their children’s health, medication use, eating times, and napping on sampling days. The questionnaires were reviewed to ensure compliance. In addition, mothers were given a phone call on the first evening of collection to review the collection procedures and answer any questions. Mothers were reminded to avoid sampling when their children were using steroid based medications or were ill. Mothers were mailed additional materials if they accidently sampled when the child was ill.

Cortisol Measures

Assay results for all three mornings and evenings were averaged to create measures of average morning and evening levels. A diurnal slope value was computed by subtracting the average evening from the average morning value. The average morning score was .29 (SD = .21), average evening was .13 (SD = .18), and average diurnal slope was .16 (SD = .20). As is common with cortisol data, values were positively skewed, and log transformations were applied to average morning and the average evening variables. Using the log-transformed morning and evening values, a diurnal pattern was recalculated. All data used in analyses were conducted with log-transformed values. To explore whether associations with low income would be detected on a continuum or at the ends of the continuum of cortisol, categorical variables for low morning and low diurnal slope were created. Low morning and low diurnal slope values were indicated at 1 SD below the mean. For low morning levels, 234 children had scores above the −1 SD cutoff and 36 children had values at or below the −1 SD cutoff. For low diurnal slope, 224 children had values above the −1 SD cutoff and 47 children had values at or below the −1 SD cutoff. To explore whether low income or poverty was related to high cortisol levels, high morning and evening values were calculated at ?1 SD above the mean. For high morning values, 235 children had values below +1 SD above the mean. For high evening values, 222 children had values below +1 SD, and 48 children had values at or above +1 SD.1

Analytic Plan

First, cortisol data were analyzed for compliance with the collection protocol and identification of covariates for analyses. Next, analyses were conducted to examine the relations of low income, cumulative family adversity and parenting to children’s cortisol levels and diurnal patterns. To address the first aim of the study, correlations among income, poverty and children’s cortisol levels were examined. Subsequently, we tested the relations of individual family risk factors and cumulative family adversity to cortisol levels, with the hypothesis that the accumulation of family adversity would relate to cortisol levels more consistently than individual risk factors. Next, multiple regression models were conducted to test whether cumulative family adversity and parenting predicted cortisol levels and

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1 Analyses also were conducted with 1.5 SD cutoffs and the pattern was identical. Thus, the variables based on 1 SD cutoffs are presented.
accounted for the effects of low income on cortisol levels to address the second and third aims of the study. Regressions were tested in Mplus 6.0 [38] using Full Information Maximum Likelihood Estimation (FIMLE). FIMLE requires estimation of means and intercepts, as well as covariances and path coefficients, using all the data available simultaneously to calculate parameter estimates. FIMLE has been found to be less biased and more efficient than other techniques for handling missing data [39]. Our examination of bias in missing data (see below) suggested that the pattern of missing data introduced minimal bias and aligned with the assumptions of FIMLE. Therefore, families with any data on the study variables were included in the path analyses for a sample size of 306.

Missing Data Analyses

Participants missing any data (n = 100) on study variables were compared with those missing no data (n = 206) to assess the extent of bias introduced by missing data. No participants were missing data on income. Complete data on cumulative risk, parenting and cortisol were available for 96, 94 and 88% of the participants, respectively. Levels of family income, cumulative risk, parenting, and cortisol were compared. Participants missing data differed from those not missing data in that they had lower income (M missing = 7.80, M no missing = 9.20, t(304) = 2.97, p < .01), higher cumulative risk (M missing = 1.22, M no missing = .92, t(304) = -2.76, p = .01), lower maternal warmth (M missing = 3.64, M no missing = 3.78, t(304) = 2.54, p = .01) and lower maternal scaffolding (M missing = 3.32, M no missing = 3.52, t(304) = 2.78, p = .01). However, the effect sizes of the associations of missingness to income (r = - .17), cumulative risk (r = .17), warmth (r = -.15), and scaffolding (r = -.16) were modest and did not reach previously cited thresholds for introducing substantial bias (i.e., r > .40) [40]. Thus, it appears that little bias was introduced due to missing data.

Results

Cortisol Collection Protocol Compliance

Prior to conducting analyses, variables addressing compliance with the cortisol collection protocol were examined to identify potential covariates of cortisol levels. Health, medication, food ingestion, and whether the child napped were examined in relation to cortisol variables. One- to two-percent of children were reported to have an illness that included 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 and evening cortisol levels (r = .14, p = .02 for both). However, excluding those cases from the sample did not alter

2 A latent approach to modeling multiple observations of morning level and diurnal slope cortisol variables was attempted. In this approach, a latent morning level factor was specified with the 3 morning values as indicators, and the daily latency to collection variables as error covariates; and a latent diurnal slope factor was specified with the 3 morning–evening values as indicators and daily latency to collection variables as error covariates. This model was not identified. Thus, to estimate this model the latent factor indicator loadings were set equal to each other as were the error-covariate loadings. Although this model was identified, there were negative residuals that resulted in the Theta-Eps matrix not being positive definite. Two residuals were set to zero, resulting in a model that produced parameter estimates and standard errors for significance tests. The pattern of findings in this model was nearly identical to the findings resulting from the multiple regression analyses. However, given the instability of the models including the latent factors, we presented the results from the regression analyses using observed variables.
the pattern of the correlations of cortisol variables with other variables, and therefore these participants’ samples were retained. Inhaler use was reported in 3.5 % of children in the sample, but none of the children were reported to have used their inhalers on the days of sampling. Similarly, 1.6 % of the children in the sample were reported to have used steroidal mediations on the days of sampling. However, use of a steroidal mediation was unrelated to any of the cortisol variables. Eating within a half hour prior to collecting saliva was reported in 10–15 % of cases on the 3 mornings of collection, and in 14–21 % of cases on the 3 evenings of collection. Eating within the 30 min timeframe on morning 3 was related to lower morning cortisol level ($r = - .15$, $p = .01$), and eating within 30 min on evening 2 was related to lower evening cortisol ($r = - .13$, $p = .04$). 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 therefore these participants’ samples were retained. Sixty-seven percent of children were reported to have taken a nap on each of the 3 days. However, napping was not related to the cortisol variables. None of the remaining variables were consistently significantly related to cortisol variables.

Wake time, bed time and latencies between these times and collection times were examined. Latency to collection for morning 1 and Child wake time on morning 1 were related to the average morning levels, $r = - .15$, $p < .05$ and $r = - .13$, $p < .05$, respectively, whereas latency to collection and wake time of the other 2 days were unrelated to the cortisol variables. Composites of collection latencies and wake times across the 3 days were computed as the averages of those variables. These were related to average morning levels $r = - .15$, $p < .05$ and $r = - .12$, $p < .05$, respectively. Neither evening collection times nor bed times were related to evening cortisol levels or diurnal patterns.

Correlations of Individual Risk Factors and Cumulative Risk with Cortisol

The associations of family income and poverty status with measures of cortisol are presented in Table 1. Lower income was related to lower average morning cortisol levels. Similarly, there were trends toward poverty status being related to lower morning levels, regardless of whether 100, 150 or 200 % of the poverty cutoff was used. However, only the continuous indicator of income was significantly related to cortisol. There were also trends towards associations of income and poverty with the categorical indicator of low diurnal slope.

Also presented in Table 1 are the correlations of the family risk factors with cortisol. The individual family risk factors were largely unrelated to the HPA indicators, with only 2 exceptions. Being an adolescent parent was related to lower morning values, and single parent status was related to a low diurnal slope. Cumulative family risk, which was a sum of the individual family risk factors, was correlated with lower morning levels and a low diurnal slope. These correlations suggest that no one risk factor accounted for the relation of cumulative risk to cortisol, but rather, points to the accumulation of risk being the operative factor in relation to cortisol. Based on the magnitude and consistency of the correlations of income and cumulative risk with cortisol variables compared to the individual family risk factors, continuous family income and cumulative family risk were retained in the remaining analyses. In addition, based on the pattern of correlations only continuous morning level and diurnal slope cortisol variables were examined as outcomes in the remaining analyses, as these were largely capturing the pattern of relations with the categorical cortisol variables, as well.
The correlations among income, cumulative risk, parenting, and cortisol indicators were examined to assess the plausibility of the proposed mediating relations (see Table 2). Lower family income was related to higher levels of cumulative risk. Parenting was related to both income and cumulative risk. Low income and cumulative risk were related to less maternal warmth and scaffolding and higher negativity. Cumulative risk also was related to lower responsiveness. Only maternal negativity demonstrated a significant zero-order correlation with the cortisol variables with higher negativity relating to lower morning levels and lower diurnal slope. Thus, cumulative risk and parenting were plausible mediators of the effects of low income on cortisol levels. Although only negativity was significantly correlated with cortisol measures, all the parenting variables were retained in subsequent analyses to assess whether the set of parenting variables accounted for the effects of income and cumulative risk, as well as to test the independent effects of parenting to identify particular parenting behaviors that are potentially relevant to cortisol functioning.

### Table 1: Correlations of family risk variables and cortisol morning levels and diurnal slope

<table>
<thead>
<tr>
<th></th>
<th>Average morning</th>
<th>Average evening</th>
<th>Diurnal slope</th>
<th>Low (+1 SD) morning</th>
<th>High (+1 SD) evening</th>
<th>Low (+1 SD) diurnal slope</th>
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<td>.06</td>
<td>-.07</td>
<td>.05</td>
<td>-.02</td>
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<td>100 % Poverty</td>
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<td>.02</td>
<td>-.11†</td>
<td>.09</td>
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<tr>
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† \( p < .10 \), * \( p < .05 \)

### Correlations Among Income, Cumulative Risk, Parenting, and Cortisol

The correlations among income, cumulative risk, parenting, and cortisol indicators were examined to assess the plausibility of the proposed mediating relations (see Table 2). Lower family income was related to higher levels of cumulative risk. Parenting was related to both income and cumulative risk. Low income and cumulative risk were related to less maternal warmth and scaffolding and higher negativity. Cumulative risk also was related to lower responsiveness. Only maternal negativity demonstrated a significant zero-order correlation with the cortisol variables with higher negativity relating to lower morning levels and lower diurnal slope. Thus, cumulative risk and parenting were plausible mediators of the effects of low income on cortisol levels. Although only negativity was significantly correlated with cortisol measures, all the parenting variables were retained in subsequent analyses to assess whether the set of parenting variables accounted for the effects of income and cumulative risk, as well as to test the independent effects of parenting to identify particular parenting behaviors that are potentially relevant to cortisol functioning.

### Regressions of Parenting, Cumulative Risk on Cortisol

Multiple regressions were conducted to test whether cumulative risk and parenting accounted for the association of low income with cortisol levels. Estimates of indirect effects were examined to test for potential intervening relations of cumulative risk and parenting. Variables were entered in hierarchical steps to test whether cumulative family adversity served as an intervening pathway between income and cortisol, and whether parenting served as an intervening pathway from income and cumulative risk to cortisol (see Table 3).
Table 2  Correlation among income, cumulative family adversity, parenting, cortisol morning levels and diurnal slope

<table>
<thead>
<tr>
<th></th>
<th>Family income</th>
<th>Cumulative adversity</th>
<th>Warmth</th>
<th>Negativity</th>
<th>Scaffolding</th>
<th>Responsiveness</th>
<th>Limit setting</th>
<th>Morning cortisol</th>
<th>Diurnal cortisol</th>
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<td>-.12*</td>
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<td>-.09</td>
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<td>-.26*</td>
<td>.40*</td>
<td>.07</td>
<td>.29*</td>
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<td>.03</td>
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<td>-.12*</td>
<td>-.23*</td>
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<td>.42*</td>
<td>.05</td>
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<td>-.03</td>
<td></td>
<td>.01</td>
<td>-.02</td>
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<tr>
<td>Limit setting</td>
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<tr>
<td>Morning cortisol</td>
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</tbody>
</table>

* < .05
First, the relation of income to morning cortisol level and diurnal cortisol slope was tested with the effects of covariates of child gender, average latency of cortisol collection and average wake time. Lower income was significantly related to lower morning levels, but not diurnal slope. Cumulative risk was entered in the second step of the regression. When cumulative risk was added to the model, it significantly predicted lower morning cortisol levels, and the magnitude of the effect of income on morning level decreased and became nonsignificant. There was a trend toward an indirect relation of income on average morning level through cumulative risk ($\beta = .09, \text{SE} = .06, p = .10$). In addition, cumulative risk significantly predicted a lower diurnal slope, indicating a flat slope.

Finally, the set of parenting variables were added to the models. The effects of cumulative risk were reduced and became nonsignificant when the set of parenting variables was added. Maternal warmth predicted higher morning cortisol levels above income and cumulative risk. Further, there was a trend toward an association of responsiveness to higher morning level. Maternal negativity, scaffolding and responsiveness predicted diurnal slope, with negativity being related to a flatter diurnal pattern, responsiveness to a steeper diurnal slope, and a trend toward an association of scaffolding with a higher diurnal slope. The indirect effect of cumulative risk on diurnal cortisol slope through maternal negativity was significant ($\beta = -.03, \text{SE} = .01, p = .05$). There was a trend toward an indirect effect of income on diurnal slope through cumulative risk and maternal negativity ($\beta = .004, \text{SE} = .002, p = .06$).

**Table 3** Standardized regression coefficient for the effects of income, cumulative family adversity, and parenting on cortisol morning level and diurnal slope

<table>
<thead>
<tr>
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<th>Morning cortisol level</th>
<th>Diurnal cortisol slope</th>
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<td>$\beta$ at entry</td>
<td>SE</td>
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<td>Cortisol child wake time day 1</td>
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<td>Scaffolding</td>
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<td>Responsiveness</td>
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<tr>
<td>Limit Setting</td>
<td>.07</td>
<td>-.02</td>
</tr>
</tbody>
</table>

* $< .05$
than dichotomous poverty cut points, captured the association of low income to low morning cortisol level. Second, the results supported the hypotheses that low income would be related to the accumulation of adversity and risk experiences rather than the individual risk factors. Finally, parenting was related to HPA-axis activity and partly accounted for the relations of low income and cumulative risk to disruptions in HPA-axis activity.

Lower income was related to lower morning cortisol levels but not to evening cortisol level or diurnal slope. Examining the poverty cut points did not enhance our understanding of the relation between income and cortisol. This suggests that the relation between income and morning level was consistent along the continuum of income, with little information gained by examining the ends of the continuum. Other studies have examined categorical variables representing the ends of the cortisol distribution so that associations occurring at either end of the distribution would not be obscured [11, 12]. However, in this study, the continuous income indicator and continuous morning cortisol level indicator captured the association between these variables without loss of information resulting from artificially dichotomized variables. This association was likely observed as a result of the study design, which employed a flat distribution of income that represented low and high-income families equally. The use of cutoffs might be particularly useful in samples for which the effects of a variable might be obscured by low representation in the “high-risk” ends of the continuum.

In this study, neither the continuous indicator of income nor the poverty indicators were related to high cortisol levels, replicating the findings of studies of preschool children [12, 15, 19], but in contrast to other studies of income and cortisol. As the field attempts to differentiate what factors contribute to the prediction of either high or low cortisol levels, age of participants may be a critical factor in formulating expectation about the pattern of disrupted cortisol to be observed. There is some evidence that very young children, starting at 12 months, may enter a stress hyporesponsive period, in which low levels of cortisol are observed. The stress hyporesponsive period is a physiological process observed in rats days 4–14. How or whether this translates to a developmental period in human children is unclear. It is possible that the lower cortisol levels in response to adversity seen in preschool children could be related to this biological phenomenon [41].

There was a trend towards an association of being at or near poverty with a flattened diurnal slope, which might reflect the significant association between cumulative risk and low diurnal slope. As poverty is a marker for a number of adverse experiences or conditions in children’s lives [42], it may be the accumulation of these experiences, and not one experience alone, accounts for the effects of low income [17]. In this study, cumulative family risk was related to lower morning cortisol levels and a low diurnal slope. Additionally, the correlations of cumulative family risk with the cortisol indicators were of greater magnitude and consistency than the correlations of the independent risk factors with cortisol, pointing to the value of examining the effects of the accumulation of risk on cortisol functioning. Nonetheless, it would be fruitful to identify which specific individual risk factors relate to disruptions in cortisol levels or functioning in the future. Further, cumulative family risk accounted for the effects of low income on morning cortisol levels. Although this finding requires replication, it suggests that a potential mechanism of the effects of poverty on disruptions of the HPA-axis occurs through the burden of stressful conditions experienced by children, consistent with hypotheses of the study and with the concept of allostatic load [2]. It should be noted, though, that flattened diurnal patterns could also reflect adaptation to the stressful circumstances. This data cannot rule out the latter possibility.
Experiences of low income and cumulative family risk tax parental resource, undermining parenting [37]. Results from the current study indicate that parenting potentially served as an intervening mechanism of the effects of income and cumulative risk on HPA-axis functioning. In particular, maternal negativity presented a pathway through which cumulative family risk related to lower morning cortisol levels. This replicates and extends prior findings showing that higher levels of maternal negativity predicted disruptions in children’s HPA-axis functioning [12]. Several human studies have found that parenting constructs similar to negativity, such as harshness, emotional unavailability [26], withdrawal and unresponsiveness [43] were related to disrupted cortisol in children. Experimental animal studies corroborate these findings in that less licking, grooming and nursing behaviors performed by rodent mothers had a negative impact on offspring’s HPA-axis [44]. The animal literature has repeatedly demonstrated that maternal responsiveness is essential for the regulatory development of the offspring’s HPA-axis. Animal separation paradigms that serve as a model for early adversity experiences show that maternal separation is detrimental to offspring HPA-axis development [45, 46]. The current findings extend beyond these studies to show that particular aspects of affective parenting behaviors partially explain the relation between cumulative risk and lower levels of morning cortisol.

Strengths and Limitations

This study has several strengths. First, by using a large sample and a flat income distribution that over-represented low-income families, a rigorous test of the effects that low income may have on children’s HPA-axis was permitted. In addition, the use of observational parenting measures afforded independent assessment of cumulative family risk and parenting, thus strengthening the conclusions that can be drawn about the pathways by which income may disrupt children’s cortisol. Finally, multiple indicators of cortisol were examined to determine whether differences in findings across studies could be partially attributed to the inconsistencies in cortisol measures used.

A limitation of this study was the use of cross-sectional data. Longitudinal data would allow more robust examination of the mediating effects of cumulative family risk and parenting in the relation between income and children’s cortisol levels. In addition, because cortisol reactivity was not measured, the association between cortisol reactivity and income was not examined. Despite these limitations, the use of multiple indicators of cortisol and observed parenting measures strengthen the overall conclusions.

Conclusions and Implications

Our understanding of how allostatic load operates is enhanced by considering the distal and proximal risk factors to which children are exposed and how they related to HPA-axis functioning. Low income exposes families to more experiences of risk and adversity and may impact children through their experiences of disruptions in their families and parenting behaviors. At the individual level, difficulties with adjustment observed in children growing up in economically disadvantaged environments may in part be explained by disruptions in their stress-physiology, rendering them less able to cope with the accumulating experiences of adversity faced within the family context. This lends further justification for prevention efforts that promote parenting behaviors that protect children from experiences of adversity and facilitate regulation of children’s physiological stress response systems to reduce the adverse impact of economic disadvantage on children’s well-being.
Summary

Although previous studies have explored the relation of family income to disrupted cortisol patterning in young children, there is little research identifying the levels of low income at which disrupted patterns of cortisol functioning emerge. There is also inconsistency in findings regarding whether low income is related to disrupted diurnal cortisol patterns that are elevated or blunted. In this study, we examined the relation of low income and poverty to cortisol levels and tested cumulative family adversity and parenting as potential pathways to account for the association. We found that lower income was related to lower morning cortisol levels. In addition, we found that cumulative family adversity predicted a flatter diurnal slope, with a significant indirect effect through maternal negativity, suggesting that negativity might mediate this effect. The implications of these findings include clarifying that disruptions to HPA-axis functioning occur on a continuum of income and may be accounted for by the burden of stress and adversity associated low income and through parenting behaviors that may shape HPA-axis regulation. Thus, low income families can be identified as potentially benefitting from prevention programs that promote parenting strategies that may facilitate HPA-axis regulation.

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