Demographic and Psychosocial factors associated with Hair Cortisol Concentrations in Preschool Children

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Abstract

Background: Early life stress has enduring effects on physical and mental health. Hair cortisol concentrations (HCC) reflect exposures to contextual stressors in early life, but are understudied in preschool children.

Methods: Hair samples from children (N=693) during clinic visits (CV) scheduled at 1–4 years (CV1-CV4) were measured using validated assay methods for HCC.

Results: HCC were highest at CV1 and decreased at CV2-CV4, with no sex differences. Black children had higher HCC than White/other children; these differences persisted even after adjusting for socioeconomic factors. Bivariable analyses showed significant effects on HCC for

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Category of Study: Population Study

Conflict of Interest:
The authors have no conflicts of interest to disclose.

Black race, with specific demographic and psychosocial factors at different ages. Multivariable analyses showed that higher HCC at CV1 were associated with Black race and male sex; at CV2 with Black race, lower maternal self-esteem, socioeconomic adversity, and the child's risk for developmental delay; at CV3 with Black race; at CV4 with maternal depression and the child's prior HCC values.

Conclusions: HCC were higher in Black children than White/other races; differences were related to maternal factors, socioeconomic adversity, and the child's risk for developmental delay. Public health measures to reduce disparities between Blacks and other races must also consider the long-term effects of chronic stress in early life.

Editor's Focus Summary:

Hair cortisol concentrations in preschool children showed significant racial differences, related to maternal factors, socioeconomic adversity, as well as their social-emotional development and risks for developmental delay.

Hypothalamic-pituitary-adrenal (HPA) axis activity following exposure to acute stress involves the release of cortisol, usually measured by salivary or serum cortisol changes. HPA-axis regulation normally limits cortisol release to the hypothalamus and anterior pituitary through negative feedback loops, which can be dampened or disrupted in children exposed to chronic or prolonged stress, leading to HPA-axis dysregulation[1].

Changes in salivary or serum cortisol levels reflect acute stress reactivity[2], but require repeated sampling to measure cumulative or chronic stress[3]. Cortisol binds to growing hair, thus hair cortisol concentrations (HCC) are increasingly accepted as cumulative measures of stress experienced over the past 3–6 months in adults[4] and children[5–7]. It is assumed that hair grows about 1 centimeter per month[8] and it incorporates the circulating non-protein bound cortisol[5], although the precise mechanisms incorporating serum cortisol into the growing hair shaft remain unknown[9].

Preclinical, clinical, and epidemiologic studies suggest that early life stress (ELS) has significant downstream effects including disrupted development, altered endocrine, immune, cardiovascular, and metabolic regulation, as well as lifespan effects on cognition, behavior, physical and mental health[1, 10]. To identify the factors associated with HCC in preschool children, we previously developed and validated a specific and sensitive assay for measuring HCC[11]. We also previously reported an association of HCC with social-emotional development in 1-year-old infants[12]. For this study, we hypothesized that demographic and psychosocial factors are associated with higher HCC in children aged 1–4 years, reflecting their exposures to ELS.

METHODS

The CANDLE Study enrolled 1,503 healthy, English-speaking women aged 16–40 years in their second trimester of singleton pregnancy at an urban hospital and from the general community in Shelby County, Tennessee. Exclusion criteria included pre-existing chronic diseases (hypertension, diabetes, sickle cell disease), known pregnancy complications (placenta previa, oligohydramnios), and women not planning to deliver at the four

participating hospitals. Following approval from the Institutional Review Board (IRB) at University of Tennessee Health Sciences Center, participants or legal guardians gave informed consent for participation in the CANDLE study and hair sampling. Stanford University IRB approved these data analyses.

Data Collection

Similar to previous studies (13), children were enrolled for a nested cohort study with hair sampling during annual clinic visits (CV) centered at ages 1–4 years (CV1, CV2, CV3, CV4). Data collection included demographic, parental, social, cognitive, and behavioral measures assessed at the CV1-CV4 time-points; mother's education, marital status, income, health insurance, pre-pregnancy weight and BMI were recorded at the first prenatal visit. Due to funding reasons, enrollment in this study was terminated before most children had reached the age of 4 years when they would have been eligible for their fourth clinic visit (CV4).

Hair Cortisol Measurements

Hair (5–50mg) samples were collected from the posterior vertex; 1–3 cm hair samples were cut as close to the scalp as possible, sealed in Zip-lock bags®, and weighed. Hair cortisol concentrations (HCC) were measured using validated hair extraction and sensitive ELISA assays(11, 12). Hair samples were minced and gently shaken in methanol overnight at 52°C alternating with acetone at 25°C for 5 minutes; this extraction process was repeated twice for each hair sample(11). Pellets from each sample were re-suspended in 70μl phosphate buffered saline for 10 mg hair. Cortisol was measured following manufacturer's instructions for salivary cortisol (Alpco Diagnostics, Salem, NH). All calibrator, control, and test specimens were measured in duplicate. Assay plates were read using an Epoch plate reader (BioTek Instruments, Winooski, VT) set at 450nm and Gen51.11 software quantified cortisol expression in unknown specimens against a standard curve. Results were normalized to the weight of each hair sample (ng cortisol/mg hair). Intra-assay and inter-assay coefficients of variability were below 7% and 10% respectively.

Statistical Analyses

Demographic, maternal, and child characteristics were examined at CV1, CV2, CV3, and CV4. Raw HCC (ng/mg) values were natural log (ln) transformed to approach normal distributions. Individuals contributed HCC data at any combination of the four time-points. Demographic and risk factors for mothers and children were summarized at each CV using means and standard deviations for continuous variables, and numbers and percentages for categorical variables. Data from non-White, non-Black participants (n=11, 2.8%) were not different from White children; thus, their data were combined with the White children. Distributions of ln-HCC were examined by clinic visit (CV) or child's age, sex, and race, grouped as Black or White/other.

To account for repeated hair sampling from 286 children (41%) in this study, we used ***mixed effects*** linear regression models(14) to test for ln-HCC changes by the child's age, race within age, sex within age, and race within sex and age. Race and socioeconomic adversity are linked in Shelby County, therefore models were fit with and without adjusting for

socioeconomic adversity, as measured by a previously validated index(15). Mothers with incomplete socioeconomic data were assigned to the lowest socioeconomic quintile, thereby maximizing the effects of socioeconomic adversity on ln-HCC and ensuring more stringent tests of our hypotheses related to age, race, and sex.

We used a variety of strategies to identify the most discriminating variables from the CANDLE Study. First, based on literature searches and expert knowledge, we selected 25–30 variables per CV as potential correlates of ln-HCC. Second, at each CV, we used bivariable linear regression models to assess the association of each candidate variable with ln-HCC (Table 2). Third, using all variables listed in Table 2, we conducted multivariable analyses to examine significant associations of candidate variables at each CV with the corresponding ln-HCC values.

To overcome limitations of randomly missing data in the CANDLE dataset and high collinearity among variables measured at each CV, we adopted two strategies. We first used the MI-LASSO (least absolute shrinkage selection operator) algorithm that replaces missing data using 'multiple imputations in chained equations' (*mice*) and then performs variable selection to create parsimonious statistical models(16). Traditional multivariable models use forwards- or backwards-stepwise selection, analyzing variable-by-variable and ending up only with statistically significant variables in the model. In contrast, the LASSO algorithm analyzes all independent variables simultaneously to select *groups of variables* and performs L1 regularization to minimize cross-validation errors(16). Second, after LASSO had selected the most important group of variables, we then re-fit the linear regression models to examine the significance of each variable, where statistical inference was performed by pooling results across five imputed datasets using Rubin's rules. As sensitivity analyses for LASSO, we also fitted conditional inference decision trees to model the relations between ln-HCC and candidate predictors at each time-point(17). All analyses were conducted in R (version 3.4.3).

RESULTS

Characteristics of the 1,503 women enrolled in the CANDLE study showed a mean age 26 years, 19.9% had college degrees, 37.5% were married, 21.2% earned >$65,000 per year, and 41.6% had private health insurance. Subsets of these mothers gave birth to live-born infants (n=1,457) with an equal split of males (49.9%) and females (50.1%) and consented for their child's hair sampling at clinic visits CV1-CV4 (Table 1). Demographic differences between those with and without hair samples are presented online (Tables S1–S4) with comparable differences identified at all clinic visits (CV1-CV4).

Hair Cortisol Concentrations (ln-HCC ng/mg, N=1060) were measured from 693 children at the CV1-CV4 time-points; 41% children (n=286) gave more than one sample, 5 children gave samples at all timepoints and 71 children gave samples at 3 timepoints. *Mixed effects* linear regression models were employed with fixed effects for CV and random effects for subjects. These models showed decreasing HCC during early childhood, with significantly higher ln-HCC at CV1 compared to CV2 (estimate −0.48; 95%CI: −0.69, −0.27), CV3 (−0.67; 95%CI: −0.91, −0.43), and CV4 (−1.98; 95%CI: −2.38, −1.57) (all p<0.0001). ln-

HCC values were also higher at CV2 (−1.50; 95%CI: −1.90, −1.10) and CV3 (−1.31; 95%CI: −1.72, −0.90) compared to CV4 (both p<0.0001); (Figure 1a). These differences remained significant even after adjusting for socioeconomic factors (Table S5). *Mixed effects* models including fixed effects for sex, age, and sex by age yielded no significant ln-HCC differences between males and females at CV1 (-0.29; p=0.0645), CV2 (0.33; p=0.0545), CV3 (0.31; p=0.1353), or CV4 (0.17; p=0.6653) (Figure 1b). *Mixed effects* models including fixed effects for race, age, and race by age revealed higher ln-HCC in Black than in White/other children at CV1 (1.10; 95%CI: 0.81, 1.39; p<0.0001), CV2 (1.35; 95%CI: 1.03, 1.66; p<0.0001), CV3 (1.44; 95%CI: 1.08, 1.81; p<0.0001), and CV4 (1.48; 95%CI: 0.67, 2.28; p=0.0001) (Figure 1c). All differences by race remained significant even after adjusting for socioeconomic adversity (Table S6). When adding a fixed effect for sex to this model, racial differences were somewhat greater among girls (estimates 1.13–2.25, p<0.0001) than among boys (estimates 0.94–1.22, p<0.002; Figure 1d). Density and scatterplots showing frequency distributions of ln-HCC values by age in months (Figure 2a) and CV (Figure 2b) also showed substantially different distributions for Black vs. White/other children, with Black children having higher ln-HCC values across all age groups.

Bivariable regression analyses:

Ln-HCC showed ***direct*** relations with Black race (CV1-CV4), mother's pre-pregnancy weight and BMI (CV1), mother's Rigidity Score (CV1-CV3) and Abuse Score (CV2) in the Child Abuse Potential Index (CAPI), and maternal depression (CV4), the child's prior ln-HCC values at CV1 (CV2, CV4) and CV2 (CV3, CV4), and social-emotional problems (CV1, CV2) from BITSEA (Brief Infant-Toddler Social-Emotional Assessment)(Table 2). Ln-HCC showed ***inverse*** relations with mother's age (CV1), education (CV1-CV3), marital status (CV1-CV3), health insurance and income (CV1-CV4), household structure (CV1-CV3), knowledge of infant development (CV1), and the child's gestational age, birthweight, and female sex (CV1) (Table 2). Of note, Black mothers had higher pre-pregnancy weight and BMI (p<0.0001) than White/other mothers; these were associated with the child's ln-HCC values at CV1, but not at CV2, CV3, or CV4.

Categorical variables for maternal education, marital status, income, health insurance, and household structure were previously combined into a composite numerical index measuring socioeconomic adversity in the perinatal, infant/toddler, and preschool periods(15). To allow their inclusion into multivariable regression analyses, we substituted these categorical variables with the validated numerical index(15), which showed significant relationships with ln-HCC at all four clinic visits (CV1-CV4)(Table 2).

Multivariable analyses:

The LASSO algorithm simultaneously analyzed all independent variables to select the *group of variables* that best explain the child's ln-HCC values at each CV (Table 3). **At CV1**, higher ln-HCC was associated with Black race (p<0.0001) and male sex (p<0.005). **At CV2**, higher ln-HCC was associated with Black race (p<0.0001), low maternal self-esteem (p<0.05), greater socioeconomic adversity (p<0.005), as well as the child's 'risk for developmental delay' (p=0.01). The CANDLE study had defined 'at risk for developmental delay' *a priori* as scores below the 15th percentile for age in the BITSEA Competence, or

Bayley-III Cognitive, or Bayley-III Language scales. *At CV3*, higher ln-HCC were associated only with Black race (p<0.0001). *At CV4*, higher ln-HCC were associated with the child's prior ln-HCC values at CV2 (p<0.005).

Black race was the strongest determinant of ln-HCC at CV1-CV3; therefore, we conducted LASSO analyses separately within each racial group to unmask other factors associated with ln-HCC (Table 3). *At CV2*, higher ln-HCC in Black children were associated with lower maternal self-esteem (p<0.03), greater socioeconomic adversity (p<0.02), prior ln-HCC at CV1 (p<0.01), and being at risk for developmental delay (p<0.05). *At CV3*, higher ln-HCC in Black children were associated with the child's poor social skills (p=0.01) and greater social-emotional problems (p<0.0005). Among White/other children *at CV4*, higher ln-HCC were associated with greater socioeconomic adversity (p=0.005).

Decision tree analyses—Decision trees analyses at each clinic visit (CV1, CV2, CV3) used recursive partitioning to split the sample into similar groups of ln-HCC as a secondary means of variable selection. At CV1, CV2, and CV3, as noted in the LASSO analyses, Black race was the most significant factor (p<0.001 at CV1, CV2, and CV3) (Figure S1). Black children with greater social-emotional problems showed higher ln-HCC than those without (p=0.002) *at CV3*, confirming our results from LASSO (Table 3).

DISCUSSION

Hair cortisol may be a promising measure of chronic stress in children(3, 5, 6, 12), although several conceptual and methodological issues remain unresolved(3, 9). This is the first study to report the psychosocial and demographic factors associated with HCC in a large, geographically defined population of preschool children. This is also the first study to include sufficient numbers of children from underrepresented minorities and to use a validated HCC assay with high sensitivity/specificity(11). Children aged 1–4 years showed decreasing HCC values with age (Fig.1a), extending results from previous studies with smaller sample sizes and less accurate assays(18, 19) (Fig.1b). Unlike all previous studies, we found major differences between the children of Black vs. White/other mothers, occurring most prominently around 1–3 years of age.

Limited data exist for HCC in preschool children. In Swedish children aged 1, 3, 5, and 8 years, higher HCC occurred in the younger children and were associated with maternal HCC, birth weight, and psychosocial stress(18). HCC were measured in 128 Dutch children aged 4–14 years, that included 27 children at 4–5 years and none below this age(19). In 3–6 year-old Canadian children, lower parental education was associated with elevated HCC, but race/ethnicity or other maternal/child factors were not reported(20). Groeneveld et al. studied children aged 4–5 years and reported that HCC increased after school entry, particularly in fearful children (6). Rippe et al. studied 6-year-old Dutch children, but none were of preschool age (13). Despite concerns about the sensitivity of hair cortisol assays in previous studies(11), small sample sizes, and failure to adjust for puberty or other known factors that affect cortisol, these are the only data available for HCC in preschool children(18, 19).

We are the first to characterize the maternal, demographic, and psychosocial correlates of HCC in minority preschool children(5). Mothers' pre-pregnancy weight and BMI were associated with the child's HCC at CV1 but not at CV2-CV4, suggesting that the child's HPA-axis may be influenced by the pro-inflammatory milieu associated with maternal obesity(21). Our bivariable analyses further suggest that children of mothers who were Black, younger, less educated, with less stable partner relationships, lower socioeconomic status, less knowledge of infant development, greater rigidity or abuse potential, or likely to be depressed may experience greater chronic stress than their corresponding comparison groups. Categorical maternal factors were included in the Socioeconomic Adversity Index(15), which also showed highly significant relationships with HCC at all ages. Chronic stress was also greater if the children were born earlier, had lower birth weights, social-emotional problems, or had experienced chronic stress at younger ages.

The LASSO algorithm allowed us to select in an agnostic, unbiased manner the most important *group of variables* that best explained the child's HCC at each clinic visit. The strongest and most consistent factor was Black race. Racial identity conveys information about many correlates of socioeconomic disadvantage, unstable housing, job or food insecurity, inadequate support systems, or other factors causing maternal stress(22). We found that these racial differences were unchanged even after adjusting for socioeconomic adversity. Recurrent or chronic maternal stress may be transmitted to her offspring via epigenetic changes or altered maternal behaviors. Indeed, ELS can upregulate(23) and social bonding can downregulate the infant's HPA axis(24).

These data extend previous findings(12) and confirm similar results in adults. In 102 adults, the highest HCC were found in Black subjects, followed by Hispanic, White, and other subjects(25). HCC were higher in adult minorities from both low and high socioeconomic status (SES) groups, even though perceived stress was similar regardless of SES classification(26). Among pregnant women, traumatic life events(27) and exposures to childhood abuse(28) were associated with elevated HCC in pregnancy, but after stratification for race/ethnicity these associations remained only for Black women.

The Family Life Project (FLP) found elevated salivary cortisol levels in 7–48 month-old children associated with poverty, poor housing quality, and low positive parenting behaviors(29). However, salivary cortisol levels were *"elevated in African-American children relative to their white counterparts even when controlling for multiple aspects of risk ….(which) may indicate an enduring intergenerational effect of social injustice on stress physiology levels"*(29). Another study of salivary cortisol in kindergarten children found that ethnic minority status, socioeconomic status, and family adversity predicted stress reactivity(30). Although disparities in socioeconomic status, or other variables for Black women in the CANDLE study(12) were similar to those in FLP and other studies(29–31), we posit that HCC is a more specific marker for chronic/recurrent stress(4, 5, 9). We found socioeconomic adversity associated with higher HCC, consistent with multiple lines of evidence linking poverty with chronic stress(20, 30–32). Socioeconomic disadvantage increased the odds of developing two or more chronic health conditions in 2-year-old children after controlling for maternal ethnicity, smoking, poor health, depressive symptoms, and child gender(33).

Elevated HCC in early childhood may also increase the long-term risks for adverse health-related consequences, given that HCC values were correlated with hemoglobin A1c in Black adults(34). Because both hyper- and hypo-reactivity of the HPA axis following ELS can increase the long-term risks of chronic diseases and/or psychopathology(1, 23, 35), we sought to identify the factors other than Black race that were associated with higher HCC in preschool children.

HCC at CV1 was associated with male sex, although our direct comparisons had not identified differences between boys and girls (Figure 1b). Rippe et al. studied 6 year-old Dutch children (N=2,484) and found slightly higher HCC in boys than girls (1.55 vs. 1.38 pg/mg)(13). Gerber et al. sampled 6–8 year-old Swiss children (N=318) and found significantly higher HCC in boys vs. girls (14.25 vs. 10.44 pg/ml), related to BMI in girls and somatic complaints in boys(36). Experiences related to male aggression in elementary school may explain greater stress in boys, although both these studies did not include preschool children. Ten-fold differences in the HCC values between these two studies highlight the variability of HCC measurements in prior research(13, 36). Using an assay with significantly greater sensitivity/specificity, we also found sex differences in the same direction, but only at CV1(12). Across childhood and adolescence, 5 studies reported similar sex differences, whereas 11 studies found no sex differences(5). From our data, we cannot substantiate the previous findings of sex differences in preschool children.

Maternal temperament and mental health are important regulators of an infant's developing HPA axis(12, 31). Our previous findings that lower maternal self-esteem, irritable temperament, and depression were associated with elevated HCC in 1 year-olds(12) support the important role of maternal psychological function in regulating HPA-axis responses during early life. We also found higher HCC associated with low maternal self-esteem at CV2 and maternal depression at CV4, supporting earlier findings(12, 18, 31, 37).

LASSO analyses selected prior HCC values as positive contributing factors for later HCC; thus, chronic stress experienced as toddlers (CV2) may influence the stress responses of preschoolers (CV4), suggesting the effects of 'allostatic load' from ELS(29, 30). We posit, however, that this isolated finding must be interpreted with caution. We strongly suggest that future research conduct longitudinal analyses examining the trajectory of HCC values across childhood to confirm long-term effects of ELS on subsequent stress responses and the potential for HPA-axis dysregulation(12).

Being at risk for developmental delay appeared to contribute to greater stress levels among all children and specifically among Black children at CV2. Social-emotional problems showed similar effects among Black children at CV1 from our previous study and also in bivariable linear regressions at CV1 and CV2 in this study. Developmental delay, social-emotional problems and chronic stress may be inter-related in complex ways, adversely affecting cognitive performance and emotional regulation in multiple domains(12, 29–31). Social-emotional problems also contribute to parenting stress, family disruptions, and externalizing behaviors in later childhood often linked to adverse behavioral and cognitive outcomes(37).

We should note four limitations of this study. First, socio-demographic differences between children with and without HCC measurements in the CANDLE study, or between Shelby County and other counties(15) limit the generalizability of these data. This is not uncommon in other cohort studies of early childhood stress(5, 12, 13). As a related point, the CANDLE study included only English-speaking mothers. Second, hair sampling at CV4 was limited because relatively fewer mothers gave consent, some 4-year-old boys had 'buzz cuts' with inadequate hair samples(6), and we had to terminate study enrollment before many children could be scheduled for their CV4 visit. Third, we did not measure hair growth rates or hair composition in this study. Arguably, slower hair growth rates(8) or higher hair lipids(38) could explain higher HCC values in Black children. Previous studies reporting HCC in children or adults have not considered these factors either but they must be addressed in future investigations. Lastly, we could not assess the effects of maternal smoking on HCC in preschool children.

Preschool children are most vulnerable to HPA-axis dysregulation from ELS, with well-known associations with obesity, chronic non-communicable diseases, or psychopathology(1, 35, 39, 40). Measuring HCC allows us to objectively probe the social psychology of early life from the child's perspective, thus building on observations from verbal reports, parent/caregiver surveys, psychometric lab testing, or home evaluations. This is especially valuable for non-verbal populations, those accompanied by unreliable historians, those experiencing social change (e.g., bereavement, migration, foster care) or contextual stressors (e.g., intimate partner violence, bullying, child maltreatment)(3, 25, 35, 39). The public health importance of our finding that Black preschool children have significantly higher HCC values than other children has bearing on the health disparities between Blacks and other demographic groups(41). Other findings that maternal psychological function, socioeconomic adversity, and the child's risk for developmental delay may accentuate chronic stress, suggest potential directions for preventing or reducing the long-term health outcomes and psychosocial effects of early life stress in preschool children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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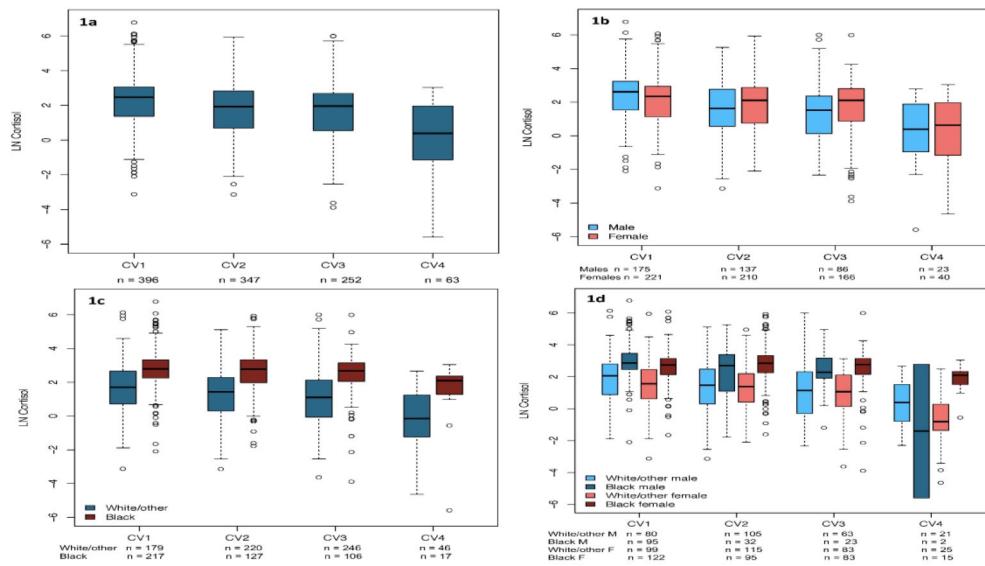
Figure 1:

Changes in log-normal hair cortisol concentrations (ln-HCC, ng /mg) compared using *mixed effects* linear regression models by: (A) clinic visits at 1–4 years (CV1-CV4), (B) sex, (C) race, and (D) race by sex. Results suggest: (A) decreasing stress during early childhood; (B) no sex differences; (C) greater stress in Black children compared to White/other children at all ages; and (D) greater stress in Black males compared to White/other males at CV1-CV3 and in Black females compared to White/other females at all ages.
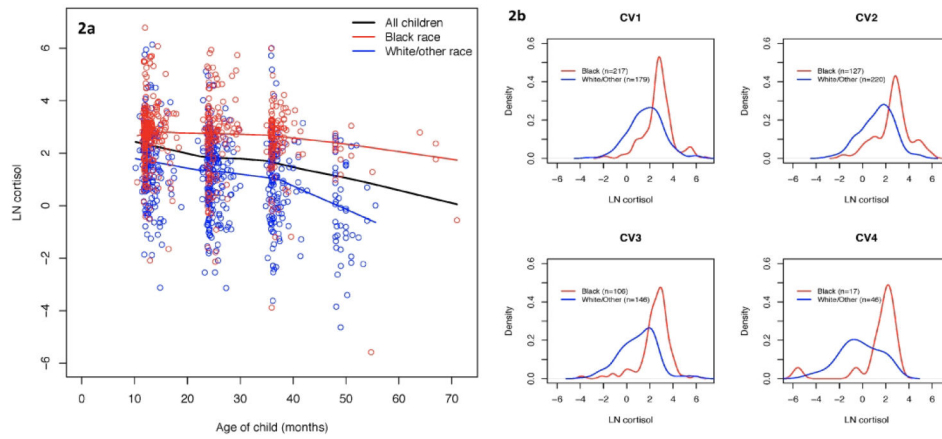
Figure 2:

Hair cortisol concentrations (ln-HCC, ng/mg) distributed by age and race. (A) Scatterplot showing individual ln-HCC values distributed by child's age in months, with Black children in red and White/other children in blue; (B) Density plots showing frequency distributions of ln-HCC in Black and White/other children at each clinic visit (CV1-CV4).

Table 1:

Demographic Characteristics from Clinic Visits (CV) at 1–4 years

|	CV1 n=396	CV2 n=347	CV3 n=252	CV4 n=63
Age (months)	13.1 (±1.9)	25.1 (±1.8)	36.8 (±1.8)	50.7 (±4.8)
Sex				
Male	175 (44.2%)	137 (39.5%)	86 (34.1%)	23 (36.5%)
Female	221 (55.8%)	210 (60.5%)	166 (65.9%)	40 (63.5%)
Race				
White/other	179 (45.2%)	220 (63.4%)	146 (57.9%)	46 (73.0%)
Black	217 (54.8%)	127 (36.6%)	106 (42.1%)	17 (27.0%)
Birthweight (grams)	3301.3 (±517.0)	3342.6 (±505.6)	3293.9 (±510.8)	3482.5 (±360.4)
Missing	2 (0.5%)	1 (0.3%)	1 (0.4%)	0 (0%)
Gestational age (weeks)	39.0 (±1.4)	39.0 (±1.3)	38.8 (±1.6)	39.1 (±1.1)
Missing	2 (0.5%)	0 (0%)	1 (0.4%)	0 (0%)
Mother's age at birth (years)	27.2 (±5.4)	27.8 (±5.2)	27.4 (±5.2)	29.5 (±4.8)
Missing	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)
Education level				
< High School	17 (4.3%)	11 (3.2%)	13 (5.2%)	1 (1.6%)
High School/GED	162 (40.9%)	117 (33.7%)	86 (34.1%)	17 (27.0%)
Technical School	35 (8.8%)	27 (7.8%)	21 (8.3%)	0 (0.0%)
College Degree	105 (26.5%)	122 (35.2%)	71 (28.2%)	18 (28.6%)
Grad/Professional	74 (18.7%)	70 (20.2%)	61 (24.2%)	27 (42.9%)
Missing	3 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Marital status				
Never married	116 (29.3%)	86 (24.8%)	72 (28.6%)	12 (19.0%)
Divorced	7 (1.8%)	7 (2.0%)	8 (3.2%)	1 (1.6%)
Separated	7 (1.8%)	9 (2.6%)	4 (1.6%)	0 (0.0%)
Living with partner	53 (13.4%)	31 (8.9%)	25 (9.9%)	6 (9.5%)
Married	209 (52.8%)	213 (61.4%)	143 (56.7%)	44 (69.8%)
Missing	4 (1.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
Income				
<25K	150 (37.9%)	103 (29.7%)	75 (29.8%)	13 (20.6%)
25–65K	122 (30.8%)	106 (30.5%)	70 (27.8%)	18 (28.6%)
>65K	108 (27.3%)	127 (36.6%)	93 (36.9%)	32 (50.8%)
Missing	16 (4.0%)	11 (3.2%)	14 (5.6%)	0 (0.0%)
Insurance				
Public	184 (46.5%)	136 (39.2%)	120 (47.6%)	23 (36.5%)
Private	212 (53.5%)	211 (60.8%)	132 (52.4%)	40 (63.5%)

Note: One child may appear at multiple time points based on data collection at each of the four clinic visits.

Table 2:

Factors associated with Hair Cortisol Concentrations (HCC) in Bivariable Regression Analyses

Variable	CV1 (n=396) Estimate (95% CI)	P-value	CV2 (n=347) Estimate (95% CI)	P-value	CV3 (n=252) Estimate (95% CI)	P-value	CV4 (n=63) Estimate (95% CI)	P-value
Black Race (ref: White/Other)	1.08 (0.81, 1.35)	<0.0001	1.36 (1.03, 1.68)	<0.0001	1.45 (1.07, 1.82)	<0.0001	1.69 (0.66, 2.71)	0.0013
Mother's age at M1	−0.03 (−0.05, 0.00)	0.043						
Mother's education at M1 (ref: <high school):								
High School/GED	−0.06 (−0.66, 0.54)		−0.63 (−1.77, 0.5)		−0.82 (−1.78, 0.13)			
Technical School	−0.15 (−0.88, 0.58)	0.014	−0.56 (−1.87, 0.75)	0.0039	−0.83 (−2.07, 0.41)	0.0678		
College Degree	−0.58 (−1.2, 0.04)		−1.13 (−2.26, 0)		−1.27 (−2.23, −0.31)			
Grad/Professional	−0.58 (−1.24, 0.07)		−1.4 (−2.57, −0.24)		−1.21 (−2.2, −0.23)			
Marital status at M1 (ref: never married)								
Divorced	−0.43 (−1.86, 1.01)		1.13 (−0.4, 2.67)		−0.08 (−1.73, 1.56)			
Separated	−0.1 (−1.2, 0.99)−	0.0014	−0.52 (−1.63, 0.59)	<0.0001	−1.22 (−2.87, 0.43)	0.0002		
Living with partner	−0.45 (−0.87, −0.03)		−0.3 (−0.96, 0.36)		−0.4 (−1.17, 0.37)			
Married	−0.71 (−1.04, −0.37)		−1.1 (−1.5, −0.7)		−1.13 (−1.62, −0.63)			
Mother's health Insurance: private (ref: public ins.)	−0.6 (−0.89, −0.32)	<0.0001	−1.04 (−1.4, −0.69)	<0.0001	−0.73 (−1.17, −0.3)	0.0009	−1.07 (−2.23, 0.08)	0.068
Income at M1 (ref: <$25K per year)								
$25K-$65K	−0.19 (−0.54, 0.16)	0.0019	−0.64 (−1.07, −0.22)	<0.0001	−0.49 (−1.05, 0.07)	0.0031	0.40 (−1.17, 1.98)	0.002
>$65K	−0.64 (−0.99, −0.28)		−1.15 (−1.56, −0.73)		−0.93 (−1.46, −0.39)		−1.46 (−2.9, −0.03)	
Household structure (ref: single parent, 5+ people in the home)								
Single parent, 2–4	−0.12 (−0.67, 0.43)	0.0018	−0.61 (−1.28, 0.06)	<0.0001	−0.36 (−1.07, 0.35)	0.0004		
Both parents, 5+	−0.62 (−1.13, −0.1)		−1.36 (−1.99, −0.74)		−1.18 (−1.86, −0.5)			
Both parents, 2–4	−0.74 (−1.2, −0.28)		−1.33 (−1.91, −0.75)		−1.08 (−1.71, −0.45)			
Mother's weight at M1 (Kg)	0.01 (0.00, 0.02)	0.004						
Mother's BMI at M1 (kg/m²)	0.03 (0.01, 0.05)	0.0052						
KIDI total score (M2)	−2.24 (−3.27, −1.21)	<0.0001						
CAPI Rigidity Score	0.02 (0.01, 0.03)	0.0001	0.03 (0.02, 0.04)	<0.0001	0.03 (0.00, 0.05)	0.0002		
CAPI Abuse Score			0 (0, 0.01)	0.0031				
Irritable temperament (TEMPS-A)					−2.93 (−6.25, 0.39)	0.0837		
Maternal depression (CESD)							0.08 (0.01, 0.15)	0.0166

Variable	CV1 (n=396) Estimate (95% CI)	P-value	CV2 (n=347) Estimate (95% CI)	P-value	CV3 (n=252) Estimate (95% CI)	P-value	CV4 (n=63) Estimate (95% CI)	P-value
Gestational age (weeks)	−0.13 (−0.23, −0.03)	0.0134						
Birthweight (kg)	−0.25 (−0.53, 0.03)	0.08						
Female sex (ref: male)	−0.37 (−0.66, −0.08)	0.0117	0.33 (−0.01, 0.67)	0.0601				
BITSEA Problem Total	0.04 (0.01, 0.06)	0.0087	0.04 (0.01, 0.06)	0.0036				
LN Cortisol at CV1			0.40 (0.19, 0.62)	0.0002			1.1 (0.25, 1.96)	0.0115
LN Cortisol at CV2					0.28 (0.09, 0.46)	0.0034	0.69 (0.26, 1.11)	0.0016
Child care at organized daycare							−1.41 (−2.9, 0.07)	0.0624
Bracken School Readiness Competency							−0.03 (−0.07, 0.00)	0.0624
SAI (ref: 1=worst)								
2	−0.09 (−0.63, 0.46)		−0.07 (−0.69, 0.55)		−0.06 (−0.76, 0.65)		2.27 (0.17, 4.37)	
3	−0.17 (−0.66, 0.32)	0.0001	−0.92 (−1.55, −0.3)	<0.0001	−0.65 (−1.38, 0.09)	0.0007	1.73 (−0.25, 3.71)	0.0006
4	−0.52 (−0.96, −0.09)		−1.31 (−1.86, −0.76)		−0.79 (−1.45, −0.14)		1.39 (−0.44, 3.21)	
5	−0.9 (−1.34, −0.46)		−1.53 (−2.06, −0.99)		−1.12 (−1.73, −0.51)		−0.29 (−1.96, 1.38)	

Note: Only bivariable relationships with p<0.1 are listed. *HCC: Hair Cortisol Concentrations, TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris & San Diego-Auto, CESD: Center for Epidemiological Studies Depression Scale, HV: Home Visit in CANDLE, BITSEA: Brief Infant-Toddler Social-Emotional Assessment scale, CAPI: Child Abuse Potential Index, KIDI: Knowledge of Infant Development Index, SAI: Socioeconomic Adversity Index.

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Table 3:

Factors associated with Hair Cortisol Concentrations (HCC) in Multivariable Analyses

Ln-HCC in All Children	Estimate (95% CI)	P-value
CV1		
Black race	1.00 (0.72, 1.27)	<.0001
Female sex (ref: male)	−0.39 (−0.66, −0.12)	0.0048
Length of NICU stay (days)	0.01 (−0.01, 0.03)	0.4494
Gestational age (weeks)	−0.08 (−0.18, 0.01)	0.0858
Maternal weight (kg)	0.01 (0, 0.01)	0.1006
CV2		
Black race	0.94 (0.5, 1.39)	<.0001
Rosenberg Self-Esteem Score	−0.04 (−0.08, 0)	0.0352
Socioeconomic Adversity Index (infant/toddler)	−0.22 (−0.36, −0.07)	0.0029
Ln-HCC at CV1	0.09 (−0.1, 0.28)	0.3772
At-risk for Developmental Delay[*]	−0.63 (-1.11, −0.15)	0.0104
CV3		
Black race	1.15 (0.69, 1.61)	<.0001
Ln-HCC at CV1	0.15 (−0.04, 0.33)	0.147
Ln-HCC at CV2	0.15 (−0.01, 0.31)	0.0754
CV4		
Black race	0.4 (−0.84, 1.64)	0.5251
Socioeconomic Adversity Index (preschool)	−0.19 (−0.59, 0.21)	0.3536
CESD Maternal depression at CV4	0.08 (0.01, 0.14)	0.0178
Ln-HCC at CV2	0.63 (0.22, 1.04)	0.0028
Child care in an organized daycare facility	−1.15 (−2.33, 0.03)	0.0604
Ln-HCC by Racial Group	**Estimate (95% CI)**	**P-value**
CV2: Black race		
Rosenberg Self-Esteem Score	−0.08 (−0.15, −0.01)	0.028
Socioeconomic Adversity Index (infant/toddler)	−0.26 (−0.47, −0.04)	0.019
Ln-HCC at CV1	0.44 (0.12, 0.77)	0.0092
At-risk for Developmental Delay[*]	−0.83 (−1.58, −0.08)	0.0316
CV3: Black race		
Poor social skills (Autism subscale, BITSEA)	−0.2 (−0.34, −0.05)	0.0102
Social-emotional Problems (BITSEA)	−0.06 (−0.1, −0.03)	0.0004
CV4: Black race		
CAPI rigidity score	−0.03 (−0.09, 0.03)	0.316
Socioeconomic Adversity Index (preschool)	0.46 (−0.26, 1.19)	0.2108
Female sex (ref: male)	3.11 (0.77, 5.45)	0.0092[**]
CV4: White/Other race		
Socioeconomic Adversity Index (preschool)	−0.70 (−1.16, −0.24)	0.0051

The most important *groups of variables* were selected using the LASSO algorithm. Following that, we re-fit linear regression models to examine the statistical significance of each individual variable on ln-HCC values within these final models. Estimates and 95% confidence intervals (CI) of all variables are listed, only those with p<0.05 were considered clinically relevant.

*
The CANDLE Study *a priori* defined being 'at risk for developmental delay' if the child's BITSEA Competence, or Bayley-III Cognitive, or Bayley-III Language scores were below the 15th percentile for age.

**
The higher ln-HCC in the Black females from this sub-group analysis was discarded because the CV4 group had only included two Black males.

Abbreviations: NHB Non-Hispanic Black, TEMPS Temperament Evaluation of Memphis, Pisa, Paris & San Diego, CESD Center for Epidemiological Studies Depression Scale, NICU Neonatal Intensive Care unit, BITSEA: Brief Infant-Toddler Social-Emotional Assessment scale.