Received: 8 July 2019 | Revised: 5 April 2020 | Accepted: 9 April 2020

DOI: 10.1002/dev.21981

RESEARCH ARTICLE

Developmental Psychobiology WILEY

# Biological sensitivity to context in Pakistani preschoolers: Hair cortisol and family wealth are interactively associated with girls' cognitive skills

Emma Armstrong-Carter[1] | Jenna E. Finch[2] | Saima Siyal[3] | Aisha K. Yousafzai[4] | Jelena Obradović[1]

[1]Stanford University, Stanford, CA, USA

[2]University of Nebraska-Lincoln, Lincoln, NE, USA

[3]Aga Khan University, Karachi, Pakistan

[4]Harvard University, Cambridge, MA, USA

**Correspondence**
Emma Armstrong-Carter, Stanford University, 520 Galvez Mall, Stanford, CA 94305, USA.
Email: emmaac@stanford.edu

**Funding information**
Grand Challenges Canada Saving Brains Initiative, Grant/Award Number: 0061-03; Institute of Education Sciences, Grant/Award Number: R305B140009; Jacobs Foundation Advanced Research Fellowship, Grant/Award Number: 2017-1261-07

[Correction added on July 17, 2020, after first online publication: The additional funder 'Jacobs Foundation Advanced Research Fellowship, Grant/Award Number: 2017-1261-07' was included in the funding information section and also in the acknowledgement section.]

## Abstract

Many young children in low- and middle-income countries (LMICs) face heightened risk for experiencing environmental adversity, which is linked with poorer developmental outcomes. Children's stress physiology can shed light on why children are differentially susceptible to adversity. However, no known studies have examined whether links between adversity and children's development are moderated by children's stress physiology in LMICs. The present study revealed significant interactive effects of hair cortisol concentrations, an index of chronic physiological stress regulation, and family wealth on preschoolers' cognitive skills in rural Pakistan. In a sample of 535 4-year-old children ($n$ = 342 girls), we found significant associations between family wealth and direct assessments of verbal intelligence, pre-academic skills, and executive functions only in girls with lower hair cortisol concentrations. Specifically, girls with lower cortisol concentrations displayed greater cognitive skills if they came from relatively wealthier families, but lower cognitive skills if they came from very poor families. There were no significant associations among boys. Results provide evidence of biological sensitivity to context among young girls in a LMIC, perhaps reflecting, in part, sex differences in daily experiences of environmental adversity.

KEYWORDS

adversity, child development, cognitive, hair cortisol, low- and middle-income country

## 1 | INTRODUCTION

Children's stress physiology can shed light on which children are more or less susceptible to contextual experiences (Obradović, 2016). Over the last decade, researchers have identified concentrations of the stress hormone cortisol in the hair shaft as a promising biomarker indexing the long-term, chronic impact of adversity on functioning of the hypothalamic–pituitary–adrenal (HPA) axis (Bates, Salsberry, & Ford, 2017). However, links between hair cortisol concentrations (HCCs), adversity, and young children's development have only been tested in a few studies of children from high-income countries. The goal of the present study was to examine whether HCCs were related to cognitive development in preschoolers from rural Pakistan, a lower middle-income country. Furthermore, we examined whether HCCs served as a marker of biological sensitivity to children's family contexts by moderating the effects of family socioeconomic resources at birth on preschoolers' verbal intelligence, pre-academic skills, and executive functions (EFs).

  

### 1.1 | Hair cortisol and family resources

HCCs are multidetermined (Bates et al., 2017), but provide some insight into chronic levels of cortisol exposure that is partially influenced by ongoing environmental circumstances (Bates et al., 2017; Gerber et al., 2017; Simmons et al., 2019). As such, HCCs represent cumulative cortisol exposure over the 3 months prior to collection and long-term activity of the HPA axis (Bates et al., 2017). Research from high-income countries has revealed significant associations between children's HCCs and their families' socioeconomic status, typically examined using measures of maternal education and family income (Rippe et al., 2016; Ursache, Merz, Melvin, Meyer, & Noble, 2017; Vaghri et al., 2013; Vliegenthart et al., 2016). Specifically, higher HCCs have been linked to lower family income in children at age 6 (Rippe et al., 2016) and to lower parental education at ages 4 to 18 (Vaghri et al., 2013; Vliegenthart et al., 2016). Additionally, elevated HCCs have been associated with less family access to social and economic resources at age 4 (Simmons et al., 2019), and greater food insecurity from ages 2 to 5 (Ling, Robbins, & Xu, 2019). However, several studies have also found null results. For example, HCCs were not related to parental education in infants and 7-year-olds (Gerber et al., 2017; Karlen et al., 2015; Liu, Snidman, Leonard, Meyer, & Tronick, 2016; Palmer et al., 2013), family income in 5- to 7-year-olds (Ursache et al., 2017), or a composite of parental education and family income in infants (Flom, St. John, Meyer, & Tarullo, 2017). These mixed results call into question which aspect of family socioeconomic resources best captures early childhood experiences that affect chronic functioning of HPA axis.

Current research linking HCCs and family socioeconomic resources has only been conducted in high-income countries with relatively restricted income ranges, representing a small fraction of children's early experiences worldwide (Rippe et al., 2016; Vaghri et al., 2013; Vliegenthart et al., 2016). We know very little about the interplay between family socioeconomic resources and HCCs in low- and middle-income countries (LMICs). Although there is considerable variation across LMICs, communities, and families, many children experience numerous, co-occurring forms of adversity that hinder cognitive development, including poverty, nutritional deficiencies, environmental toxins, and inadequate cognitive stimulation (Black et al., 2013; Obradović & Willoughby, 2019; Walker et al., 2007). In this context, parental education is a poor proxy for family socioeconomic status, because most women are likely to never complete primary education (UNICEF, 2013). Instead, family environmental socioeconomic status is better represented by family wealth and resources that reflect tangible household assets and dwelling characteristics, such as access to drinking water and home sanitation facilities (Vyas & Kumaranayake, 2006), that show considerable variability across families in LMICs.

### 1.2 | Hair cortisol and child development

Hyper- and hypo-secretion of stress hormones have been hypothesized to undermine child development (Blair & Raver, 2012; Gunnar & Vasquez, 2001). While a large body of work links dysregulated salivary cortisol responses to children's maladaptive outcomes (Blair, Granger, & Razza, 2005; Bruce, Davis, & Gunnar, 2002; Gunnar & Vazquez, 2001; Shirtcliff, Granger, Booth, & Johnson, 2005), there is limited empirical evidence of associations between HCCs and children's development (Bates et al., 2017; Gray et al., 2018). A few recent studies demonstrated that HCCs is related to adult reports of children's behavior. In community samples, higher HCCs have been linked with more maternal-reported socio-emotional problems in infants (Palmer et al., 2013) and greater teacher-reported behavioral problems in elementary and middle school-aged children (Fuchs et al., 2018), but there have also been null results (Kao, Doan, St. John, Meyer, & Tarullo, 2018). Conversely, lower HCCs have also been linked with more ADHD symptoms in one sample of kindergarten boys (Pauli-Pott et al., 2017; Schloß et al., 2018). This association was most pronounced among boys with relatively greater psychosocial risk, suggesting that potential links between HCCs and child development may be stronger in contexts of elevated adversity (Pauli-Pott et al., 2017).

Acute cortisol response to environmental events is related to cognition in an inverted U-shape function, such that moderate levels of salivary cortisol response are associated with optimal cognitive performance, while too little or too much cortisol tends to undermine cognitive performance (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Willoughby, Blair, Wirth, & Greenberg, 2010; Obradović, 2016). Limited studies have examined links between chronic cortisol response, indexed by HCCs, and children's cognitive skills. These have shown mixed results. Lower HCCs at age 5 were linked with higher levels of directly assessed working memory and inhibitory control, in a sample of middle-class expatriate families in the United Arab Emirates (von Suchodoletz & Barza, 2015). In contrast, two more studies did not find links between HCCs and a direct assessment of cognitive development in 1-year-olds (Palmer et al., 2013) or direct assessments of IQ and EFs in 7-year-olds (Chau, Cepeda, Devlin, Weinberg, & Grunau, 2017). Given the paucity of evidence, more research is needed to understand how children's HCCs, a cumulative measure of HPA axis' activation, are related to cognition. It is particularly important to examine if there are associations during preschool age, a sensitive period for cognitive development (Jensen, Berens, & Nelson, 2017).

### 1.3 | Stress response as a measure of differential susceptibility

Physiological stress response has long been identified as important moderator of associations between children's environmental experiences and development. This has been observed both cross-sectionally and longitudinally (e.g., Essex, Armstrong, Burk, Goldsmith, & Boyce, 2011; Obradovic et al., 2016). Two theories, Differential Susceptibility (Pluess & Belsky, 2007) and Biological Sensitivity to Context (BSC; Blair, 2002; Boyce, 2007; Ellis, Essex, & Boyce, 2005; Klein Velderman, Bakermans-Kranenburg, Juffer,

& van Ijzendoorn, 2006), have advanced our understanding of how individual differences in physiological response help to identify children who are more or less susceptible to contextual influences. Consistent with BSC theory which originally identified children with *higher* reactivity as more sensitive to their environments, numerous studies have shown that children who exhibit heighted salivary cortisol response to laboratory challenges show better adaptation if they are exposed to more advantageous experiences, and worse adaptation if exposed to greater adversity. For example, cortisol response moderated the effect of family income on children's performance on EF tasks (Obradović, Portilla, & Ballard, 2016), and cortisol response moderated the effect of contextual influences on multi-informant ratings of child behaviors (Obradović, Bush, Stamperdahl, Adler, & Boyce, 2010) and mental health (Essex et al., 2011).

Empirical work has also demonstrated that the specific biological profile marking greater sensitivity may differ by biological system, context, timing, and measure. For example, several studies have also found that *lower* physiological reactivity can mark heightened sensitivity. Children who displayed low physiological reactivity in response to hearing adults argue were more susceptible to the detrimental effects of marital conflict compared to children who displayed high physiological reactivity (El-Sheikh & Whitson, 2006; Katz, Hessler, & Annest, 2007). Thus, while theory and empirical work indicate that some children are more or less biologically susceptible to contextual influences, whether a pattern of low or high reactivity marks greater sensitivity depends substantially on the biological marker and environment context. It is important to note that all of this research has come from children in high-income countries with restricted ranges of experiences of family adversity. There is a need to extend this work by examining whether HCCs—at either higher or lower levels—can also mark biological sensitivity in contexts of greater adversity.

### 1.4 | Sex differences in hair cortisol and cognitive development

In LMICs, such as India, Kenya, Tanzania, Ethiopia, and Cambodia, there are significant disparities in boys' and girls' daily experiences, ranging from household chores to family responsibilities and expectations of vocation and differential investments in boys and girls education (Escueta, Whetten, Ostermann, & O'Donnell, 2014). Boys and girls coming from families of similar wealth may have very different day-to-day experiences, with girls on average being more disadvantaged than boys (Escueta et al., 2014). Similarly, in rural Pakistan, although progress has been made, considerable sex disparities remain (Najam & Bari, 2017). Just 15% of poor rural 10-year-old girls complete primary education, compared to 40% of their male peers (Najam & Bari, 2017), and girls tend to receive a smaller share of scarce household resources (UNDP, 2014).

It is feasible that these differential experiences may put girls in LMICs at greater developmental risk, in that they are impacted more strongly by adverse contextual influences. Supporting this notion, girls have been shown to exhibit lower cognitive performance compared to boys in LMICs (Escueta et al., 2014). Indeed, a recent study from the current sample suggests that in rural Pakistan, girls' and boys' experience may also be differentially biologically embedded. Specifically, EEG gamma power, a marker of neural maturity, was related to higher verbal IQ for girls, but not for boys (Tarullo et al., 2017).

Furthermore, studies from high-income samples have found both mean-level sex differences in HCCs and some sex-specific links between HCCs and measures of children's development. Specifically, young boys have higher HCCs compared to girls (Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Gerber et al., 2017; Grunau et al., 2013; Maurer et al., 2016; Rippe et al., 2016; Simmons et al., 2019). One recent sample has revealed that sex moderated the association between HCCs and attention, in that HCCs were associated with attention problems in kindergarten boys, but not in girls (Pauli-Pott et al., 2017; Schloß et al., 2018). Together, these studies suggest that contextual experiences of boys and girls differ, and HCCs may operate differently among boys compared to girls. Rather than studying sex as a moderator, contextual experiences should be examined separately in boys and girls to avoid conflating these many differences in experiences and biological processes.

### 1.5 | Current study

The goals of the present study were to examine (a) whether HCCs are uniquely related to children's early cognitive skills in the context of rural Pakistan, and (b) whether HCCs are a marker of children's biological sensitivity to context, by moderating the relation between family wealth and cognitive skills. We examined these associations separately in boys and girls for four reasons. First, in LMICs, boys and girls have differently daily experiences of resource allocation, home activities, and educational opportunities. A girl and a boy from homes with identical levels of socioeconomic status may experience very different home experiences, due to their sexes. Second, sex-specific disparities in experiences and resources in LMICs (Escueta et al., 2014) may be differentially embedded in boys' and girls' stress physiology (Tarullo et al., 2017). Third, previous studies showed sex differences in mean levels of HCCs, and these differences could confound observed associations between HCCs and cognitive development (Gerber et al., 2017; Simmons et al., 2019). Finally, associations between HCCs and children's development have been observed to vary by sex (Pauli-Pott et al., 2017; Schloß et al., 2018). In light of this theoretical and empirical evidence that the interplay between family resources, HCCs and cognitive skills may differ between boys and girls, we split the sample by sex, a priori, for analysis. Moreover, we decided this was the most effective approach because as our sample included more girls than boys (discussed further in the Section 7). Given that there is a paucity of research investigating HCCs among LMIC children, we did not have a hypothesis for the direction of effects, and our research questions were exploratory.

We employed HCC as a biomarker of stress physiology. Although multidetermined, HCCs partially capture the physiological impact of long-term, chronic adversity in children (Vanaelst et al., 2013). To assess cognitive skills, we employed developmentally appropriate and culturally adapted direct assessments of verbal intelligence, pre-academic skills, and EFs. We used a culturally relevant index of family wealth via the mother or head of household's report of tangible household assets and dwelling characteristics. Our multi-method approach, which included biological samples, parent report, and direct assessments, minimized potential shared-method bias. Furthermore, our longitudinal design enabled us to measure family wealth at child's birth, capturing the socioeconomic resources available during the child's early life. To increase robustness of our findings, we controlled for salient child and family characteristics that have been shown to relate to early cognitive development in LMICs.

## 2 | METHOD

### 2.1 | Participants

Study participants came from the largely agricultural Naushero Feroze District of Sindh province, Pakistan. The sample was drawn from a larger study of 1,302 children (46% girls) and primary caregivers (99% mothers) who had previously participated in the Pakistan Early Child Development Scale-Up (PEDS) Trial, a community-based, cluster-randomized controlled trial with a 2 × 2 factorial design (Yousafzai, Rasheed, Rizvi, Armstrong, & Bhutta, 2014).

The cohort was recruited at birth from local community centers and every infant born in the study area between April 1, 2009, and March 31, 2010, was eligible for enrollment. Children were screened for signs of severe cognitive impairments during the first 2 to 5 months of life, and if they did not show signs of impairment, were considered eligible and participated in the PEDS Trial during their first 2 years of life (Yousafzai et al., 2014, 2016).

The PEDS Trial consisted of two intervention arms designed to promote healthy child development. The responsive stimulation intervention promoted positive and responsive parenting practices via individualized coaching, support, and feedback during monthly home visits and community group meetings. The enhanced nutrition intervention provided additional education in health, hygiene, and nutrition, and also delivered micronutrient supplements for the children. There was an additional control group who received routine health and nutrition services. Families were randomly assigned to the control condition ($n$ = 368), the responsive stimulation intervention ($n$ = 383), the enhanced nutrition intervention ($n$ = 364), or both the responsive stimulation and enhanced nutrition interventions ($n$ = 374). More details on the intervention design and its effects are reported elsewhere (see Yousafzai et al., 2014, 2016). As reported previously, the responsive stimulation intervention was found to improve children's EFs, IQ, pre-academic skills and prosocial behavior, while enhanced nutrition improved children's motor development (Yousafzai et al., 2016).

The current study analytic sample was limited to 535 boys and girls who had valid hair cortisol data collected at age 4 ($M_{age}$ = 4.02 years, $SD$ = 0.03 years, range 3.92–4.27 years). This was a convenience subsample of children. We collected hair samples until we had reached 40% of children in each of the intervention groups. Boys with valid HCC data had higher maternal education, height for age, school readiness, and verbal IQ, compared to boys without valid HCC data, but did not differ based on any other study variables ($ps$ = .09–.72). Girls with valid HCC data did not differ on any analysis variables, compared to girls without valid HCC data ($ps$ = .28–.89).

On average, the participants in the full study were exposed to high levels of poverty and adversity (Yousafzai et al., 2014, 2016), and the current analytic sample was largely reflective of the larger cohort. In the current analytic sample and larger cohort, average monthly household income was approximately $100 USD (PKR 9,821; $SD$ = PKR 14,283, range PKR 0–200,000). In the current analytical sample, 30% of families experienced food insecurity, compared to 32% in the full cohort (Yousafzai et al., 2014, 2016) and 60% in Pakistan nationally (USAID, 2019). Ten percent of children in the current analytic sample were underweight, compared to 11% in the full cohort and 32% of children under age 5 nationally (USAID, 2019). In addition, 14% of children in the current analytic sample were stunted, compared to 16% in the full cohort and 44% of children under age 5 in Pakistan nationally (Asim & Nawaz, 2018; UNICEF, 2013). Similarly, 6% of children in the current analytic sample were wasted compared to 5% in the full cohort and 15% in Pakistan nationally (Asim & Nawaz, 2018; UNICEF, 2013). Primary school attendance in the area was low and 67% of mothers in the current analytic sample were illiterate, compared to 68% in the full cohort and 45% national average (Unesco, 2017). Differences between our study and national averages may partially be due to differences in measures.

### 2.2 | Procedures

This longitudinal study employed data collected at three time points: at the child's birth (within 0 to 2 months), at 2 years, and at 4 years of age. All data were collected by a group of Community-based Child Development Assessors who were specifically trained to interact with families and administer the assessments in the local language, Sindhi. At the child's birth, the mother or head of household reported maternal educational attainment, family wealth, and family structure including number of children. When the child was 2 years old, mothers reported their family's experience of food insecurity and a study team member directly measured children's height to capture children's early nutrition and growth. At age 4 years, children participated in a longitudinal follow-up study that consisted of a 3-hr home visit and a 3-hr center visit (Yousafzai et al., 2016). Most of these visits (98.6%) occurred within a month of the child's fourth birthday. At this time, the study staff collected children's hair samples to assay for HCCs and directly assessed cognitive performance: verbal intelligence, pre-academic skills, and EFs. A multidisciplinary team of experts and local staff spent 6 months adapting all measures for administration in the Pakistani context (Yousafzai et al., 2016). Family income and children's pre-academic skills were recorded at home. Children's growth measurements, hair collection, and assessments of EFs and verbal IQ took place at a community-based assessment center. This space was free of distraction and was specially setup for child assessments close to the villages of families. All mothers gave written informed consent (or a thumb print for consent) and could decline or decide not to participate at any time. Ethics approval for this study was obtained from the ethical review committee of the Aga Khan University in Pakistan (Protocol 2265-Ped-ERC-12) and from the institutional review board at Stanford University (Protocol ID 26174; study title: Early Childhood Cognitive Stimulation and Successful Transition to Preschool in a Disadvantaged Population in Rural Pakistan).

## 2.3 | Measures

### 2.3.1 | Family wealth

To assess family wealth, the mother or head of household reported on 44 items that indicate the presence or absence of various assets, including ownership of property, livestock, and household assets (e.g., car, bike, TV); living conditions (e.g., access to water, sanitation facilities, type of flooring material); and number of bedrooms in the home at the time of the child's birth. A binary score was assigned to individual items as 0 (absent) and 1 (present). We used an established procedure (Vyas & Kumaranayake, 2006) that employs principal components analysis to weigh various assets according to their relative importance in this population and calculate a single standardized composite factor score that represented a comprehensive measure of family wealth.

### 2.3.2 | Hair cortisol concentrations

HCCs indexed children's stress physiology. Hair cortisol offers a major advantage for examining chronic stress and adversity, as it reflects cumulative cortisol output rather than daily fluctuations like salivary or urinary concentrations (Russell, Koren, Rieder, & Van Uum, 2012; Sauvé, Koren, Walsh, Tokmakejian, & Uum, 2007; Stalder & Kirschbaum, 2012). Hair strands of a diameter of approximately 3 mm were extracted as close as possible to the scalp from a posterior vertex position (back of the head) using fine, steel scissors. Two samples of hair (each containing multiple hair strands) were collected from the back of the head from each child. This ensured that there was enough hair to assay and minimized the noticeability of missing hair. Following extraction, hair samples were carefully placed on foil, and were not folded to avoid damage or breakage. They were stored at room temperature until analysis.

Biological assays were conducted in the Kirschbaum laboratory in Dresden, Germany. Cortisol concentrations were determined from the first 3 cm hair segment proximal to the scalp and 50 mg of hair was considered sufficient for cortisol assay. This hair segment reflects hair growth over the 3-month period prior to hair sampling, based on a hair growth rate of approximately 1 cm/month (Wennig, 2000). During analysis, washing and steroid extraction followed the protocol described in detail in Stalder and Kirschbaum (2012, study II) with 7.5 mg of whole, non-pulverized hair being incubated in 1,800 ml methanol for 18 hr at room temperature. Cortisol concentrations were determined using a commercially available immunoassay with chemiluminescence detection (CLIA, IBL-International). The intra- and inter-assay coefficients of variation of this assay were below 8%. HCC values were log-transformed to adjust for positive skew.

When collecting hair samples, several challenges emerged. Many of the children in the original full sample, particularly boys, had hair that was too short to collect the necessary 3 cm sample close to the scalp. Thus, we were unable to collect HCCs in equal number of boys and girls. Some families also shaved their children's heads in the summer. In addition, some families chose not to provide hair samples because they did not want to cut the child's hair, or children were afraid of the scissors. When children did choose to provide hair samples, two assessors aided with the hair cortisol collection to reduce fear and make the child more comfortable. One assessor distracted the child with conversation while the other gently extracted the hair sample.

## 2.4 | Cognitive skills

### 2.4.1 | Verbal IQ

To assess children's verbal ability, we used the Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III; Wechsler, 2012) after culturally adapting the measure for the Pakistani context (see Rasheed et al., 2018). A *verbal IQ composite* (VIQ) was calculated from scale scores from Information, Vocabulary, and Word Reasoning subtests (Cronbach's $\alpha$ = 0.88, $M$ = 77.47, $SD$ = 9.95), each of which required spoken responses to the presentation of pictures or information. Items were scored as correct (1) or incorrect (0). The subtest was assigned a raw score by summing the scores on all items and then converted to a t-score using normed tables provided in the WPPSI-III manual.

### 2.4.2 | Pre-academic skills

To assess pre-academic skills, we used the Bracken School Readiness Assessment mastery score, a standardized measure of school readiness (BSRA-3; Bracken, 2007). The BSRA-3 is nonverbal and was administered as a table-top task during a home visit. Children were asked to respond to the assessor's questions by selecting a correct picture response from four or more options. The *pre-academic skills* measure was a composite of the five dimensions of emergent pre-literacy (i.e., letters) and mathematical knowledge (i.e., numbers, sizes, shapes). The BSRA is normed for 3–6-year-old children (Bracken, 2007), and has been reported to be a reliable measure of school readiness in young children living in India (Shah, Schaefer, & Clark, 2013).

### 2.4.3 | Executive functions

To assess EFs, we used a composite measure of six developmentally and culturally appropriate tasks which have undergone extensive cultural adaptation and have been successfully validated on this sample (see Obradović et al., 2019). The tasks assessed children's inhibitory control (ability to suppress a dominant response in favor of a subdominant response), short-term memory (ability to hold, update, and manipulate information in the mind over short periods of time), and cognitive flexibility (ability to switch flexibly between two different dimensions). Specifically, the Fruit Stroop (inhibitory control task) assessed the child's ability to focus on a subdominant perceptual feature of an image, rather than on a dominant feature (Carlson, 2005). The Knock-Tap Game (inhibitory control task) assessed children's ability to implement a set of rules and suppress an imitation of the assessor's actions (Molfese et al., 2010). The Big/Little Game (inhibitory control task) assessed children's ability to state a contradictory rather than a salient perceptual feature of an image (Carlson, 2005). The Go/NoGo Game (inhibitory control task) assessed children's ability to perform an action following a frequent "go" stimulus and to inhibit that same action following a less frequent "no-go" stimulus (Willoughby et al., 2010). The Forward Word Span (short-term memory task) measured children's ability to repeat verbally presented word sequences of increasing length (Noël, 2009). The Separated Dimensional Change Card Sort (cognitive flexibility task) measured children's ability to switch attention between two different dimensions, using a set of colored cards (green or yellow) featuring the black silhouette of a common shape (e.g., star or truck; Carlson, 2005). Comprehension of task rules was determined by performance on practice trials. Children who did not pass task-specific comprehension criteria did not receive a valid test score. A final *Executive Functions* composite score (EF) was calculated as an average of valid scores across the six tasks (Cronbach's $\alpha = 0.60$). The EF composite included scores for children who passed comprehension criteria for three or more tasks (89% of children), since a three-task battery is considered acceptable to measure children's overall EF (Willoughby, Pek, & Blair, 2013). Please see Obradović et al. (2018) for more information regarding the reliability and descriptive statistics for each task, as well as details on the comprehension criteria used for each task.

#### 2.4.4 | Covariates

We controlled for child and family characteristics that have been shown to covary with family wealth and to predict early cognitive development (Black et al., 2013) and have been previously used in numerous publications of this study (e.g., Obradović, Yousafzai, Finch, & Rasheed, 2016; Yousafzai et al., 2014, 2016). Specifically, *maternal education* indexed the number of years mothers attended formal school ($M = 2.48$, $SD = 3.79$) and was included as extant research demonstrates that maternal education is linked with improved child-care practices related to health and nutrition and reduced odds of stunting which both impact cognitive development (Black et al., 2013). Children's *number of older siblings* ($M = 2.55$, $SD = 2.43$) was included as a covariate since it has been previously linked with cognition in this sample (Obradović et al., 2018).

Consistent with previous work, we employed two binary variables to control for the published effects of the responsive stimulation (1 = *responsive stimulation intervention exposure*) and the enhanced nutrition interventions (1 = *enhanced nutrition intervention exposure*) on children's Verbal IQ, pre-academic skills and EFs (Obradović, Yousafzai, et al., 2016; Yousafzai et al., 2016).

Food insecurity and height for age enabled us to control for children's access to adequate nutrition in the first 2 years of life. *Family food insecurity* (Coates, Swindale, & Bilinsky, 2007) was assessed using a binary measure of whether the family had access to safe and nutritionally adequate food (0 = food secure, 1 = food insecure). Food security is an important predictor of children's growth and development (Black et al., 2013). Children's *height for age* reflected nutrition during the first 2 years of a child's life, which has been shown to represent a longitudinal risk factor for poor cognitive development (Black et al., 2017; Grantham-McGregor et al., 2007). Trained assessors measured children's height to the nearest 0.1 cm in accordance with standardized guidelines (Cogill, 2003). Height was converted into a standardized height-for-age index ($M = -2.33$, $SD = 1.12$) using WHO Anthro software V3.2.2. All these variables were included as covariates in the models.

### 2.5 | Data and analysis

The percentage of missing data was small, ranging from 1% to 4%, except for the child EF composite (10%), which was largely due to children's inability to understand task rules. Other reasons for missing data included external disruptions that caused the assessment to be cut short (e.g., no electricity in the room), lack of permission from the head of household to stay for the full duration, challenging behavior, and obvious disabilities (e.g., unable to walk or speak). Missing data on all predictors were multiply imputed using chained equations with 20 datasets. Imputation models included a robust set of covariates: all demographic variables used in analyses, as well as all other covariates collected as a part of the larger study including measures of children's cognition, language, and motor skills, prosocial behavior, socio-emotional behaviors, inhibition, positive and negative affect, maternal engagement, and home environment. All continuous variables were standardized on the full, larger sample of children in the longitudinal follow-up of the PEDS trial. All variables exceeding ± 4 *SD* in the full sample were considered outliers and were truncated to 4 *SD* (HCCs: $n_{boys} = 2$, $n_{girls} = 0$, wealth: $n_{boys} = 1$, $n_{girls} = 0$, cognitive skills, $ns_{boys} = 0–2$, $ns_{girls} = 0–2$). Variables were then re-standardized on the smaller subsample of children in the current study to make coefficients interpretable as effect sizes. Analyses were conducted in Stata Version 13.1 (StataCorp, 2013).

We used multiple regression models to examine whether HCCs were directly or interactively associated with boys' and girls' cognitive skills (verbal IQ, pre-academic skills, EFs), after controlling for demographic covariates. Model 1 examined direct associations of HCCs with cognitive skills, controlling for factors at birth (family wealth, maternal education, number of older siblings), at 2 years (food insecurity and height for age), and exposure to the responsive stimulation and enhanced nutrition interventions during the first 2 years of the child's life. Model 2 additionally examined how an interaction between HCCs and family wealth was related to cognitive skills. The regression models accounted for the clustering of data within the 80 community-based team members who administered the original intervention trial (Yousafzai et al., 2014) with robust clustered standard errors. Significant interactions were further probed using the simple slopes technique (Aiken, West, & Reno, 1991) by testing the associations between family wealth and cognitive skills for girls with high (1 *SD* above the mean) and low (1 *SD* below the mean) HCC.

## 3 | RESULTS

Table 1 provides descriptive statistics for key study variables for the full sample and separately by sex. Boys and girls did not differ significantly from each other on study variables (*ps* = .06–.84). Importantly, boys had similar levels of HCCs ($M$ = 9.01 pg/mg, $SD$ = 10.56) compared to girls ($M$ = 11.34 pg/mg, $SD$ = 25.53), $t(533) = 1.52$, $p = .126$). On average for boys and girls together in our sample, HCCs ($M$ = 9.55 pg/mg, $SD$ = 10.68) were lower than average levels reported in children of similar age ranges from previous studies in high-income countries, in which average HCCs range from 12.22 to 29.55 pg/mg (e.g., Gerber et al., 2017; Ursache et al., 2017; Vaghri et al., 2013) and even in low-income samples in the United States and Australia, in which average HCCs range from 32.02 to 46.00 pg/mg (Ling et al., 2019; Simmons et al., 2019). However, mean levels cannot be definitively compared due to variable measurement error across different laboratories and assays, as well as potential ethnic differences in mean levels of HCCs (Ling et al., 2019; Rippe et al., 2016). We elaborate on this further in the discussion.

### 3.1 | Bivariate correlations

Bivariate correlations among all study variables are presented in Table 2, separately for girls (below diagonal) and boys (above diagonal). Family wealth and maternal education were positively associated with boys' and girls' verbal IQ and pre-academic skills. Maternal education was also linked with higher EFs for girls only. Furthermore, higher HCCs were associated with higher levels of maternal education, family wealth, and pre-academic skills for girls only. Exposure to the responsive stimulation and enhanced nutrition interventions in first 2 years of life were not significantly linked with HCCs for girls or boys.

### 3.2 | Regressions

Regression results are presented for boys in Table 3 and for girls in Table 4. Significant covariate effects on children's cognitive skills are largely consistent with previously published studies (Obradović, Yousafzai, et al., 2016; Tarullo et al., 2017; Yousafzai et al., 2016); minor deviations are likely due to smaller sample size of children with valid HCC data.

As shown in Table 3, we found no direct (Model 1) or interactive (Model 2) associations between HCCs and cognitive skills for boys. However, as shown in Table 4, HCCs were associated with cognitive skills for girls. Model 1 demonstrated that HCCs were positively associated with girls' pre-academic skills ($\beta = 0.19$, $p = .002$), but were not significantly associated with girls' verbal IQ or EFs. Model 2 revealed that HCCs and wealth were significantly and interactively associated with girl's verbal IQ ($\beta = -0.09$, $p = .04$), pre-academic skills ($\beta = -0.09$, $p = .03$) and EFs ($\beta = -0.10$, $p = .03$).

**TABLE 1** Descriptive statistics for all study variables

| | Full sample M/% (*SD*) | Boys M/% (*SD*) | Girls M/% (*SD*) | Range |
|---|---|---|---|---|
| Covariates | | | | |
| Number of siblings | 2.55 (2.43) | 2.51 (2.30) | 2.49 (2.31) | 0 to 11 |
| Food insecurity | 0.30 (0.46) | 0.34 (0.48) | 0.31 (0.46) | 0 to 1 |
| Maternal education (years) | 2.48 (3.79) | 2.24 (3.79) | 2.13 (3.56) | 0 to 16 |
| Height for age | −0.82 (1.10) | −0.76 (1.11) | −0.85 (1.09) | −3.88 to 3.06 |
| Predictors | | | | |
| HCCs (pg/mg) | 9.55 (10.68) | 9.01 (10.56) | 11.34 (25.53) | 0.10 to 96.06 |
| Family wealth | 0.02 (0.96) | −0.05 (0.94) | 0.06 (0.97) | −1.02 to 4.25 |
| Cognitive skills | | | | |
| Verbal IQ | 77.22 (9.81) | 77.41 (10.02) | 77.11 (9.70) | 55 to 137 |
| Pre-academic skills | 12.93 (7.54) | 12.08 (6.23) | 13.42 (8.25) | 0 to 48 |
| EF composite | −0.05 (0.62) | −0.08 (0.58) | −0.03 (0.64) | −2.61 to 2.76 |
| *N* | 535 | 193 (36%) | 342 (64%) | |

Abbreviations: EF, executive function; HCCs, hair cortisol concentrations; pg/mg, picogram/milligram.

**TABLE 2** Bivariate correlations among girls (below diagonal, *n* = 342) and boys (above diagonal, *n* = 193)

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HCCs | | 0.17* | −0.04 | 0.02 | −0.09 | −0.16* | −0.04 | 0.05 | 0.08 | 0.03 | −0.05 |
| 2 | Number of siblings | −0.14* | | 0.10 | −0.23** | −0.17* | −0.16* | −0.03 | 0.02 | 0.14 | 0.13 | 0.08 |
| 3 | Food insecurity | −0.07 | 0.10 | | −0.26*** | −0.19** | −0.11 | −0.12 | −0.13 | −0.12 | −0.09 | 0.01 |
| 4 | Maternal education | 0.19*** | −0.15** | −0.20*** | | 0.30*** | 0.24*** | 0.25*** | 0.29*** | 0.13 | 0.04 | 0.01 |
| 5 | Family wealth | 0.17** | −0.09 | −0.25*** | 0.34*** | | 0.13 | 0.28*** | 0.23** | 0.08 | −0.03 | −0.05 |
| 6 | Height for age | 0.05 | −0.05 | −0.20*** | 0.13* | 0.18*** | | 0.23** | 0.15* | 0.08 | −0.01 | −0.08 |
| 7 | Verbal IQ | 0.03 | −0.03 | −0.13* | 0.11* | 0.13* | 0.21*** | | 0.41*** | 0.46*** | 0.20** | −0.11 |
| 8 | Pre-academic skills | 0.17** | −0.11* | −0.06 | 0.18*** | 0.17** | 0.13* | 0.29*** | | 0.23** | 0.09 | −0.06 |
| 9 | EF composite | −0.03 | −0.02 | −0.09 | 0.20*** | 0.09 | 0.17** | 0.54*** | 0.30*** | | 0.15* | 0.01 |
| 10 | Intervention RS | −0.05 | 0.02 | −0.07 | −0.04 | 0.05 | 0.01 | 0.09 | 0.14** | 0.09 | | 0.08 |
| 11 | Intervention EN | −0.08 | −0.11* | −0.15** | 0.06 | −0.03 | 0.01 | 0.05 | 0.18*** | 0.09 | −0.03 | |

Abbreviations: EF, executive functions; EN, enhanced nutrition intervention; HCCs, hair cortisol concentrations; RS, responsive stimulation intervention.

***$p < .001$,

**$p < .01$,

*$p < .05$.

Probing these significant interactions revealed that greater family wealth was significantly associated with greater verbal IQ and pre-academic skills only for girls with low HCCs (verbal IQ: $\beta = 0.17$, $p = .02$; pre-academic skills: $\beta = 0.20$, $p = .02$), and not for girls with high HCCs (verbal IQ: $\beta = -0.03$, $p = .66$; pre-academic skills: $\beta = 0.03$, $p = .72$). Figures 1 and 2 demonstrate disordinal interactions between wealth and HCCs, such that girls with low HCCs displayed higher verbal IQ and pre-academic skills if they came from relatively wealthier families, but lower verbal IQ and pre-academic skills if they came from poorer families. As shown in Figure 3, a similar pattern was found for EFs, however, the simple slopes were not significant ($\beta = 0.09$, $p = .26$ for girls with low HCCs and $\beta = - 0.08$, $p = .28$ for girls with high HCCs).

## 4 | DISCUSSION

The goal of the present study was to understand how young children's hair cortisol concentrations (HCCs), which partially reflect chronic activation of the HPA axis, are associated with cognitive development in the context of rural Pakistan. In a large sample of preschoolers, we first examined whether HCCs were related to direct assessments of children's verbal IQ, pre-academic skills, and EFs, after controlling for salient individual and family characteristics. Second, we explored whether the associations between family wealth and young children's cognitive skills varied by the level of children's HCCs. We separately analyzed subsamples of boys and girls for a priori reasons discussed above. Our results revealed divergent patterns of HCC associations. Namely, there were no significant direct or interactive associations between HCCs and cognitive skills for boys. In girls, HCCs were directly associated with children's pre-academic skills. HCCs also moderated the association between family wealth and all three measures of cognitive development, in that girls with low HCCs showed higher cognitive skills if they came from relatively wealthier families and lower cognitive skills if they came from very poor families. In contrast, family wealth was not associated with cognitive skills among young girls who had high HCCs. These findings indicate that HCCs may be a marker of heightened biologically sensitivity to family socioeconomic status for young girls from a rural LMIC setting, such that the cognitive skills of girls who had lower levels of HCC were more sensitive to variability in family environmental resources.

### 4.1 | Hair cortisol concentrations and cognitive development

Bivariate correlations revealed that girls' HCCs were positively associated with pre-academic skills, indexing greater knowledge of colors, shapes, and sizes. In contrast, HCCs were not directly correlated with verbal intelligence or EFs. Although our study cannot address the mechanisms behind this divergent finding, it is feasible that the association between HCC and cognitive skills may differ for crystallized knowledge, deduced from exposure to information and facts, versus fluid cognitive skills, which reflect children's capacity to process and integrate information (Obradović & Willoughby, 2019). Future research should continue to investigate the associations between stress hormones and different aspects of early cognitive development in children from LMIC settings. Alternatively, this discrepancy may have been driven by the assessment-specific

**TABLE 3** Regression results associated with boys' cognitive skills

| | Verbal IQ | | | | Pre-academic skills | | | | EF composite | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Model 1 | | Model 2 | | Model 1 | | Model 2 | | Model 1 | | Model 2 | |
| | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* |
| HCCs | −0.05 (0.07) | .51 | −0.03 (0.07) | .68 | 0.03 (0.05) | .58 | 0.04 (0.06) | .50 | 0.06 (0.06) | .33 | 0.05 (0.06) | .42 |
| Number of Siblings | 0.04 (0.07) | .53 | 0.04 (0.07) | .58 | 0.08 (0.05) | .16 | 0.07 (0.05) | .18 | 0.17 (0.07) | .01 | 0.18 (0.07) | .01 |
| Food Insecurity | −0.02 (0.14) | .90 | −0.02 (0.14) | .90 | −0.06 (0.10) | .58 | −0.06 (0.10) | .58 | −0.12 (0.18) | .50 | −0.12 (0.18) | .50 |
| Maternal Education | 0.14 (0.08) | .08 | 0.13 (0.08) | .09 | 0.18 (0.08) | .03 | 0.18 (0.08) | .03 | 0.08 (0.07) | .27 | 0.09 (0.08) | .25 |
| Family Wealth | 0.16 (0.07) | .02 | 0.18 (0.07) | .01 | 0.12 (0.06) | .06 | 0.14 (0.06) | .04 | 0.03 (0.08) | .70 | 0.02 (0.08) | .76 |
| Height for Age | 0.18 (0.07) | .01 | 0.17 (0.06) | .01 | 0.09 (0.05) | .08 | 0.09 (0.05) | .09 | 0.17 (0.06) | .01 | 0.17 (0.07) | .01 |
| Intervention RS | 0.41 (0.15) | .01 | 0.42 (0.16) | .01 | 0.15 (0.12) | .21 | 0.15 (0.12) | .20 | 0.23 (0.16) | .15 | 0.22 (0.16) | .17 |
| Intervention EN | −0.21 (0.15) | .17 | −0.21 (0.15) | .16 | −0.10 (0.11) | .39 | −0.10 (0.11) | .37 | 0.02 (0.14) | .90 | 0.02 (0.14) | .90 |
| Wealth * HCCs | | | 0.09 (0.09) | .33 | | | 0.06 (0.07) | .41 | | | −0.04 (0.09) | .61 |
| Constant | −0.05 (0.07) | .69 | −0.03 (0.07) | .74 | 0.03 (0.05) | .40 | 0.04 (0.06) | .43 | 0.06 (0.06) | .47 | 0.05 (0.06) | .48 |
| $R^2$ | .17 | | .17 | | .14 | | .14 | | .11 | | .11 | |
| *N* | 193 | | 193 | | 193 | | 193 | | 173 | | 173 | |

Abbreviations: EFs, executive functions; EN, enhanced nutrition intervention; HCCs, hair cortisol concentrations; RS, responsive stimulation intervention.

**TABLE 4** Regression results associated with girls' cognitive skills

| | Verbal IQ | | | | Pre-academic skills | | | | EF composite | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Model 1 | | Model 2 | | Model 1 | | Model 2 | | Model 1 | | Model 2 | |
| | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* | *β (SE)* | *p* |
| HCCs | 0.02 (0.06) | .73 | 0.04 (0.06) | .54 | 0.17 (0.06) | .00 | 0.19 (0.06) | .00 | −0.07 (0.06) | .26 | −0.05 (0.06) | .40 |
| Number of Siblings | 0.03 (0.05) | .56 | 0.03 (0.05) | .61 | −0.05 (0.04) | .26 | −0.05 (0.04) | .24 | 0.03 (0.06) | .59 | 0.03 (0.07) | .62 |
| Food Insecurity | −0.07 (0.13) | .57 | −0.06 (0.13) | .64 | 0.18 (0.14) | .19 | 0.20 (0.14) | .15 | 0.00 (0.13) | .99 | 0.02 (0.12) | .85 |
| Maternal Education | 0.04 (0.05) | .39 | 0.05 (0.05) | .31 | 0.10 (0.05) | .06 | 0.11 (0.05) | .04 | 0.19 (0.06) | .00 | 0.20 (0.06) | .00 |
| Family Wealth | 0.03 (0.06) | .57 | 0.06 (0.06) | .32 | 0.08 (0.07) | .20 | 0.11 (0.07) | .10 | −0.02 (0.07) | .78 | 0.01 (0.07) | .93 |
| Height for Age | 0.26 (0.06) | .00 | 0.26 (0.06) | .00 | 0.19 (0.06) | .00 | 0.19 (0.06) | .00 | 0.20 (0.06) | .00 | 0.20 (0.06) | .00 |
| Intervention RS | 0.21 (0.12) | .08 | 0.22 (0.12) | .07 | 0.37 (0.09) | .00 | 0.38 (0.09) | .00 | 0.22 (0.12) | .07 | 0.23 (0.12) | .06 |
| Intervention EN | 0.06 (0.11) | .58 | 0.06 (0.11) | .60 | 0.42 (0.09) | .00 | 0.42 (0.09) | .00 | 0.15 (0.12) | .21 | 0.15 (0.12) | .21 |
| Wealth * HCCs | | | −0.08 (0.04) | .05 | | | −0.09 (0.04) | .03 | | | −0.09 (0.04) | .03 |
| Constant | 0.02 (0.06) | .12 | 0.04 (0.06) | .13 | 0.17 (0.06) | .00 | 0.19 (0.06) | .00 | −0.07 (0.06) | .15 | −0.05 (0.06) | .14 |
| $R^2$ | .09 | | .10 | | .15 | | .16 | | .09 | | .10 | |
| *N* | 342 | | 342 | | 342 | | 342 | | 309 | | 309 | |

Abbreviations: EFs, executive functions; EN, enhanced nutrition intervention; HCCs, hair cortisol concentrations; RS, responsive stimulation intervention.

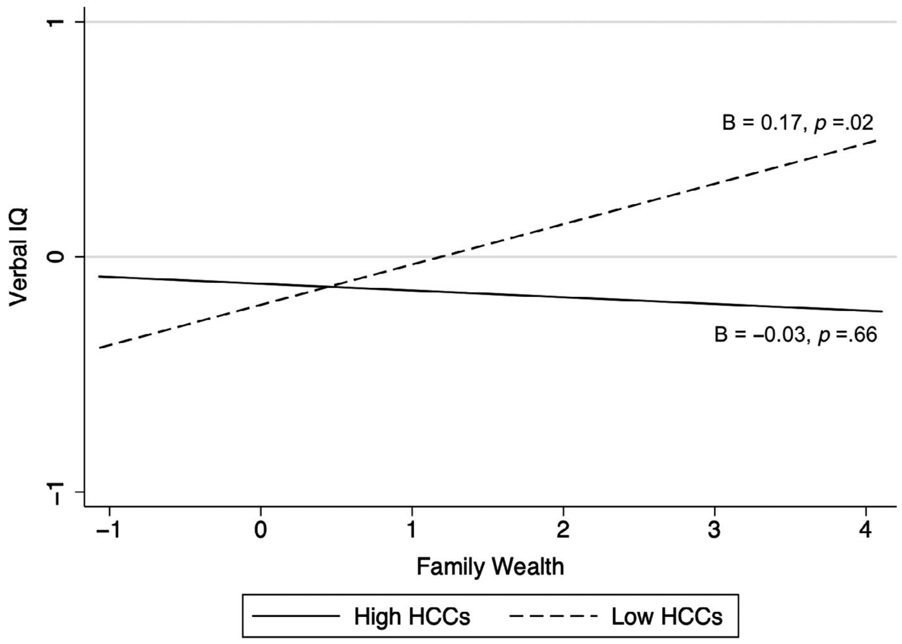


**FIGURE 1** Family wealth and hair cortisol concentrations (HCCs) were interactively associated with girls' verbal intelligence

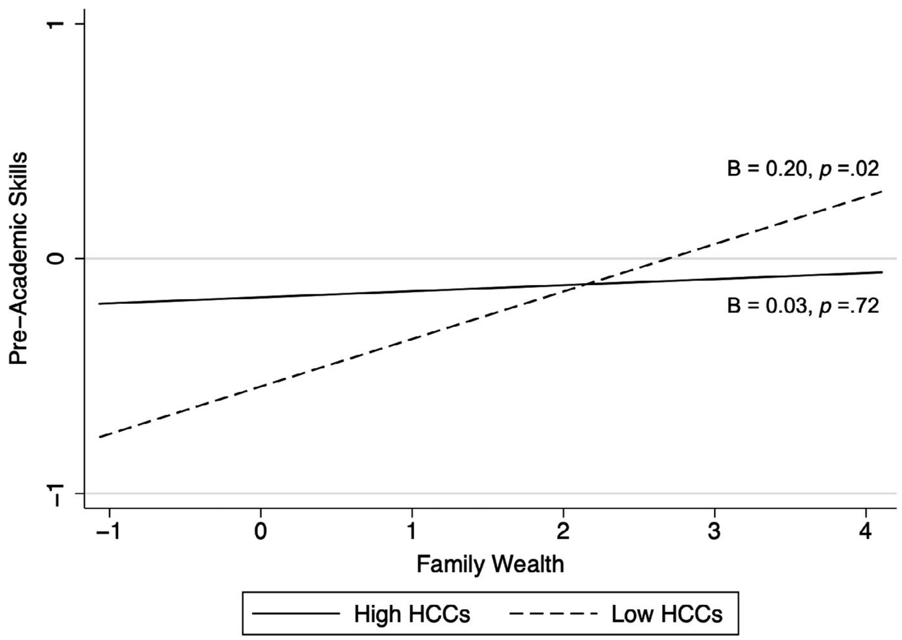


**FIGURE 2** Family wealth and hair cortisol concentrations (HCCs) were interactively associated with girls' pre-academic skills

differences (e.g., distribution of scores), highlighting the need to employ multiple cognitive assessments. While in our study these discrepant bivariate correlations were further qualified by significant interactions with family wealth in our regression models, future research should consider how the link between HCCs and cognitive skills may vary by the specific type or measure of cognitive skill.

Most importantly, our findings revealed the associations of family wealth with all three types of cognitive skills were significantly moderated by HCCs in girls. For pre-academic skills and verbal IQ, the association between family wealth and cognitive skills was significant only for girls with *lower* HCCs. Specifically, a cross-over interaction pattern indicated that girls with lower HCCs displayed lower levels of pre-academic skills and verbal IQ if they came from poorer families, but higher levels of these skills if they came from relatively wealthier families. In contrast, girls who had higher HCCs did not differ in levels of pre-academic skills and verbal IQ across the spectrum of family wealth. For EF, the significant interaction revealed an analogous pattern, despite the non-significant simple slopes. Girls with lower HCCs displayed higher average EFs than those with higher HCC at high levels of family wealth.

Lower HCCs appear to mark young girls' biological sensitivity to family resources. This finding contributes to a growing body of

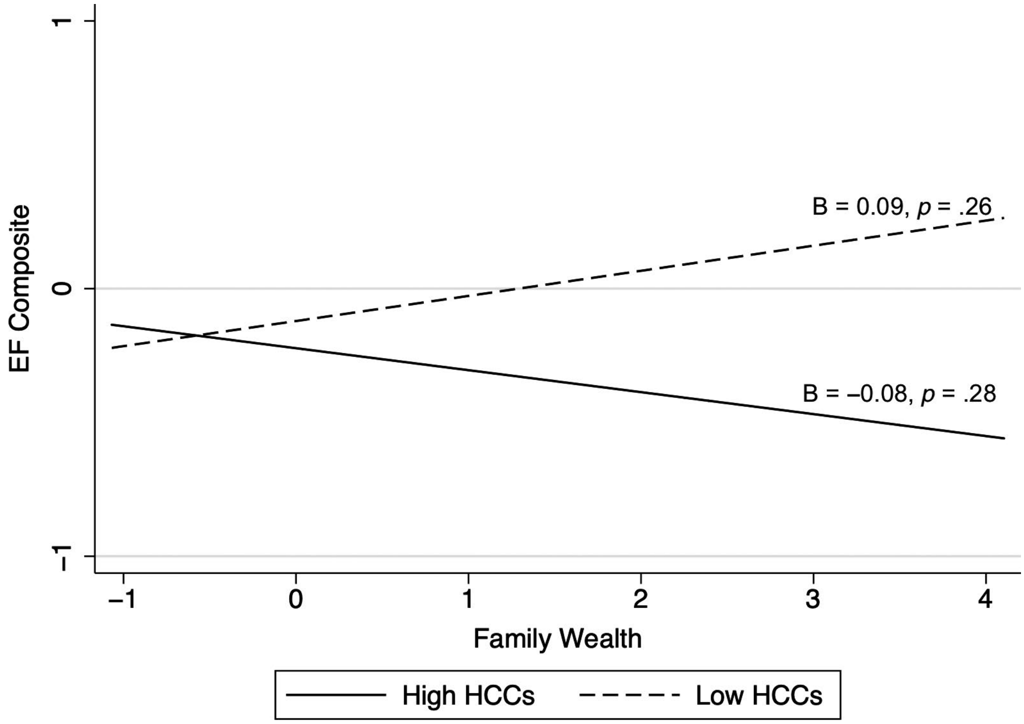


**FIGURE 3** Family wealth and hair cortisol concentrations (HCCs) were interactively associated with girls' executive functions

evidence that markers of biological sensitivity vary across different biological systems, contextual experiences, and studied populations (Obradović, 2016). For example, while some previous work has shown that higher physiological reactivity marks greater biological sensitivity to family resources (Ellis, & Boyce, 2011; Essex et al., 2011; Obradović et al., 2010; Obradović, Yousafzai, et al., 2016), others have shown that children who displayed low physiological reactivity were more susceptible to detrimental effects of family conflict (El-Sheikh & Whitson, 2006; Katz et al., 2007). Among our sample of rural Pakistani girls, lower levels of HCCs seem to identify girls whose cognitive skills are linked with family resources "for better and for worse," as differential susceptibility hypothesis posits (Pluess & Belsky, 2007).

Our study does not indicate how, or why, low HCCs indexes greater susceptibility to family wealth for cognitive development only in girls, and not in boys, in rural Pakistan. However, next, we discuss these findings in the context of other secondary results in order to offer future research directions that could advance understanding of how and why the chronic activation of HPA axis moderates the link between family socioeconomic resources and early cognitive development in similar settings.

### 4.2 | Possible mechanisms

Although boys and girls did not differ in their average levels of HCCs, statistical comparison of bivariate correlations of HCCs with other child and family measures revealed divergent patterns of findings. First, in girls but not in boys, HCCs were positively associated with family wealth and maternal education. We need more research to understand why the level of girls' HCCs increases as their family socioeconomic resources increase. This association is in contrast with the findings from high-income countries that reveal higher levels of young children's HCCs are associated with higher exposure to socioeconomic risks and adversity (Rippe et al., 2016; Ursache et al., 2017; Vaghri et al., 2013). This discrepancy raises a question of what levels of HCCs are sufficient or optimal.

Average levels of both boys' and girls' HCCs in the current sample are lower than average levels of HCCs reported in other studies of preschool children from high-income countries (e.g., Groeneveld et al., 2013; Ursache et al., 2017; Vaghri et al., 2013), even in those from socioeconomic disadvantaged communities in high-income countries (Ling et al., 2019; Simmons et al., 2019). Although we cannot determine statistical significance of these mean-level differences because cross-assay and cross-ethnic comparisons can be methodologically biased (Ling et al., 2019; Rippe et al., 2016; Stalder & Kirschbaum, 2012), our findings suggest a potential chronic hypo-responsivity of the HPA axis among children growing up in rural Pakistan. Blunted HCCs response in our sample is consistent with prior work showing blunted or down-regulated acute salivary measures of HPA axis activity among children who have experienced chronic neglect (Gunnar & Vazquez, 2001; Koss, Hostinar, Donzella, & Gunnar, 2014; Zalewski, Lengua, Kiff, & Fisher, 2012). Given that moderate levels of salivary cortisol are considered optimal for cognitive performance (Blair & Raver, 2012; Gunnar & Vazquez, 2001; Lupien et al. 2007), it is possible that higher HCCs in our sample may represent more optimal HPA axis functioning. If this assumption is true, lower HCCs in our sample may be seen as a physiological vulnerability for cognitive development in context of poverty. Future studies should investigate non-linear associations of HCCs and cognitive development in socioeconomically diverse samples to determine if indeed there are sufficient or optimal levels of HCCs for emerging cognitive skills if those levels vary as a function of contextual resources.

Future research should also address how these distal SES measures are translated into daily experiences that are linked to HCCs in girls, but not in boys. In our sample, we found that having more siblings was associated with significantly lower HCCs in girls, but significantly higher HCCs in boys. Does this divergent link between HCCs and family size among boys and girls reflect inequalities in how family resources are allocated? Relative to boys, girls in impoverished families may shoulder more of the daily burden of poverty by taking on more household responsibilities (i.e., domestic labor) or receiving a smaller share of scant resources (Escueta et al., 2014; UNICEF, 2013). Thus, daily experiences of extreme poverty may be more stressful for young girls compared to boys. At the same time, girls may also benefit more than boys in conditions of relatively greater family wealth. Boys' daily experiences of stressors and challenges that could affect chronic HPA axis functioning may not vary as much as a function of family wealth, as they may receive larger share of scant resources. Future studies of young children in LMICs should also examine how HCCs, a chronic stress marker, relates to salivary cortisol, which fluctuates daily, in order to understand how stress and adversity becomes biologically embedded.

Alternatively, HCCs may reflect different biological processes in boys and girls. For example, HCCs were related to physical growth (i.e., standardized height-for-age index) in boys, but not in girls, despite no significant differences in boys' and girls' physical growth. Similar sex differences have been found in a previous study finding examining links between EEG power and cognitive skills in a smaller subsample of 105 preschoolers drawn from the same PEDS study as current sample. Namely, higher gamma power, an index of temporal integration of sensory input with higher-level cognitive processes, was related to greater verbal ability only among girls (Tarullo et al., 2017).

The reasons why low HCCs marks heightened biological sensitivity to family wealth only among girls still remain to be addressed. Sex differences in susceptibility to environmental influences have been observed in nutritional interventions across LMICs, which were found to be more protective against girls' risk of anemia compared to boys (Balarajan, Ramakrishnan, Özaltin, Shankar, & Subramanian, 2011). Understanding both biological and social mechanisms underlying differences in how stress physiology moderates the link between family resources and emerging cognitive skills is important next step for addressing boys' and girls' inequalities in early educational outcomes i!n rural LMICs settings.

### 4.3 | Limitations and future directions

We acknowledge several limitations and highlight future directions for study. Our sample included more girls than boys because of the difficulties of collecting hair samples from boys with very short hair. It is possible that the observed sex differences may be in part explained by greater statistical power to detect potential effects among girls. This is unlikely, however, as our sample includes almost 200 boys and over 300 girls, and is larger than many prior studies examining cortisol in children. New advances in science may allow smaller amounts of hair to be used for assay (Stalder & Kirschbaum, 2012) and allow for increased sample sizes of boys. Our study is also limited in that we measure children's stress physiology concurrently with children's cognitive skills. However, since HCCs are believed to reflect cumulative HPA axis functioning over the previous 3 months, HCCs can partially be understood as a marker of stress-physiological response indexed before cognitive skills were tested. Finally, sociocultural elements that are unique to Pakistan may limit the generalizability of the findings to other LMIC settings; future research should investigate generalizability. By demonstrating the feasibility and relevance of measuring hair cortisol in this highly disadvantaged context, we hope to encourage researchers to probe associations between children's stress physiology and development in other LMICs.

## 5 | CONCLUSION

The current study examined cortisol extracted from hair as a measure partially reflecting chronic exposure to adversity, in a population of children from a rural Pakistan where many children experience heightened risk of adversity. The handful of prior studies examining links between children's HCCs and development have revealed mostly null results, but these have only included children high-income contexts who have a restricted range of family incomes and experiences. We extend this work to highlight how HCCs operate among boys and girls in rural Pakistan, where, although there is considerable variation across families and regions, children on average are more economically disadvantaged compared to relatively wealthier countries. Our results suggest that girls with lower HCCs may be biologically more sensitive to contextual influences on cognitive development. This work highlights the complex interplay between stress physiology, socioeconomic status, and sex for preschoolers' cognitive development in LMICs.

**ACKNOWLEDGEMENTS**

Data collection for this research was supported by a research grant from the Grand Challenges Canada Saving Brains Initiative (Grant number 0061-03). The preparation of this manuscript is supported by a grant from the Institute of Education Sciences (IES), U.S. Department of Education (R305B140009) to Emma Armstrong-Carter and by a Jacobs Foundation Advanced Research Fellowship (2017-1261-07) to Jelena Obradović. The authors also thank the children and families who participated and made this research possible, and the staff who helped collect and process the data. The findings, conclusions, and opinions here are those of the authors and do not represent views of the funding agencies.

## DATA AVAILABILITY STATEMENT

All data and syntax are available upon request.

## ORCID

*Emma Armstrong-Carter* https://orcid.org/0000-0002-5847-9486

## REFERENCES


Aiken, L. S., West, S. G. (1991). *Multiple regression: Testing and interpreting interactions*. Newbury Park: Sage.

Asim, M., & Nawaz, Y. (2018). Child malnutrition in Pakistan: Evidence from literature. *Children*, *5*(5), 60. https://doi.org/10.3390/children5050060

Balarajan, Y., Ramakrishnan, U., Özaltin, E., Shankar, A. H., & Subramanian, S. V. (2011). Anaemia in low-income and middle-income countries. *The Lancet*, *378*(9809), 2123–2135. https://doi.org/10.1016/S0140-6736(10)62304-5

Bates, R., Salsberry, P., & Ford, J. (2017). Measuring stress in young children using hair cortisol: The state of the science. *Biological Research for Nursing*, *19*(5), 499–510. https://doi.org/10.1177/1099800417711583

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., De Onis, M., ... Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet*, *382*(9890), 427–451. https://doi.org/10.1016/S0140-6736(13)60937-X

Blair, C. (2002). School readiness: Integrating cognition and emotion in a neurobiological conceptualization of children's functioning at school entry. *American Psychologist*, *57*(2), 111–127. https://doi.org/10.1037//0003-066X.57.2.111

Blair, C., Granger, D., & Peters Razza, R. (2005). Cortisol reactivity is positively related to executive function in preschool children attending Head Start. *Child Development*, *76*(3), 554–567. https://doi.org/10.1111/j.1467-8624.2005.00863

Blair, C., & Raver, C. C. (2012). Child development in the context of adversity: experiential canalization of brain and behavior. *American Psychologist*, *67*(4), 309. https://doi.org/10.1037/a0027493

Boyce, W. T. (2007). A biology of misfortune: Stress reactivity, social context, and the ontogeny of psychopathology in early life. *Multilevel Dynamics in Developmental Psychopathology: Pathways to the Future*, *34*, 45–82.

Bracken, B. (2007). *School readiness assessment: Examiner's manual*. San Antonio, TX: Pearson.

Bruce, J., Davis, E. P., & Gunnar, M. R. (2002). Individual differences in children's cortisol response to the beginning of a new school year. *Psychoneuroendocrinology*, *27*(6), 635–650. https://doi.org/10.1016/S0306-4530(01)00031-2

Carlson, S. M. (2005). Developmentally sensitive measures of executive function in preschool children. *Developmental Neuropsychology*, *28*(2), 595–616. https://doi.org/10.1207/s15326942dn2802_3

Chau, C. M., Cepeda, I. L., Devlin, A. M., Weinberg, J., & Grunau, R. E. (2017). The Val66Met brain-derived neurotrophic factor gene variant interacts with early pain exposure to predict cortisol dysregulation in 7-year-old children born very preterm: Implications for cognition. *Neuroscience*, *342*, 188–199. https://doi.org/10.1016/j.neuroscience.2015.08.044

Coates, J., Swindale, A., & Bilinsky, P. (2007). *Household Food Insecurity Access Scale (HFIAS) for measurement of food access: Indicator guide* (p. 34). Washington, DC: Food and Nutrition Technical Assistance Project, Academy for Educational Development.

Cogill, B. (2003). *Anthropometric indicators measurement guide*. Washington, DC: Academy for Educational Development.

Dettenborn, L., Tietze, A., Kirschbaum, C., & Stalder, T. (2012). The assessment of cortisol in human hair: Associations with sociodemographic variables and potential confounders. *Stress*, *15*(6), 578–588. https://doi.org/10.3109/10253890.2012.654479

Ellis, B. J., & Boyce, W. T. (2011). Differential susceptibility to the environment: Toward an understanding of sensitivity to developmental experiences and context. *Development and psychopathology*, *23*(1), 1–5. https://doi.org/10.1017/S095457941000060X

Ellis, B. J., Essex, M. J., & Boyce, W. T. (2005). Biological sensitivity to context: II. Empirical explorations of an evolutionary–developmental theory. *Development and Psychopathology*, *17*(2), 303–328. https://doi.org/10.1017/S0954579405050157

El-Sheikh, M., & Whitson, S. A. (2006). Longitudinal relations between marital conflict and child adjustment: Vagal regulation as a protective factor. *Journal of Family Psychology*, *20*(1), 30. https://doi.org/10.1037/0893-3200.20.1.30

Escueta, M., Whetten, K., Ostermann, J., & O'Donnell, K. (2014). Adverse childhood experiences, psychosocial well-being and cognitive development among orphans and abandoned children in five low income countries. *BMC International Health and Human Rights*, *14*(1), 6. https://doi.org/10.1186/1472-698X-14-6

Essex, M. J., Armstrong, J. M., Burk, L. R., Goldsmith, H. H., & Boyce, W. T. (2011). Biological sensitivity to context moderates the effects of the early teacher-child relationship on the development of mental health by adolescence. *Development and Psychopathology*, *23*(1), 149–61. https://doi.org/10.1017/S0954579410000702

Flom, M., St. John, A. M., Meyer, J. S., & Tarullo, A. R. (2017). Infant hair cortisol: Associations with salivary cortisol and environmental context. *Developmental Psychobiology*, *59*(1), 26–38. https://doi.org/10.1002/dev.21449

Fuchs, A., Jaite, C., Neukel, C., Dittrich, K., Bertsch, K., Kluczniok, D., ... Kaess, M. (2018). Link between children's hair cortisol and psychopathology or quality of life moderated by childhood adversity risk. *Psychoneuroendocrinology*, *90*, 52–60. https://doi.org/10.1016/j.psyneuen.2018.02.003

Gerber, M., Endes, K., Brand, S., Herrmann, C., Colledge, F., Donath, L., ... Zahner, L. (2017). In 6-to 8-year-old children, hair cortisol is associated with body mass index and somatic complaints, but not with stress, health-related quality of life, blood pressure, retinal vessel diameters, and cardiorespiratory fitness. *Psychoneuroendocrinology*, *76*, 1–10. https://doi.org/10.1016/j.psyneuen.2016.11.008

Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., Strupp, B., & Group, I. C. D. S. (2007). Developmental potential in the first 5 years for children in developing countries. *The Lancet*, *369*(9555), 60–70. https://doi.org/10.1016/S0140-6736(07)60032-4

Gray, N. A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N. P., & Stein, D. J. (2018). Determinants of hair cortisol concentration in children: A systematic review. *Psychoneuroendocrinology*, *87*, 204–214. https://doi.org/10.1016/j.psyneuen.2017.10.022

Groeneveld, M. G., Vermeer, H. J., Linting, M., Noppe, G., van Rossum, E. F., & van IJzendoorn, M. H. (2013). Children's hair cortisol as a biomarker of stress at school entry. *Stress*, *16*(6), 711–715. https://doi.org/10.3109/10253890.2013.817553

Grunau, R. E., Cepeda, I. L., Chau, C. M., Brummelte, S., Weinberg, J., Lavoie, P. M., ... & Van Uum, S. (2013). Neonatal pain-related stress and NFKBIA genotype are associated with altered cortisol levels in preterm boys at school age. *PloS one*, *8*(9). https://doi.org/10.1371/journal.pone.0073926

Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology*, *13*(3), 515–538. https://doi.org/10.1017/S0954579401003066

Jensen, S. K., Berens, A. E., & Nelson, C. A. 3rd (2017). Effects of poverty on interacting biological systems underlying child development. *The Lancet Child & Adolescent Health*, *1*(3), 225–239. https://doi.org/10.1016/S2352-4642(17)30024-X

Kao, K., Doan, S. N., St. John, A. M., Meyer, J. S., & Tarullo, A. R. (2018). Salivary cortisol reactivity in preschoolers is associated with hair cortisol and behavioral problems. *Stress*, *21*(1), 28–35. https://doi.org/10.1080/10253890.2017.1391210

Karlen, J., Ludvigsson, J., Hedmark, M., Faresjo, A., Theodorsson, E., & Faresjo, T. (2015). Early psychosocial exposures, hair cortisol levels, and disease risk. *Pediatrics*, *135*(6), e1450–e1457. https://doi.org/10.1542/peds.2014-2561

Katz, L. F., Hessler, D. M., & Annest, A. (2007). Domestic violence, emotional competence, and child adjustment. *Social Development*, *16*(3), 513–538. https://doi.org/10.1111/j.1467-9507.2007.00401.x

Klein Velderman, M., Bakermans-Kranenburg, M. J., Juffer, F., & Van Ijzendoorn, M. H. (2006). Effects of attachment-based interventions on maternal sensitivity and infant attachment: Differential susceptibility of highly reactive infants. *Journal of Family Psychology*, *20*(2), 266–274. https://doi.org/10.1037/0893-3200.20.2.266

Koss, K. J., Hostinar, C. E., Donzella, B., & Gunnar, M. R. (2014). Social deprivation and the HPA axis in early development. *Psychoneuroendocrinology*, *50*, 1–13. https://doi.org/10.1016/j.psyneuen.2014.07.028

Ling, J., Robbins, L. B., & Xu, D. (2019). Food Security Status and Hair Cortisol among Low-income Mother-Child Dyads. *Western journal of nursing research*, *41*(12), 1813–1828. https://doi.org/10.1177/0193945919867112

Liu, C. H., Snidman, N., Leonard, A., Meyer, J., & Tronick, E. (2016). Intra-individual stability and developmental change in hair cortisol among postpartum mothers and infants: Implications for understanding chronic stress. *Developmental Psychobiology*, *58*(4), 509–518. https://doi.org/10.1002/dev.21394

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*, *65*(3), 209–237. https://doi.org/10.1016/j.bandc.2007.02.007

Maurer, N., Perkinson-Gloor, N., Stalder, T., Hagmann-von Arx, P., Brand, S., Holsboer-Trachsler, E., ... Lemola, S. (2016). Salivary and hair glucocorticoids and sleep in very preterm children during school age. *Psychoneuroendocrinology*, *72*, 166–174. https://doi.org/10.1016/j.psyneuen.2016.07.003

Molfese, V. J., Molfese, P. J., Molfese, D. L., Rudasill, K. M., Armstrong, N., & Starkey, G. (2010). Executive function skills of 6–8 year olds: Brain and behavioral evidence and implications for school achievement. *Contemporary Educational Psychology*, *35*(2), 116–125. https://doi.org/10.1016/j.cedpsych.2010.03.004

Najam, A., & Bari, F. (2017). Unleashing the potential of a young Pakistan. Pakistan National Human Development Report. Published for the United Nations Development Programme (UNDP).

Noël, M.-P. (2009). Counting on working memory when learning to count and to add: A preschool study. *Developmental Psychology*, *45*(6), 1630–1643. https://doi.org/10.1037/a0016224

Obradović, J. (2016). Physiological responsivity and executive functioning: Implications for adaptation and resilience in early childhood. *Child Development Perspectives*, *10*(1), 65–70. https://doi.org/10.1111/cdep.12164

Obradović, J., Bush, N. R., Stamperdahl, J., Adler, N. E., & Boyce, W. T. (2010). Biological sensitivity to context: The interactive effects of stress reactivity and family adversity on socioemotional behavior and school readiness. *Child Development*, *81*(1), 270–289. https://doi.org/10.1111/j.1467-8624.2009.01394.x

Obradović, J., Finch, J. E., Portilla, X. A., Rasheed, M. A., Tirado-Strayer, N., & Yousafzai, A. K. (2018). Early executive functioning in a global context: Developmental continuity and family protective factors. *Developmental Science*, e12795, https://doi.org/10.1111/desc.12795

Obradović, J., Portilla, X. A., & Ballard, P. J. (2016). Biological sensitivity to family income: Differential effects on early executive functioning. *Child Development*, *87*(2), 374–384. https://doi.org/10.1111/cdev.12475

Obradović, J., & Willoughby, M. T. (2019). Studying executive function skills in young children in low-and middle-income countries: Progress and directions. *Child Development Perspectives*, *13*(4), 227–234. https://doi.org/10.1111/cdep.12349

Obradović, J., Yousafzai, A. K., Finch, J. E., & Rasheed, M. A. (2016). Maternal scaffolding and home stimulation: Key mediators of early intervention effects on children's cognitive development. *Developmental Psychology*, *52*(9), 1409–1421. https://doi.org/10.1037/dev0000182

Palmer, F. B., Anand, K. J. S., Graff, J. C., Murphy, L. E., Qu, Y., Völgyi, E., ... Tylavsky, F. A. (2013). Early adversity, socioemotional development, and stress in urban 1-year-old children. *The Journal of Pediatrics*, *163*(6), 1733–1739. https://doi.org/10.1016/j.jpeds.2013.08.030

Pauli-Pott, U., Schloss, S., Ruhl, I., Skoluda, N., Nater, U. M., & Becker, K. (2017). Hair cortisol concentration in preschoolers with attention-deficit/hyperactivity symptoms – Roles of gender and family adversity. *Psychoneuroendocrinology*, *86*, 25–33. https://doi.org/10.1016/j.psyneuen.2017.09.002

Pluess, M., & Belsky, J. (2010). Differential susceptibility to parenting and quality child care. *Developmental psychology*, *46*(2), 379. https://doi.org/10.1037/a0015203

Rasheed, M. A., Pham, S., Memon, U., Siyal, S., Obradović, J., & Yousafzai, A. K. (2018). Adaptation of the Wechsler Preschool and Primary Scale of Intelligence-III and lessons learned for evaluating intelligence in low-income settings. *International Journal of School & Educational Psychology*, *6*(3), 197–207. https://doi.org/10.1080/21683603.2017.1302851

Rippe, R. C., Noppe, G., Windhorst, D. A., Tiemeier, H., van Rossum, E. F., Jaddoe, V. W., ... van den Akker, E. L. (2016). Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, sex and other child characteristics with hair cortisol and cortisone. *Psychoneuroendocrinology*, *66*, 56–64. https://doi.org/10.1016/j.psyneuen.2015.12.016

Russell, E., Koren, G., Rieder, M., & Van Uum, S. (2012). Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology*, *37*(5), 589–601. https://doi.org/10.1016/j.psyneuen.2011.09.009

Sauvé, B., Koren, G., Walsh, G., Tokmakejian, S., & Van Uum, S. H. (2007). Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clinical & Investigative Medicine*, *30*(5), 183–191. https://doi.org/10.25011/cim.v30i5.2894

Schloß, S., Müller, V., Becker, K., Skoluda, N., Nater, U. M., & Pauli-Pott, U. (2018). Hair cortisol concentration in mothers and their children: Roles of maternal sensitivity and child symptoms of attention-deficit/hyperactivity disorder. *Journal of Neural Transmission*, *126*(9), 1135–1144. https://doi.org/10.1007/s00702-018-1944-7

vonSuchodoletz, A. V., & Barza, L. (2015). *Individual differences in children's self -regulation in response to classroom activities and interactions*. Paper presented at the Biennial Meeting of the European Association for Research on Learning and Instruction, Limassol, Cyprus.

Shah, M. B., Schaefer, B. A., & Clark, T. P. (2013). Reliability of bracken school readiness assessment, third edition scores with young children in Mumbai, India. *International Journal of School & Educational Psychology*, *1*(1), 58–66. https://doi.org/10.1080/21683603.2013.782595

Shirtcliff, E. A., Granger, D. A., Booth, A., & Johnson, D. (2005). Low salivary cortisol levels and externalizing behavior problems in youth. *Development and Psychopathology*, *17*(1), 167–184. https://doi.org/10.1017/S0954579405050091

Simmons, J. G., Azpitarte, F., Roost, F. D., Dommers, E., Allen, N. B., Havighurst, S., & Haslam, N. (2019). Correlates of hair cortisol concentrations in disadvantaged young children. *Stress and Health*, *35*(1), 104–111. https://doi.org/10.1002/smi.2842

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair – State of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

StataCorp. (2013). *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP.

Tarullo, A. R., Obradović, J., Keehn, B., Rasheed, M. A., Siyal, S., Nelson, C. A., & Yousafzai, A. K. (2017). Gamma power in rural Pakistani children: Links to executive function and verbal ability. *Developmental Cognitive Neuroscience*, *26*, 1–8. https://doi.org/10.1016/j.dcn.2017.03.007

UNDP (2014). *Human Development Report 2013: Pakistan The Rise of the South: Human Progress in a Diverse World*. Washington, D.C.: United Nations Development Programme.

Unesco Pakistan. (2017, April 12). Pakistan. Retrieved from http://uis.unesco.org/en/country/pk

UNICEF. (2013). *Out-of-school children in the Balochistan, Khyber Pakhtunkhwa, Punjab and Sindh provinces of Pakistan*. Islamabad, Pakistan: UNICEF Pakistan.

Ursache, A., Merz, E. C., Melvin, S., Meyer, J., & Noble, K. G. (2017). Socioeconomic status, hair cortisol and internalizing symptoms in parents and children. *Psychoneuroendocrinology*, *78*, 142–150. https://doi.org/10.1016/j.psyneuen.2017.01.020

USAID. (2019, September 30). Food Assistance Fact Sheet—Pakistan. Retrieved from https://www.usaid.gov/pakistan/food-assistance

Vaghri, Z., Guhn, M., Weinberg, J., Grunau, R. E., Yu, W., & Hertzman, C. (2013). Hair cortisol reflects socio-economic factors and hair zinc in preschoolers. *Psychoneuroendocrinology*, *38*(3), 331–340. https://doi.org/10.1016/j.psyneuen.2012.06.009

Vanaelst, B., Michels, N., De Vriendt, T., Huybrechts, I., Vyncke, K., Sioen, I., … De Henauw, S. (2013). Cortisone in hair of elementary school girls and its relationship with childhood stress. *European Journal of Pediatrics*, *172*(6), 843–846. https://doi.org/10.1007/s00431-013-1955-1

Vliegenthart, J., Noppe, G., Van Rossum, E. F. C., Koper, J. W., Raat, H., & Van den Akker, E. L. T. (2016). Socioeconomic status in children is associated with hair cortisol levels as a biological measure of chronic stress. *Psychoneuroendocrinology*, *65*, 9–14. https://doi.org/10.1016/j.psyneuen.2015.11.022

Vyas, S., & Kumaranayake, L. (2006). Constructing socio-economic status indices: How to use principal components analysis. *Health Policy and Planning*, *21*(6), 459–468. https://doi.org/10.1093/heapol/czl029

Walker, S. P., Wachs, T. D., Gardner, J. M., Lozoff, B., Wasserman, G. A., Pollitt, E., Carter, J. A., International Child Development Steering Group. (2007). Child development: Risk factors for adverse outcomes in developing countries. *The Lancet*, *369*(9556), 145–157. https://doi.org/10.1016/S0140-6736(07)60076-2

Wechsler, D. (2012). *Wechsler preschool and primary scale of intelligence – Fourth edition*. San Antonio, TX: The Psychological Corporation.

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1–3), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Willoughby, M. T., Blair, C. B., Wirth, R. J., & Greenberg, M. (2010). The measurement of executive function at age 3 years: Psychometric properties and criterion validity of a new battery of tasks. *Psychological Assessment*, *22*(2), 306. https://doi.org/10.1037/a0018708

Willoughby, M. T., Pek, J., & Blair, C. B. (2013). Measuring executive function in early childhood: A focus on maximal reliability and the derivation of short forms. *Psychological assessment*, *25*(2), 664. https://doi.org/10.1037/a0031747664

Yousafzai, A. K., Obradović, J., Rasheed, M. A., Rizvi, A., Portilla, X. A., Tirado-Strayer, N., … Memon, U. (2016). Effects of responsive stimulation and nutrition interventions on children's development and growth at age 4 years in a disadvantaged population in Pakistan: A longitudinal follow-up of a cluster-randomised factorial effectiveness trial. *The Lancet Global Health*, *4*(8), e548–e558. https://doi.org/10.1016/S2214-109X(16)30100-0

Yousafzai, A. K., Rasheed, M. A., Rizvi, A., Armstrong, R., & Bhutta, Z. A. (2014). Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial. *The Lancet*, *384*(9950), 1282–1293. https://doi.org/10.1016/S0140-6736(14)60455-4

Zalewski, M., Lengua, L. J., Kiff, C. J., & Fisher, P. A. (2012). Understanding the relation of low income to HPA-axis functioning in preschool children: Cumulative family risk and parenting as pathways to disruptions in cortisol. *Child Psychiatry & Human Development*, *43*(6), 924–942. https://doi.org/10.1007/s10578-012-0304-3

**How to cite this article:** Armstrong-Carter E, Finch JE, Siyal S, Yousafzai AK, Obradović J. Biological sensitivity to context in Pakistani preschoolers: Hair cortisol and family wealth are interactively associated with girls' cognitive skills. *Developmental Psychobiology*. 2020;00:1–16. https://doi.org/10.1002/dev.21981