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Longitudinal hair cortisol in low-income young children: A useful biomarker of behavioral symptom change?

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ABSTRACT

Early childhood is a developmental period characterized by significant plasticity, heterogeneity in behaviors and biological functioning. Yet, cumulative cortisol secretion, as measured by hair cortisol, has not been examined longitudinally in relation to change in behavioral problems in young children. The current study examined cross-sectional and longitudinal associations between hair cortisol and changes in behavior problems in a combined sample (N = 88) of two groups of young children from low-income families: 1) A trauma-exposed sample that participated in Child-Parent Psychotherapy (CPP) (n = 43; Mean Age = 4.31, SD = 1.16; 53% Female; 77% Hispanic), and 2) A community sample of children from families experiencing high stress (n = 45; Mean Age = 3.20, SD = 0.29; 67% Female; 58% Hispanic). Cortisol was assayed from hair collected from children at baseline and, on average, one year later. Mothers completed the Child Behavior Checklist at the same time hair samples were collected. Baseline hair cortisol in children was not associated with maternally-reported child behavioral problems at baseline and did not predict change in behavior problems over time. In contrast, increases in cortisol were associated with greater improvement in child behavior problems ($b = -2.98$, $p < 0.05$), controlling for group status and relevant covariates. Subgroup analyses showed that cortisol change across one year significantly differed between the two groups ($p = 0.043$): on average, community children exhibited a decrease, whereas CPP children demonstrated no change. Hair cortisol concentration was similarly related to improvements in mother-reported behavior problems across both CPP and community groups over time. In summary, there were no cross-sectional associations with hair cortisol, whereas increases were associated with improved child well-being. Findings demonstrate an important link between this increasingly common biomarker and child health, but suggest that changes over time may be more informative than cross-sectional associations.

1. Introduction

Dysregulation of the hypothalamic-pituitary adrenal (HPA) axis has been implicated in multiple mental and physical health disorders, particularly in individuals exposed to early adversity (Pan et al., 2018; Wichmann et al., 2017). Hair samples are increasingly being used as a measure of cortisol secretion over a prolonged period of time (several weeks to several months) that is robust to short-term environmental changes (Stalder et al., 2017). Early childhood is a developmental period characterized by significant plasticity and heterogeneity in behaviors and biological functioning; however, very few longitudinal studies have been conducted to examine *changes* in this biomarker early in life (Bryson et al., 2021; Gray et al., 2018). Moreover, despite the significant upsurge in peer-reviewed articles on the use of hair cortisol concentration (HCC) as a biomarker of stress and psychopathology in recent years, very little is known about its association with behavior problems in young children or its potential "malleability" in the context of psychological treatment.

At least two systematic reviews of child HCC research have been conducted recently, one focused on associations between social

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adversity and HCC among children ages 0–8 (Bryson et al., 2021) and the other focused on various psychosocial and physical correlates of HCC in children ages 0–18 (Gray et al., 2018). In both reviews, the studies included were primarily cross-sectional, samples of children were relatively low risk and the nature of the association between HCC and either social adversity or behavioral problems varied considerably. Although a review of 9 studies of hair cortisol in young children suggested a positive association between HCC and socioemotional problems (Bates et al., 2017), a few investigations suggested no association between HCC and behavioral problems in preschoolers (Kao et al., 2018; Simmons et al., 2019; Ursache et al., 2017), and other studies have found that both lower and higher HCC may indicate risk of behavioral problems (Anand et al., 2020; Fuchs et al., 2018; Golub et al., 2019; Pauli-Pott et al., 2019; Schloß et al., 2018). Importantly, not one of these studies employed repeated measures of both HCC *and* behavioral problems to examine change over time. Longitudinal research in which HCC and behavioral problems are measured repeatedly over time is likely to offer much needed clarity on HCC as a biomarker of behavioral risk in early childhood.

In addition, assessing changes in HCC and behavior problems among young children receiving psychotherapeutic treatment may provide insight into whether HCC can be employed as a useful biomarker of psychological adjustment in early childhood. Interventions aimed at treating or preventing behavioral problems in children may exert their behavioral effects via or in accordance with positive changes to the HPA axis as a result of the intervention (Slopen et al., 2014). We identified only one published intervention study that assessed hair cortisol and followed youth over a period of time (Dajani et al., 2018). In this study, war-affected Syrian and Jordanian adolescents participated in group-based stress management activities, and HCC was assessed prior to and following the 8-week intervention and changes were compared to a waitlisted control group. Three trajectories of HCC change were identified (i.e., hypersecretion, medium secretion, and hyposecretion), and intervention effects on HCC varied by trajectory: the intervention increased HCC levels in youth who exhibited a hyposecretion trajectory and decreased HCC levels in youth with hypersecretion or medium secretion profiles, suggesting that rather than absolute increases or decreases, a return to normative variation might be optimal. Notably, repeated measures of psychological symptoms by group (intervention vs. control) were not reported, nor were associations between symptom change and HCC change or between baseline HCC and symptom change. There is some evidence from studies using salivary measures of cortisol that greater cortisol secretion pre-treatment may be related to greater symptom improvement following trauma-focused therapy (Zantvoord et al., 2019). To our knowledge, no study has examined potential intervention-related HCC changes or HCC as a predictor of intervention-related symptom improvement in young children.

In sum, there is only a nascent literature on HCC as a biomarker of behavioral problems in low-income young children, specifically, and findings have been inconsistent (Bates et al., 2017). Further, associations between HCC and behavior problems in early childhood might be elucidated by assessing the changes after effective interventions known to improve psychological functioning. Although randomized controlled trials provide optimal methodologies for such inquiries, randomizing traumatized children from families seeking therapy is unnecessary and unethical, particularly when effective treatments are available. The current study had two aims. The primary aim was to leverage harmonized longitudinal data from two low-income, ethnically-diverse samples consisting of 88 children from the same metro area to examine baseline and longitudinal changes in HCC and their association with behavior problems across a roughly one-year period. Based on the few prospective studies of associations between HCC and behavior problems in young children (e.g., Golub et al., 2019; Pauli-Pott, 2019; Schloß et al., 2018), we expected that increases in HCC would be associated with decreases in behavior problems overall. The full sample was comprised of two subgroups of children ages 2–6 years old—a sample of young children exposed to trauma who received Child-Parent Psychotherapy (CPP), which has demonstrated efficacy in 5 randomized trials (Cicchetti et al., 2006; Lieberman et al., 2005; Lieberman et al., 1991; Toth et al., 2002), and a demographically similar community sample of low-income children from highly-stressed families. Therefore, as a secondary aim, given the scant literature on intervention-related HCC changes, particularly in young children or diverse samples, subgroup analyses were conducted to examine whether HCC change or the association between HCC change and behavior change differed between the CPP and community groups. Given the potential for psychological interventions to increase HPA axis activity, with corresponding symptom improvement, in youth exposed to adversity (Luecken et al., 2014), we expected CPP children would exhibit a greater increase in HCC and a stronger link between change in HCC and change in behavior problems relative to the community sample.

2. Materials and methods

2.1. Participants

The current study is based on data drawn from two groups of children: a sample of children who received Child-Parent Psychotherapy (CPP) and a demographically-similar community group of young children from the same region (see Table 1 for descriptives).

The CPP sample included 43 children who, along with their biological mother, had been referred for outpatient mental health services at one of two campuses of the University of California, San Francisco due to

Table 1
Descriptives of demographics, hair cortisol, and behavior symptoms, by group.

	CPP		Community			
Variable	n	%, M (SD)	n	%, M (SD)	t	p
Sociodemographics						
Female	43	53%	45	67%	1.26	0.212
Hispanic	43	77%	45	58%	-1.92	0.059
Maternal age	42	32.36 (6.27)	45	31.0 (5.32)	-1.09	0.280
Married or living w/ partner	42	36%	44	68%	3.15	0.002
Maternal education (⩾High school)	43	60%	45	18%	-4.50	0.000
Child BMI (baseline)	41	17 (1.74)	43	16.93 (1.9)	-0.18	0.860
Age-corrected BMI % ile	41	0.74 (0.23)	43	0.69 (0.28)	-1.05	0.298
Child age (baseline)	43	4.31 (1.16)	45	3.2 (0.29)	-6.04	0.000
Time between assessments	43	0.75 (0.22)	45	1.31 (0.47)	7.12	0.000
Hair cortisol						
HCC baseline (pg/mg)	43	104.07 (241.15)	45	276.61 (729.82)	1.50	0.139
HCC change (pg/mg)	43	-17.8 (211.68)	45	-190.19 (783.12)	-1.42	0.161
HCC baseline $\log_{10}$	43	3.14 (1.66)	45	3.35 (1.96)	0.54	0.593
HCC change ratio $\log_{10}$	43	-0.13 (1.54)	45	-0.71 (1.41)	-1.83	0.071
HCC baseline (Blom)	43	-0.09 (0.96)	45	0.08 (1.01)	0.80	0.427
HCC change (Blom)	43	0.22 (0.96)	45	-0.21 (0.98)	-2.06	0.043
Child behavior						
CBCL-T baseline	43	63.44 (10.22)	40	45.95 (10.54)	-7.67	0.000
CBCL-T change	38	-13.82 (14.12)	38	0.05 (8.96)	5.11	0.000

Note: BMI = child body mass index; BMI percentiles based on CDC 2000 growth chart; HCC = hair cortisol concentration; HCC change ratio = $\log_{10}$ of HCC follow-up minus $\log_{10}$ of HCC baseline; CBCL-T = child behavior checklist total problems (t-score); CPP = Child-Parent Psychotherapy.

the child's exposure to interpersonal trauma (e.g., community violence, domestic violence, caregiver death) between 2013 and 2015. Biological mother-child dyads who provided consent to receive Child-Parent Psychotherapy (CPP) were invited to participate in a sub-study to examine treatment-related changes in neurobiological psychological functioning (the CPP-Health study). Dyads were eligible to participate in CPP-Health if the dyad spoke either Spanish or English, neither mother nor child been diagnosed with a chronic medical condition (asthma, diabetes, heart/thyroid/growth problems, cancer, high blood pressure, autoimmune disease or epilepsy) and neither mother nor child were taking daily medications used to treat asthma, heart or thyroid problems or seizures. Mothers reporting active interparental violence in the home or substance abuse of any kind were ineligible. Of the 80 mothers approached about the CPP-Health study, 70 provided consent and enrolled. Participating families received $100 in compensation for each assessment. Following the initial assessment, 21 dropped out of treatment and an additional 5 children were missing a hair sample at baseline (n = 1) or follow-up (n = 4), and one hair sample resulted in a biologically implausible value of cortisol and was discarded (n = 1). The final sample with two time points of valid hair collected included 43 children (53.5% female; Mean Age = 4.31 years, SD = 1.16; 77% Hispanic (any race); 9% non-Hispanic White; 5% Asian American; 9% other or more than one race) who participated in up to twenty sessions of Child-Parent Psychotherapy with their biological mother (Mean sessions = 17.6, SD = 4.0; Mean duration between baseline and follow-up = 0.75 years, SD = 0.22). A majority (54%) of mothers reported annual income of $15,999 or less, 12 (28%) between $16,000 and $34,999, and 4 (9%) reported more than $50,000. Twenty-six (60%) mothers reported having a high school degree or less. Of the 43 dyads, 29 spoke primarily Spanish.

The community-based group included 45 children (67% female; Mean age = 3.20 years, SD = 0.29; 58% Hispanic (any race); 11% non-Hispanic Black; 7% non-Hispanic White; 2% Asian; 22% other or more than one race) of low-income women, based in the same metro area, who had enrolled in the Stress, Eating, and Early Development (SEED) study, which was designed to assess the effects of prenatal factors, including high levels of stress, on children's behavioral, physiologic and anthropometric development (See Bush et al., 2017 for details). Women who were between 18 and 45 years of age, fluent in English, 8–23 weeks pregnant with a singleton gestation, were overweight or obese at the time (i.e. had a BMI of 25–40), reported incomes less than 500% of the federal poverty level (75% of the participants lived at or less than 200%, which is notable given the sample is drawn from one of the highest cost-of-living regions in the country), and had live births were eligible to participate in SEED. Women received $60 in research compensation at each study visit.

Previous research in this cohort has demonstrated the high levels of social adversity, including exposure to traumatic events, faced by families in this sample (Bush et al., 2017). Of 129 dyads participating at the 3-year assessment, child hair samples were collected from 63 when children were 3 years old. Of these children, 45 provided hair samples approximately one year later (Mean = 1.29 years, SD = 0.49) when children were 4 years old. At the 3-year assessment, 12 mothers (27%) reported annual income between $0 and $15,999, 13 (29%) between $16,000 and $34,999, and 14 (31%) reported more than $50, 000. Eight mothers (18%) reported having a high school degree or less. All dyads in the community group spoke English.

2.2. Procedures

The research was approved by the Committee for Human Research at the University of California, San Francisco, the Institutional Review Board of San Francisco General Hospital, and the Institutional Review Board of San Francisco General Hospital and the Benioff Children's Hospitals Oakland (CHORI). Mothers provided written consent and were compensated for their time. Procedures for collection of hair were identical, however cortisol assays were batched as indicated below.

2.2.1. Behavioral problems

Child behavior problems were assessed using parent report on the Child-Behavior Checklist (CBCL; Achenbach and Rescorla, 2000). The CBCL was self-administered in paper/pencil format or on a computer, unless literacy was an issue, in which case an interview was conducted. The CBCL is a 99-item parent-report measure of children's behavioral symptoms. Although the identical items were used by both studies, in SEED, parents were asked to report on their child's behavior over the past two weeks, whereas in CPP-Health, they reported on the past month. Standardized t-scores were used in all analyses.

2.2.2. Hair cortisol concentration

Hair was cut using thinning shears at the posterior vertex of the scalp as close to the scalp as possible. The hair samples were taped with painter's tape to aluminum foil for protection and storage and mailed to the Behavioral Immunology and Endocrinology Lab at the Anschutz Medical Campus – University of Colorado at Denver for analyses. Hair cortisol concentration (HCC) was assessed using the procedures described in detail elsewhere (Hoffman et al., 2017) and only briefly summarized here. The 3 cm proximal from scalp section of each hair sample was weighed, washed three times in 100% isopropanol alcohol, and re-weighed on a high sensitivity electronic balance. Following freezing, the hair was ground for 4–5 min using a ball mill (Retsch, Haan, Germany) with one 3/16-in. stainless steel ball bearing, and the powdered hair was extracted in HPLC grade methanol overnight while gently shaking. The hair was pelleted by spinning the cryovial for three minutes in a centrifuge at 1700 g and the supernatant was removed and dried under a stream of nitrogen. The extracts were reconstituted with assay diluent based on hair weight (3–43 mg). Steroid levels were determined using a commercial high sensitivity EIA kit (Salimetrics LLC, State College, PA, USA) per manufacturer's protocol. Results are reported as pg/mg, which corrects for the weight of hair extracted. All baseline and follow-up samples within individuals were plated together and assayed. Due to study timing, hair was assayed in two batches such that the majority of the CPP group (n = 40) was assayed in one batch, and the SEED (n = 45) and remaining 3 CPP individual's samples were assayed in a second batch. A pooled control of previously ground hair was extracted as above and included on each EIA plate in duplicate for determination of inter-assay coefficients of variation. Inter-assay coefficient of variation (CV) for the low control hair pool for cortisol for the two batches were 11.9% and 7.0%, and intra-assay CVs were 2.2% and 2%, respectively. All analyses controlled for study sample, and the primary focus on within-group change-over-time scores (which were run simultaneously) should limit influence of batch effects on cortisol levels. As noted earlier, one sample resulted in a biologically implausible value and thus was not included in the final sample of N = 88.

2.2.3. Covariates

In addition to maternal-reported demographic characteristics (i.e., race/ethnicity, income, education, sex), children's body mass index (BMI) was considered as a potential covariate. Child BMI was assessed at baseline by a trained research assistant. Weight was measured using SECA scale model 876 and height was measured using a Charder HM200P stadiometer. Measurements were repeated twice and a third measurement was taken if the first two were incongruent (e.g. >0.5 cm or 0.5 kg difference); the resulting measures for weight and height were each averaged, and CDC 2000 growth charts were used to calculate age and sex adjusted BMI (kg/m^2) (Flegal and Cole, 2013).

2.3. Statistical analyses

Due to the modest sample size and skewed distribution of HCC values, we applied a rank-based normalization using Blom's formula (1958) to cortisol data before all statistical tests. Rank-based normalization was chosen because it offers an excellent alternative to logarithmic or square-root transformations that are not always capable of normalizing certain distributions.[1] Following assessment of descriptives and zero-order correlations, associations between HCC and child behavior problems were examined using multiple regression. First, focusing on the full sample of children, we examined the association between baseline HCC and baseline behavior problems as well as the association between baseline HCC/change in HCC and change in behavior problems. These models adjusted for demographic covariates, including child ethnicity, sex, and maternal education, as well as child age and duration between samples due to variability in sample collection and timing across cohorts. Second, conducting a subgroup sensitivity analysis, we examined HCC, behavior problems and associations between HCC and behavior problems stratified by CPP and community group.

3. Results

3.1. Demographic and covariate descriptives stratified by group

Demographic characteristics and descriptives of primary variables of interest are included in Table 1 by group, to facilitate comparability. The two groups did not differ significantly in terms of distribution of child sex, annual family income, maternal age, or child BMI. The CPP group was significantly older than the community group on average; however, this was expected given a planned longitudinal assessment design for the SEED study, in which children were assessed a priori at age 3 years. In addition, maternal education was significantly lower in the CPP group relative to the community group, and mothers in the community group were more likely to report being married or living with a partner. Children in the CPP group were more likely to be Hispanic.

3.2. Bivariate correlations among study variables

Bivariate associations between demographic characteristics, baseline and follow-up HCC and mother-reported child behavior problems for the combined sample are provided in Table 2. As noted above, all bivariate and multivariate analyses were based on Blom-transformed HCC values, therefore interpretations of level and simple-difference-score change are normalized and relative only to the sample distribution. Greater HCC rank at baseline was correlated with a greater decreased HCC over time; greater behavior problems at baseline were associated with a greater decrease in behavioral problems across the year. Although age was not associated with HCC, we included it as a covariate in longitudinal models because hair cortisol has been shown to decrease across early childhood (Anand et al., 2020; Karlén et al., 2013). Time duration between assessments was also not associated with HCC rank change, but time was positively correlated with CBCL change. Female children had significantly lower HCC at baseline, whereas a higher BMI at baseline was associated with lower HCC at baseline. Hispanic children and children whose mothers had less education exhibited greater mother-reported child behavioral problems at baseline. Age was positively associated with behavior problems at baseline only.

3.3. Cross-sectional and longitudinal covariate-adjusted associations between HCC and behavior problems in the combined sample

First, we examined whether there was an association between HCC and maternal report of behavior problems at baseline in the full sample, when statistically adjusting for group, child ethnicity, maternal education, duration between assessments (see Model 1 in Table 3). There was no cross-sectional association between HCC and problems at baseline in the combined sample. Next, we examined associations between HCC at baseline and changes in problems across the year (see Model 2 in Table 3). HCC at baseline did not predict changes in behavior problems in the combined sample. Finally, we examined the association between change in HCC and change in behavior problems over the year in the combined sample (see Table 3, Model 3). We found a significant negative association, controlling for group, demographics, and duration between assessments: on average, as HCC increased across the year, behavior problems decreased in the sample containing both groups of children (Fig. 2). Adding an interaction term between HCC change and group did not reveal significant group differences in this negative association (n.s., $p = 0.57$). Post-hoc follow-up analyses revealed that the direction and significance of the association between HCC change and symptom change was similar for both internalizing ($b = -3.5$, $p = 0.01$) and externalizing ($b = -2.5$, $p = 0.07$) type symptoms.

3.4. Subgroup analyses of HCC and behavior problems at baseline and over time

HCC at baseline and HCC difference scores across time, by group, are also reported in Table 1. To allow for comparison to other published studies, we report the raw, log10 transformed, and Blom-transformed HCC values in Table 1; however, analyses and figures are based on Blom-transformed values only. Although, baseline HCC rank did not differ between the two groups, Blom-transformed rank change in cortisol from baseline to one year later was significantly different across the two groups ($p = 0.043$): in the community group, HCC rank values decreased on average, whereas the CPP group demonstrated no significant change in rank cortisol. Fig. 1 displays average baseline level and change in HCC by group (top panel) and within-individual baseline level and change (lower panel) plotted across child's age and by group. Also shown in Table 1, at baseline, the CPP group had significantly higher mother-reported behavior problems on average than the community group of children, and as expected, the CPP group exhibited a significant decrease in behavior problems across time, whereas there was relatively no change on average in behavior problems in the community group.

Finally, we report associations between HCC at baseline, baseline behavior problems, and HCC and behavior problem change by group in Table 3. When stratified by group, there were no statistically significant cross-sectional or longitudinal associations between HCC and mother-reported behavior problems. Fig. 2 illustrates the change-with-change associations in the full sample and by group, highlighting similar patterns of association despite the notably larger decrease in symptoms seen for children who received CPP.

4. Discussion

Hair cortisol is increasingly used as a measure of hypothalamic-pituitary-adrenal (HPA) axis activity over a prolonged period of time and a biomarker of exposure to adversity or presence of psychopathology. However, very little is known about the association between hair cortisol concentrations (HCC) and behavior problems in early childhood, particularly among children from low socioeconomic backgrounds (Bates et al., 2017). Leveraging a prospective, quasi-experimental design, the current study evaluated baseline levels and longitudinal changes in HCC and associations between HCC and behavioral problems in a highly stressed, low-income sample of young children that was comprised of two subgroups: traumatized children

[1] The complex positive and negative feedback loops in biological systems can result in more extreme statistical outliers that are nonetheless biologically plausible values: transformations such as Blom are particularly useful in this case, as they essentially maintain the *rank order* of those extreme values within the distribution, but, by normalizing the transformed variable, they prevent biases in the statistical estimates or coefficients. Effect sizes, such as the standardized regression coefficients will then also better reflect the effect size when equally weighted across the participants, rather than allowing certain participants to exert stronger influence, which then tends to result in overfitting and poorer generalization.

Table 2
Correlations for primary variables and covariates in full sample of children.

Variable	H.	F.	Inc.	Edu.	BMI	Age	HCC bl	HCC chg	CBCL bl	CBCL chg
Hispanic										
Female	-0.13									
Family Income	-0.34*	0.14								
Maternal Education	0.41*	-0.07	-0.24*							
BMI	0.19	-0.26*	-0.09	0.08						
Age	-0.03	0.01	0.07	0.20	-0.08					
HCC bl	-0.05	-0.29*	-0.20	-0.02	0.24*	-0.15				
HCC change	0.14	-0.03	-0.01	0.13	0.00	0.14	-0.5*			
CBCL T bl	0.23*	-0.15	-0.12	0.45*	0.04	0.31*	0.02	0.12		
CBCL T change	-0.30*	0.04	0.12	-0.37*	0.10	-0.10	0.10	-0.31*	-0.62*	
Time between assessments	-0.03	-0.02	-0.18	-0.06	0.00	-0.42*	0.07	-0.21	-0.34*	0.25*

*$p < 0.05$.
Note: Maternal education was coded as more than high school (0) or less than or equal to 12th grade (1). HCC = Blom-transformed hair cortisol concentration; bl = Baseline.

Table 3
Simple and covariate-adjusted regressions with unstandardized coefficients (standard error) predicting behavior change CBCL total problems, by group and across total combined sample.

	Model 1: baseline CBCL			Model 2: change CBCL			Model 3: change CBCL		
	Both	CPP	Comm.	Both	CPP	Comm.	Both	CPP	Comm.
Baseline HCC	0.70 (1.25)	0.29 (1.63)	1.83 (1.77)	1.03 (1.45)	2.46 (2.29)	-0.33 (1.56)			
Change HCC							-2.98* (1.36)	-4.51 (2.33)	-1.95 (1.47)
Group	15.79** (3.05)			-16.87** (4.57)			-16.97** (4.42)		
Hispanic	0.45 (2.69)			-2.52 (3.18)			-2.09 (3.10)		
Female	-1.84 (2.45)			-1.28 (2.92)			-1.91 (2.69)		
Maternal Education	4.88^{+} (2.81)			-3.12 (3.30)			-2.60 (3.21)		
Child Age	-0.58 (1.38)			3.17^{+} (1.78)			2.97^{+} (1.71)		
Time between assessments				-3.07 (4.23)			-4.35 (4.12)		
N	83	43	40	76	38	38	76	38	38
R2	0.46	0.00	0.03	0.34	0.03	0.00	0.38	0.09	0.05

*$p < 0.05$; **$p < 0.01$; $^{+}p < 0.1$.
Note: HCC = Blom-transformed hair cortisol concentration; CPP = Child-Parent Psychotherapy.

participating in one year of empirically-validated Child-Parent Psychotherapy (CPP) and a demographically-similar community sample also followed for one year. To our knowledge, this is the first study to examine longitudinal changes in HCC relative to changes in behavior problems over a one-year period in early childhood. In addition, it is the first investigation to explore whether HCC levels or associations between HCC and behavior problems differ between a treatment-involved and community-based group of very young children.

4.1. Level and change of HCC in two groups of children

As evidenced by a recent review of hair cortisol is young children (Bryson et al., 2021), a growing number of studies are examining HCC in early life, but few have focused specifically on those from low-income families, and the majority of studies have been cross-sectional (Bates et al., 2017; Kao et al., 2018; Ling et al., 2020; Simmons et al., 2019; Ursache et al., 2017). Although difficult to compare HCC levels across labs due to differing procedures and assay methods (Stalder et al., 2017), it is worth noting that the mean level of HCC at baseline for both groups of children in the current study (M_{Int} = 104 pg/mg and M_{comp} = 276 pg/mg) appeared to be higher than averages published in many other studies of preschool-aged children that analyzed HCC using immunoassays (between 19 and 55 pg/mg; Kao et al., 2018; Lehto et al., 2018; Ling et al., 2020; Vaghri et al., 2013). The literature on HCC in highly stressed, low-income children, like the sample in the current study, suggests that there may be a positive association between social adversity and HCC in early life, with 34 of 142 (24%) studies reviewed by Bryson et al. (2021) showing higher levels of HCC in the context of social adversity. That said, the variability in average HCC levels across studies of young children is remarkable, ranging from 1 pg/mg to 10, 000 pg/mg (Bryson et al., 2021); as such, future research should be undertaken to evaluate what might constitute "normal" levels versus relatively high or low levels of HCC.

Despite some demographic differences between the two groups as well as differences in level of behavioral problems at baseline, HCC levels in the two groups did not differ significantly at baseline. Given the small sample size, we are careful not to over-interpret this null result. There were, however, differences in HCC change in the two groups of children. The community sample of children showed a rank decrease in cortisol across one year, which is consistent with other studies that have shown that HCC values decrease with age throughout early childhood (Anand et al., 2020; Karlén et al., 2013). In contrast, the CPP group showed no change, on average. We are aware of only one other study that has examined treatment-related changes in HCC (Dajani et al., 2018), and while the group-based intervention in that study did affect HCC in participating youth, the effects varied depending on the particular HCC profile youth exhibited across an 8-week period (hypo-, hyper-, or moderate secretion). It may be that with a larger sample of

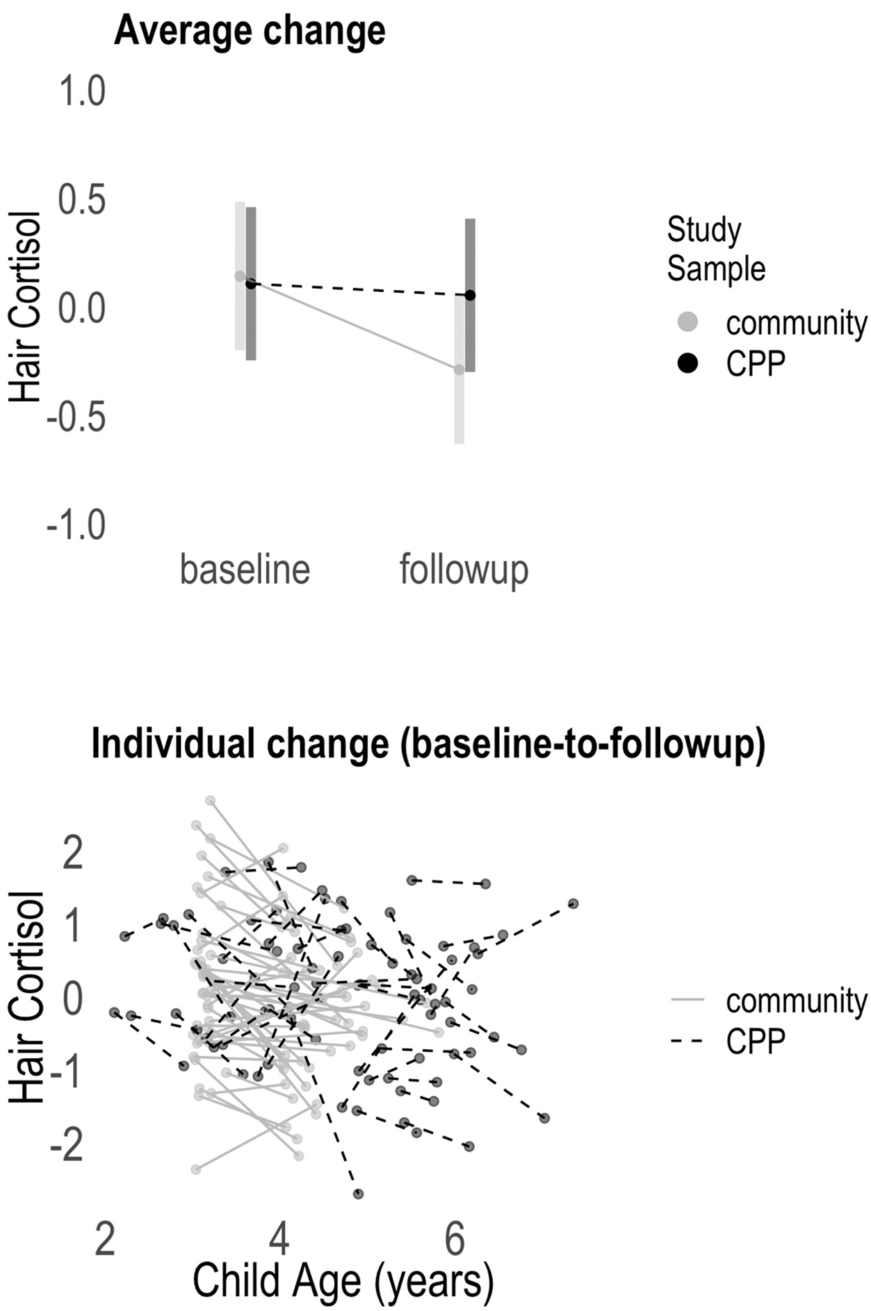

Fig. 1. Hair cortisol concentration values at baseline and follow-up by group and within individual. Hair Cortisol = Blom transformation of hair cortisol concentration (pg/mg). CPP = Child-Parent Psychotherapy. Upper panel presents group-average estimates of baseline and slope between baseline and follow-up, adjusted for child chronological age and duration between hair samples. Lower panel presents unadjusted, within-individual hair cortisol level and change data, graphed at child's age during sample collection; one line is drawn from baseline-to-follow-up timepoint for each participant.

treatment-involved children, with sufficient power to ascertain unique longitudinal profiles, intervention effects would be observed but only among those with a particular profile of HCC change.

4.2. Relations between HCC and behavior problems

Interpretation of the implications of particular HCC levels can be gleaned from examining associations between HCC, HCC change, and other indicators of adaptation – such as mental health symptoms. Relatively fewer studies of HCC and behavior problems in child populations have been conducted and findings have been inconsistent, such that direction of HCC and psychopathology associations differ depending on the behavior problem assessed, age, maternal history of adversity, or maternal psychopathology (Bates et al., 2017; Fuchs et al., 2018; Gray et al., 2018). In the current study, HCC at baseline was not associated with concurrent behavior problems. This contrasts with previous studies that found lower HCC in 4–5-year-old boys was associated with greater symptoms of attention-deficit-hyperactivity disorder concurrently and several years later at age 8 (Pauli-Pott et al., 2019). However, that study did not examine changes in HCC and findings did not hold for any other behavioral outcome examined (i.e., depression, anxiety, oppositionality). If the present null finding is replicated in larger samples of young children, it may be that HCC is less informative as a correlate of behavioral problems if examined cross-sectionally. Future research is warranted given that researchers, clinicians, and policy-makers are interested in employing hair cortisol as a biomarker of risk for current problems in pre-school aged children (for example, see Bhushan et al., 2020; Bush and Aschbacher, 2020; Center on the Developing Child at Harvard University, 2019).

Findings from longitudinal comparisons of HCC and symptom change were more compelling. HCC at baseline did not prospectively predict changes in behavior problems over time. Instead, increases in HCC across one year were significantly associated with reductions in behavior problems across both groups of children, even after adjusting

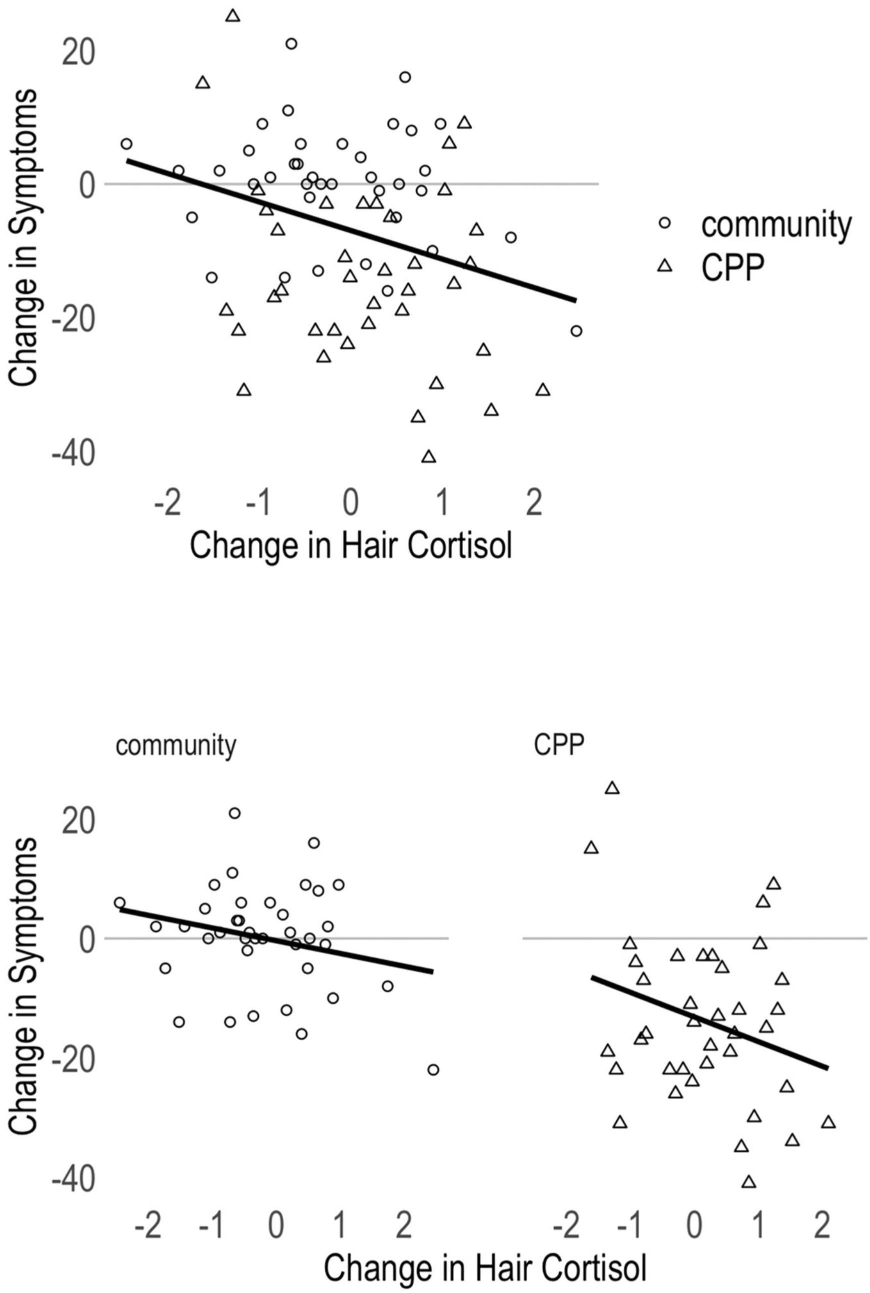

Fig. 2. Associations between change in hair cortisol and change in symptoms for all children (top panel) and by study group (bottom panel). Illustration of the associations found in Model 3, stratified by study group. Change in Symptoms = simple difference change in CBCL Total Problems T score, Follow-up minus Baseline. Change in Hair Cortisol = Blom transformation of the simple difference change in hair cortisol concentration (pg/mg), Follow-up minus Baseline. CPP = Child-Parent Psychotherapy.

for important covariates including child sex, ethnicity, age, maternal education, duration between the baseline and follow-up assessment, and study group. Specifically, for every one standard deviation increase in HCC rank, child behavior problems decreased 2.98 t-score units (roughly 1/3 of a standard deviation in CBCL t-score, based on both the population norming sample and the study sample distributions seen here), and an exploratory post hoc analysis suggested that this association was the same across both internalizing and externalizing behavior problems. Thus, in two samples of young children from highly stressed low-income families, one with clinically significant behavioral problems on average at baseline and the other without this level of impairment, we found rises in HCC across one-year could predict meaningful improvements in behavioral problems. While the direction of the association may seem counter-intuitive given the traditional interpretation of higher cortisol indicating greater risk, the pattern is consistent with previous work showing that lower HCC may confer greater risk for behavioral problems (Pauli-Pott et al., 2019; Schloß et al., 2018), particularly in children from highly stressed families (Fuchs et al., 2018).

Given the plasticity and variability of behavior in early childhood, a longitudinal assessment of biological and behavioral changes is arguably more informative than a cross-sectional design. Cortisol plays a role in a number of critical bodily processes in both resting and stressful contexts including but not limited to metabolism, memory consolidation, immunity, and pain thresholds (Staufenbiel et al., 2013). As such, higher levels of HCC might be indicative of engagement in effective coping processes that rely on memory and attention (De Kloet et al., 2005) and that are likely to support behavioral and emotional regulation, particularly in the context of high adversity. The association between increasing levels of HCC and decreasing behavior problems is consistent with the Cognitive and Activation Theory of Stress (Ursin and Eriksen, 2004), whereby positive behavioral functioning, such as coping efforts, are expected to be associated with greater secretion of cortisol. The present finding that increased HCC was associated with decreases in behavior problems is consistent with recent research showing a strong positive association between HCC and coping in high-risk, low-income populations of mothers and children (e.g., Ling et al., 2020).

In contrast to expectations, the association between change in HCC and change in behavioral problems was not stronger in the CPP group relative to community children, despite the fact that children in the CPP group demonstrated clinically and statistically significant reductions in behavior problems following one year of participation in treatment. Given that maternal nurturing and emotion coaching have been associated with lower levels of HCC in low-income early school-aged children (Simmons et al., 2019), one might expect that a therapeutic intervention aimed at supporting the parent-child relationship would affect neurobiology as well. A more recent study found that HCC level was only an indicator of concurrent emotional problems in traumatized young children if mothers' HCC or maternal emotional problems were also taken into account (Lembcke et al., 2020). If intervention effects on biological functioning are similarly dependent upon another factor in the present study, such as the nature of the HCC trajectory or parental psychopathology, a larger sample size of children is needed to investigate these possible moderators.

4.3. Limitations

The study findings need to be considered in the context of study limitations. First, the sample size was relatively small, albeit similar to other studies of HCC in low-income children (e.g., Lembcke et al., 2020; Ling et al., 2020; Simmons et al., 2019), and it is the only known study of repeated measures of HCC and behavior problems in young children to examine change. There is some indication that HCC may be related to symptomatology in youth in a non-linear fashion (Ford et al., 2019), and the current study did not have sufficient power to test this possibility. However, the longitudinal HCC data within two understudied, difficult to recruit and retain populations experiencing significant adversity is noteworthy. In addition to the size of the sample, it is important to highlight that the two groups of children were not matched a priori on demographic factors known to be associated with cortisol levels, and there were some study design differences. For example, exclusion criteria for the community group did not include whether children had chronic medical conditions and these children were born to mothers who had a BMI over 24 during pregnancy, whereas maternal BMI during pregnancy was not known for children in the CPP group. However, baseline HCC was not different on average across the two groups, suggesting limited impact of this study design, and key covariates were included in final models, increasing confidence that sampling differences were accounted for. It is important to note that families of children in both groups were low-income and, in line with the demographics of the metro area, very few of the children were African-American: as such, findings from the current study may not generalize to more economically diverse populations, or samples with different distributions of individuals from other racial categories. Finally, child behavior problems were assessed using mother report. This is a common limitation in studies of mental health in young children owing to the inability of children to report on their own behavior and a lack of validated observational measures of behavior problems in very young children.

5. Conclusion

The current study expands the limited body of research on changes in HCC over time during early childhood by examining HCC over a one-year period and evaluating cross-sectional and longitudinal associations between HCC and behavioral problems in a sample consisting of children who received a trauma-intervention and a community-based sample of young children. The lack of cross-sectional associations between HCC and behavioral problems for either group of children point to the possibility that, at least in isolation or without considering moderators, concurrent hair cortisol may not be an optimal indicator of young children's current behavioral and emotional problems, although replication of this null result is needed. As clinicians and scientists enhance their pursuit of biomarkers of child health problems (see Bush and Aschbacher, 2020), such findings provide important data for consideration, at least in terms of behavioral outcomes. The longitudinal finding that increases in HCC over time were associated with decreases in behavior problems in all children suggest that HCC change may serve as a useful biomarker of symptom improvement, particularly among high-risk children. Importantly, this evidence for pairing between cortisol and symptoms was consistent across both subgroups of children, suggesting its utility in both contexts. The notable variability in HCC level and change for all children highlight the need to carefully consider heterogeneity of 'normative' values and of within-individual change (Parent et al., 2019) as well as individual- and family-level factors that contribute to variability and associations with functioning (Boyce et al., 2021). In sum, these findings add much-needed longitudinal data to a nascent literature on HCC as a biomarker of stress and functioning in low-income young children exposed to high levels of stress (Bates et al., 2017) and offer information on the potential for HCC to serve as a useful biomarker of symptoms and symptom change in clinical and non-clinical child populations.

CRediT authorship contribution statement

MJH contributed to data collection, data analysis and interpretation of results, and wrote the manuscript. NRB designed the research study, oversaw the research, analyses and interpretation of results, and contributed to manuscript writing. AFL and EE co-designed the research with NRB and contributed to manuscript writing. AFL oversaw the intervention delivered to the intervention group. MC provided statistical expertise, analyzed the data, and assisted with the writing and presentation of results. LR performed the research and contributed to the manuscript. KJM contributed to the interpretation of results and writing of the manuscript. ML was involved in the original biomarker planning, oversaw hair sample collection and hormonal assays, and contributed to interpretation of the results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Achenbach, T.M., Rescorla, L.A., 2000. Manual for the ASEBA preschool forms and profiles, 30. University of Vermont, Research Center for Children, Youth, & Families.

Anand, K.J.S., Rovnaghi, C.R., Rigdon, J., Qin, F., Murphy, L.E., Barr, D.A., Gotlib, I.H., Tylavsky, F.A., Unit, S., 2020. Demographic and psychosocial factors associated with hair cortisol concentrations in preschool children. Pediatr. Res. 87, 1119–1127. https://doi.org/10.1038/s41390-019-0691-2.

Bates, R., Salsberry, P., Ford, J., 2017. Measuring stress in young children using hair cortisol: the state of the science. Biol. Res. Nurs. 19, 499–510. https://doi.org/10.1177/1099800417711583.

Bhushan, D., Kotz, K., McCall, J., Wirtz, S., Gilgoff, R., Dube, S., Powers, C., Olson-Morgan, J., Galest, M., Patterson, K., Harris, L., Mills, A., Bethell, C., Burke Harris, N., 2020. Roadmap for Resilience: The California Surgeon General's Report on Adverse Childhood Experiences, Toxic Stress, and Health. ⟨https://doi.org/10.48019/PEAM8812⟩.

Boyce, W.T., Levitt, P., Martinez, F.D., McEwen, B.S., Shonkoff, J.P., 2021. Genes, environments, and time: the biology of adversity and resilience. Pediatrics 147, e20201651. https://doi.org/10.1542/peds.2020-1651.

Bryson, H.E., Price, A.M., Goldfeld, S., Mensah, F., 2021. Associations between social adversity and young children's hair cortisol: a systematic review. Psychoneuroendocrinology 127. https://doi.org/10.1016/j.psyneuen.2021.105176.

Bush, N.R., Aschbacher, K., 2020. Immune biomarkers of early-life adversity and exposure to stress and violence—searching outside the streetlight. JAMA Pediatr. 174, 17–19. https://doi.org/10.1001/jamapediatrics.2019.3882.

Center on the Developing Child at Harvard University, 2019. The JPB Research Network on Toxic Stress (WWW Document).

Bush, N.R., Jones-Mason, K., Coccia, M., Caron, Z., Alkon, A., Thomas, M., et al., 2017. Effects of pre- and postnatal maternal stress on infant temperament and autonomic nervous system reactivity and regulation in a diverse, low-income population. Development and Psychopathology 29, 1553–1571.

Cicchetti, D., Rogosch, F., Toth, S., 2006. Fostering secure attachment in infants in maltreating families through preventive interventions. Development and Psychopathology 18, 623–650.

Dajani, R., Hadfield, K., van Uum, S., Greff, M., Panter-Brick, C., 2018. Hair cortisol concentrations in war-affected adolescents: a prospective intervention trial. Psychoneuroendocrinology 89, 138–146. https://doi.org/10.1016/j.psyneuen.2017.12.012.

De Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475. https://doi.org/10.1038/nrn1683.

Flegal, K.M., Cole, T.J., 2013. Construction of LMS parameters for the Centers for Disease Control and Prevention 2000 growth charts. Nat Health Stat Report 63, 1–3.

Ford, J.L., Boch, S.J., Browning, C.R., 2019. Hair cortisol and depressive symptoms in youth: an investigation of curvilinear relationships. Psychoneuroendocrinology 109, 104376. https://doi.org/10.1016/j.psyneuen.2019.104376.

Fuchs, A., Jaite, C., Neukel, C., Dittrich, K., Bertsch, K., Kluczniok, D., Möhler, E., Attar, C.H., Brunner, R., Bödeker, K., Resch, F., Bermpohl, F., 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.

Golub, Y., Kuitunen-Paul, S., Panaseth, K., Stonawski, V., Frey, S., Steigleder, R., Grimm, J., Goecke, T.W., Fasching, P.A., Beckmann, M.W., Kornhuber, J., Kratz, O., Heinrich, H., Moll, G.H., Eichler, A., 2019. Salivary and hair cortisol as biomarkers of emotional and behavioral symptoms in 6–9 year old children. Physiol. Behav. 209, 112584 https://doi.org/10.1016/j.physbeh.2019.112584.

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.

Hoffman, M.C., D'Anna-Hernandez, K., Benitez, P., Ross, R.G., Laudenslager, M.L., 2017. Cortisol during human fetal life: characterization of a method for processing small quantities of newborn hair from 26 to 42 weeks gestation. Dev. Psychobiol. 59, 123–127. https://doi.org/10.1002/dev.21433.

Kao, K., Doan St., S.N., 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, 28–35. https://doi.org/10.1080/10253890.2017.1391210.

Karlén, J., Frostell, A., Theodorsson, E., Faresjö, T., Ludvigsson, J., 2013. Maternal influence on child hpa axis: a prospective study of cortisol levels in hair. Pediatrics 132. https://doi.org/10.1542/peds.2013-1178.

Lehto, E., Ray, C., Vepsalainen, H., Korkalo, L., Lehto, R., Kaukonen, R., Suhonen, E., Nislin, M., Nissinen, K., Skaffari, E., Koivusilta, L., Sajaniemi, N., Erkkola, M., Roos, E., 2018. Increased Health and Wellbeing in Preschools (DAGIS) Study: differences in children's energy-balance-related behaviors (EBRBs) and in long-term stress by parental educational level. Int. J. Environ. Res. Public Health 15, 1–17. https://doi.org/10.3390/ijerph15102313.

Lembcke, H., Buchmüller, T., Leyendecker, B., 2020. Refugee mother-child dyads' hair cortisol, post-traumatic stress, and affectionate parenting. Psychoneuroendocrinology 111, 1–11. https://doi.org/10.1016/j.psyneuen.2019.104470.

Lieberman, A.F., Van Horn, P., Ghosh Ippen, C., 2005. Toward evidence-based treatment: Child-Parent Psychotherapy with preschoolers exposed to marital violence. Journal of the American Academy of Child and Adolescent Psychiatry 44 (12), 1241–1248.

Lieberman, A.F., Weston, D., Pawl, J.H., 1991. Preventive intervention and outcome with anxiously attached dyads. Child Development 62, 199–209.

Ling, J., Xu, D., Robbins, L.B., Meyer, J.S., 2020. Does hair cortisol really reflect perceived stress? Findings from low-income mother-preschooler dyads. Psychoneuroendocrinology 111, 104478. https://doi.org/10.1016/j.psyneuen.2019.104478.

Luecken, L.J., Hagan, M.J., Sandler, I.N., Tein, J.Y., Ayers, T.S., Wolchik, S.A., 2014. Longitudinal mediators of prevention program effects on cortisol activity among youth from parentally-bereaved families. Prevention Science.

Pan, X., Wang, Z., Wu, X., Wen, S.W., Liu, A., 2018. Salivary cortisol in post-traumatic stress disorder: a systematic review and meta-analysis. BMC Psychiatry 18, 1–10. https://doi.org/10.1186/s12888-018-1910-9.

Parent, S., Lupien, S., Herba, C.M., Dupéré, V., Gunnar, M.R., Séguin, J.R., 2019. Children's cortisol response to the transition from preschool to formal schooling: a review. Psychoneuroendocrinology 99, 196–205. https://doi.org/10.1016/j.psyneuen.2018.09.013.

Pauli-Pott, U., Schloß, S., Skoluda, N., Nater, U.M., Becker, K., 2019. Low hair cortisol concentration predicts the development of attention deficit hyperactivity disorder. Psychoneuroendocrinology 110, 104442. https://doi.org/10.1016/j.psyneuen.2019.104442.

Schloß, S., Ruhl, I., Müller, V., Becker, K., Skoluda, N., Nater, U.M., Pauli-Pott, U., 2018. Low hair cortisol concentration and emerging attention-deficit/hyperactivity symptoms in preschool age. Dev. Psychobiol. 60, 722–729. https://doi.org/10.1002/dev.21627.

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 Health 35, 104–111. https://doi.org/10.1002/smi.2842.

Slopen, N., McLaughlin, K.A., Shonkoff, J.P., 2014. Interventions to improve cortisol regulation in children: a systematic review. Pediatrics 133, 312–326. https://doi.org/10.1542/peds.2013-1632.

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., Kirschbaum, C., Miller, R., 2017. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology 77, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017.

Staufenbiel, S.M., Penninx, B.W.J.H., Spijker, A.T., Elzinga, B.M., van Rossum, E.F.C., 2013. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 38, 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015.

Toth, S.L., Maughan, A., Manly, J.T., Spagnola, M., Cicchetti, D., 2002. The relative efficacy of two interventions in altering maltreated preschool children's representational models: Implications for attachment theory. Development and Psychopathology 14, 877–908.

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.

Ursin, H., Eriksen, H.R., 2004. The cognitive activation theory of stress. Psychoneuroendocrinology 29, 567–592. https://doi.org/10.1016/S0306-4530(03)00091-X.

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, 331–340. https://doi.org/10.1016/j.psyneuen.2012.06.009.

Wichmann, S., Kirschbaum, C., Böhme, C., Petrowski, K., 2017. Cortisol stress response in post-traumatic stress disorder, panic disorder, and major depressive disorder patients. Psychoneuroendocrinology 83, 135–141. https://doi.org/10.1016/J.PSYNEUEN.2017.06.005.

Zantvoord, J.B., Ensink, J.B.M., op den Kelder, R., Wessel, A.M.A., Lok, A., Lindauer, R.J.L., 2019. Pretreatment cortisol predicts trauma-focused psychotherapy response in youth with (partial) posttraumatic stress disorder. Psychoneuroendocrinology 109, 104380. https://doi.org/10.1016/j.psyneuen.2019.104380.