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Hair cortisol and dehydroepiandrosterone concentrations: Associations with executive function in early childhood

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ABSTRACT

Chronic stress during childhood negatively impacts cognition and physical and mental health. Exposure to stressors over time can cause hypothalamic-pituitary-adrenal (HPA) axis dysregulation, leading to abnormal stress hormone levels, which can be reflected in hair cortisol concentration (HCC) and hair dehydroepiandrosterone (DHEA) concentration. Although the use of HCC and DHEA to measure chronic stress in children is increasing, their effects on cognition (as indexed by executive function) remain unexplored. Accordingly, we aimed to investigate the associations of HCC, DHEA, and their ratio with measures of executive function (cognitive flexibility and working memory) in a sample of kindergarten children (N=100). We found that the expected negative association between HCC and working memory approached significance, and DHEA was significantly and positively related to cognitive flexibility. We discuss possible interpretations of our findings. Our results suggest promising areas for future investigation and encourage further exploration into HCC and DHEA as measures of chronic stress.

1. Introduction

The deleterious impacts of chronic stress on children are a prime focus of many research and intervention efforts. Research on chronic stress has centered on the concepts of *allostasis,* the process of regulating stressors to maintain homeostasis, and *allostatic load*, an index of the physiological consequences of chronic or repeated stressors (McEwen & Stellar, 1993). One of the primary stress responsive systems is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a key neuroendocrine system involved in many functions such as regulating body temperature, the immune system, and digestion (Pariante & Lightman, 2008). Exposure to a stressor triggers HPA axis activation and the subsequent release of adrenocorticotropic hormone (ACTH). This results in a cascade of physiological responses, including the release of cortisol from the adrenal cortex, to regulate stress response and ultimately return to baseline (Smith & Vale, 2006).

Given that these hormones have been associated with chronic exposure to elevated stress and its serious consequences, there is growing interest in examining its biological embedding in order to identify those at-risk for negative outcomes and intervene early (Bush & Boyce, 2014). Accordingly, research has focused on hormonal end-products of HPA axis activity as biomarkers of allostatic load (Juster, McEwen, & Lupien, 2010), specifically hair cortisol and dehydroepiandrosterone (DHEA).

1.1. Hair cortisol

Cortisol is a glucocorticoid hormone released by the adrenal cortex as a result of HPA axis activity and provides a negative feedback loop to the HPA axis. Cortisol production increases as a result of physical or

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psychological stress, and this response is adaptive in mobilizing physiological systems to recover from or adapt to the stressor (de Kloet, 2008). However, as a result of prolonged HPA axis activation such as in a context of chronic stress, cortisol production may become permanently elevated (hypercortisolism; Stalder & Kirschbaum, 2012). Alternatively, in the context of certain types of extreme stress (e.g. child maltreatment), diminished cortisol levels (hypocortisolism) have been observed (Doom & Gunnar, 2013).

Studies that have measured cortisol to further investigate such patterns have primarily done so through collection of blood plasma, saliva, or urine (Russell, Koren, Rieder, & Van Uum, 2012). However, cortisol obtained from such methods fluctuates with circadian rhythmicity, is vulnerable to confounding by environmental factors, and only measures cortisol production from a single point in time (Meyer & Novak, 2012). Addressing this limitation, more recent work has focused on using hair cortisol concentration (HCC) as a more long-term index of HPA axis activity (Stalder et al., 2017). Cortisol is deposited into hair as it grows at a rate of approximately 1 cm per month (Wennig, 2000), though the exact mechanism of how cortisol becomes incorporated is still unclear (Meyer & Novak, 2012). With this growth rate, HCC can provide a retrospective account of cortisol levels over a certain period – for example, the most proximal 3 cm of hair would index average cortisol accumulation over the past 3 months (Russell et al., 2012). Research has shown this measure is a valid reflection of cortisol production for up to 6 months (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009; Noppe et al., 2014).

A growing number of studies have used HCC as a biomarker for stress in children, summarized in 2 systematic reviews (Bates, Salsberry, & Ford, 2017; Gray et al., 2018). The reviews focused on characterizing potential factors that were associated with hair cortisol, such as age, sex, socio-economic status (SES) and anthropometric measurements. The reviews generally showed that HCC correlated with SES, although the directionality of this relationship remains unclear (Gray et al., 2018), and that HCC correlated with poverty and other chronic stress co-factors in children (Bates et al., 2017), but that "due to the limited use of HCC in this population, much research is still needed" (Bates et al., 2017, p. 499). A better understanding of how HCC and related measures are associated with mental health, specifically their potential effects on cognitive performance, is warranted. The current study expands upon current literature to investigate the influence of HCC on EF in children to further address this gap.

1.2. DHEA

Dehydroepiandrosterone (DHEA) is a steroid hormone produced by the adrenal glands. Much research on DHEA has focused upon its implications in the aging process (Kamin & Kertes, 2017). Levels of DHEA increase with the onset of puberty (Dismukes et al., 2016), and then steadily decline with age (Hennebert, Chalbot, Alran, & Morfin, 2007). In line with this variation over the lifespan, the concentration of DHEA has also been implicated in degenerative brain diseases (Stárka, Duskova, & Hill, 2015).

DHEA is released concurrently with cortisol by adrenal glands in times of stress as a result of HPA axis activity. Complicating the interpretation of changes in DHEA as a marker for stress, DHEA has been shown to have an antagonistic effect to cortisol (Buoso et al., 2011), has neuroprotective and anti-inflammatory effects (Juster et al., 2010; Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009), and plays a key role in neurite growth and neurogenesis (Pluchino et al., 2015; Stárka et al., 2015). DHEA has also been targeted as a biomarker for neuropsychiatric diseases such as posttraumatic stress disorder (PTSD) and anxiety (Maninger et al., 2009), although its use as an indicator of allostatic load is less widespread than that of cortisol. Still, elevated DHEA levels have been observed in certain populations with exposure to chronic stress or trauma, such as individuals with PTSD (van Zuiden et al., 2017).

Although DHEA levels have primarily been investigated in blood and saliva, its measurement in hair for retrospective assessment is becoming increasingly common. Efforts to characterize potential confounds for DHEA in hair, however, still lag behind that of HCC (Gao et al., 2013). Additionally, given the role of DHEA in neural development in early life and in pubertal stages, it is unclear whether it plays as strong of a role in stress response and subsequent outcomes in early childhood as HCC. Indeed, a recent review of hair cortisol and DHEA in development highlighted "a notable lack of studies employing DHEA in young children, leav[ing] several basic questions regarding activity of the HPA axis in childhood unanswered" (Kamin & Kertes, 2017, p. 75). The current study aims to address this dearth in research by examining hair concentrations of DHEA in children and its relation to executive function (EF; see details about EF and its relation to stress in Section 1.4, below).

1.3. The HCC/DHEA ratio

Much research investigating DHEA as a proxy for chronic stress has focused on its anti-glucocorticoid effects. Cortisol's negative effects under conditions of chronic stress are well documented (Diamond et al., 2006; de Quervain, 2006), however research has found DHEA to be neuroprotective in offsetting some of these consequences (Alhaj, Massey, & McAllister-Williams, 2006; Kimonides, Spillantini, Sofroniew, Fawcett, & Herbert, 1999). In other words, it largely opposes the functions of cortisol (Kamin & Kertes, 2017) and is important for regulating glucocorticoid activity (Maninger et al., 2009). Given this, examining these two hormones as a ratio has become increasingly popular, presumably because the ratio may indicate the net effects of cortisol (Kamin & Kertes, 2017; Sollberger & Ehlert, 2016). A higher cortisol to DHEA ratio, for instance, might signal susceptibility to dysregulation of HPA axis activity. Considering the synchronous balance of cortisol and DHEA, the ratio might be a more sensitive indicator of allostatic load in representing the relative effects of the two hormones. However, there are still gaps in the literature regarding the ratio's predictive power and utility in children.

Several recent reviews have highlighted this uncertainty and called for further investigations (Gray et al., 2018; Kamin & Kertes, 2017). So far, it is unclear how the interplay of these two hormones manifests in early life and relates to other variables. Since research has suggested that the two hormones may have antagonistic effects, it is also possible that DHEA may oppose the potential negative impact of cortisol on cognitive outcomes (Kamin & Kertes, 2017). This evidence supports investigating cortisol and DHEA simultaneously as a ratio.

Measuring cortisol and DHEA in hair is therefore likely a promising method to examine the effects of chronic stress hormones on cognitive outcomes in children. The present study will examine the effects of HCC, DHEA, and their ratio on executive function in early childhood to characterize their uses as stress biomarkers at this young age.

1.4. Stress biomarkers and EF

The impact of acute stress upon EF is well documented. EF is a collection of goal-oriented processes that direct mental functions such as problem solving and decision making. EF taps into an individual's ability to inhibit irrelevant information and select options in their mind to generate a response; measures of EF are often used to assess cognitive abilities. In humans, studies employing the Trier Social Stress Test (TSST) to temporarily raise levels of cortisol in participants (Birkett, 2011) show that after being exposed to the stressor, participants experienced impairment in EF, specifically in cognitive flexibility (Alexander, Hillier, Smith, Tivarus, & Beversdorf, 2007) and working memory (WM; Quesada, Wiemers, Schoofs, & Wolf, 2012). Studies have also shown DHEA to be related to acute psychosocial stress; in response to the TSST, levels of salivary DHEA were significantly elevated (Lennartsson, Kushnir, Bergquist, & Jonsdottir, 2012). Interestingly, salivary DHEA has been shown to correlate positively with performance on EF tasks after a stressor, specifically decision making competency (Shields,

Lam, Trainor, & Yonelinas, 2016).

Further studies have suggested that the relation between EF and stress hormones can be best described with an inverted U-shaped curve (Arnsten, 2009); moderate increases in stress hormones are associated with better performance on measures of EF (Mattay et al., 2003; Qin, Hermans, van Marle, & Fernández, 2012). However, once these hormones rise above a moderate level, performance on EF measures will decrease, as the catecholamines and glucocorticoids reduce the neural activity necessary to successfully complete these tasks (Arnsten, 2015). In accordance with this research, studies on cortisol levels in young children have shown similar patterns, with moderate, acute increases in cortisol being associated with better performance on EF measures (Blair, Granger, & Razza, 2005; Blair, 2010; Davis, Bruce, & Gunnar, 2002).

Although the effects of acute cortisol increases on EF have been heavily studied, the impact of chronic elevations are not yet fully understood. Several studies have documented the negative effects of prolonged stress exposure on the brain. In rodent models, chronic stress exposure caused damage to the prelimbic and infralimbic prefrontal cortex (PFC) (Leuner, Fredericks, Nealer, & Albin-Brooks, 2014), a region heavily involved in WM and other measures of EF (Arnsten, 2009). In humans, studies have shown detrimental impacts on EF by a variety of chronic stress factors including childhood poverty (Evans & Schamberg, 2009; Noble, Norman, & Farah, 2005; Noble, McCandliss, & Farah, 2007) and childhood maltreatment (Beers & De Bellis, 2002; DePrince, Weinzierl, & Combs, 2009; Fay-Stammbach, Hawes, & Meredith, 2017).

1.4.1. HCC and executive function

Research linking HCC and EF is limited and findings are mixed. One study found that lower HCC was associated with poorer EF in older adults (Pulopulos et al., 2014), while another found no relation between HCC and EF in working-age adults (aged 21–62; McLennan, Ihle, Steudte-Schmiedgen, Kirschbaum, & Kliegel, 2016). These studies are somewhat inconsistent with prior literature linking higher (acute) cortisol increases with better EF. To our knowledge, there have been no studies examining the relation between HCC and EF in children. Examining these relations in early childhood is essential for early detection and ultimately developing targeted interventions that can mitigate the potentially long-lasting effects of chronic exposure to stress hormones upon EF.

1.4.2. DHEA and executive function

The relation between DHEA and EF has recently become a subject of interest, especially in elderly populations. Studies examining this relation have found positive associations between serum DHEA and EF in men (Hildreth et al., 2013) and both salivary and serum DHEA and EF in women (do Vale et al., 2014; Davis et al., 2008). A recent review focused on DHEA in the elderly (Maggio et al., 2015) found a positive cross-sectional association between DHEA and cognitive domains. Given the increasing interest in a potential relation between DHEA and cognition during aging in later life, it is surprising that few studies have attempted to make this link in children. Furthermore, considering the detrimental effect prolonged cortisol exposure may have on EF, it is also curious that DHEA, the potential "opposer" to these effects, has not been widely examined. One study found a positive relation between salivary DHEA and WM and found that this relation was mediated by insular-hippocampal structural covariance (Nguyen et al., 2016). However, past research on cortisol (Stalder et al., 2017) has shown that hair concentrations are not directly comparable to momentary biomarkers (blood and saliva), as hair is a measure of hormone concentration over a certain period. All previous studies measured DHEA concentration in either saliva or blood and not in hair.

1.5. The current study

In the current study, using data from healthy kindergarten children aged 5–6, we examine cross-sectional associations of hair cortisol, DHEA, and the cortisol/DHEA ratio with measures of EF as an index of cognitive outcomes. Although it is possible to measure many different hormones in hair, we chose to focus on cortisol and DHEA, in line with previous literature examining chronic stress and because of the prevalence of glucocorticoid receptors in the PFC and implications for EF development. We examine EF as an outcome given strong, prior theoretical relations with stress, as well as the relevance of EF for academic and socio-emotional outcomes in childhood (Blair & Razza, 2007; Bull, Espy, & Wiebe, 2008).

We expect the effect to be similar for cognitive flexibility and WM, given the importance of the PFC in both processes. Although research on hair biomarkers and EF is fairly limited in children, given other previous studies on the impact of cortisol on EF, we tested 2 competing hypotheses. One hypothesis is that HCC and EF will show negative and linear associations if we expect chronic elevations in cortisol to negatively impact EF. With regards to DHEA, the directionality is less clear though evidence suggests a more positive association with EF. Alternatively, if chronic cortisol elevations and EF show an inverted U-shaped curve as is observed in studies of acute stress and EF, we would expect curvilinear associations between HCC and EF. More specifically, EF performance will be poor when HCC is on either of the two extremes. Because this relation has so far been specific to EF and cortisol, we did not hypothesize an inverted U-shaped relation between EF and DHEA or EF and the HCC/DHEA ratio.

2. Methods

2.1. Participants and sample

Participants were recruited as part of a larger study run by the Precision Learning Center out of the University of California, San Francisco, which aims to address disparities in health outcomes and education. The larger study aimed to validate a dyslexia screener application known as APPRISE, by comparing results of traditional neuropsychological assessments to the results of a child's performance on the APPRISE modules. Early childhood is both a period of rapid development of EFs (Zelazo, Blair, & Willoughby, 2016) and an age at which children's stress physiology is susceptible to environmental influences (McLaughlin et al., 2015), yet has so far been overlooked by research examining hair concentrations of stress hormones.

A total of 216 kindergarteners (aged 5–6) participated in the larger project; of this sample, at the time of analysis, 122 children were consented for hair collection, and 113 hair samples were collected and analyzed. The other 9 samples were not collected because of hair length or the child's objection. The analysis sample did not differ from the overall sample in age, sex, or SES as indexed by parental education (all $ps > .05$). Of these samples, 5 were removed due to insufficient hair length for accurate analysis (< 3 cm), and 8 outliers (with cortisol values +/- 3 SD from the mean) were removed from statistical analysis, leaving a total of 100 hair samples subject to further analyses (see Fig. 1).

Although studies focused on HCC/DHEA in children have ranged widely in terms of sample size (from N=18 to 2484), the median sample size in the meta-analysis by Gray et al. (2018) is 81 with the majority of the studies (27 of 36) being N<200 and 19 of these being N<100. The analysis sample was balanced in terms of sex (52 % female). Reported racial composition was 45 % White, 42 % Asian, 7 % Multiracial, 2 % American Indian/Alaska Native, and 4 % unreported. The children were on average 5.75 years old ($M_{age\ (months)}$=69.0, SD=3.6), see Table 1. The mean amount of years of parental education was M=16.43, SD=1.85, see Table 1.

2.2. Procedures

After informed consent was collected and a verbal explanation of the procedure was given, children were assessed on all measures (see sections 2.4 and 2.5 below) by trained research assistants. Parents were sent

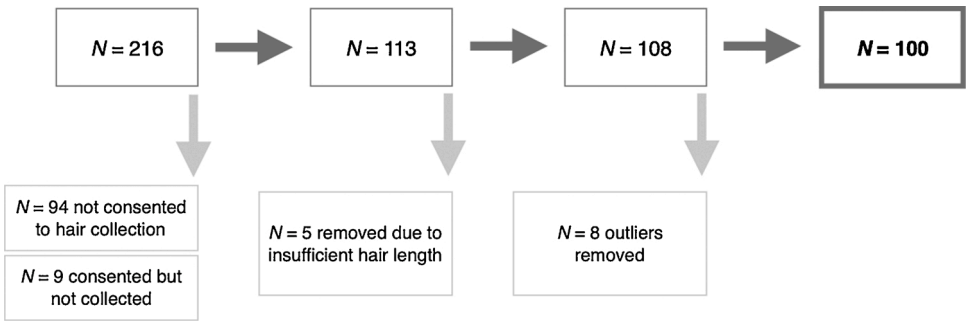

Fig. 1. Flow chart of sample inclusion and exclusion.

Table 1
Descriptive statistics of the variables of interest.

	N	Mean	SD	Min-Max	Skew	Kurtosis
1. HCC (pg/mg)*	100	0.718	.291	.18–1.7	0.84	1.06
2. DHEA (pg/mg)*	100	1.358	.235	.83–2.1	0.51	1.14
3. HCC/DHEA Ratio (pg/mg)	100	−0.641	.333	−1.2–.66	0.85	1.32
4. Age (mos)	100	69.03	3.56	62–77	0.10	−0.84
5. Gestational age at birth (wks)	91	38.81	1.84	29–44	−1.62	8.38
6. SES (parental education, yrs)	100	16.43	1.83	12–21	0.08	0.21
7. BMI	98	15.56	1.68	12–24	1.56	6.28
8. Cognitive flexibility (DCCS)	100	96.37	12.7	59–120	−0.52	0.09
9. Working memory (WMTB-C)	100	99.99	16.6	63–138	−0.16	−0.30

* log-transformed.

an online demographic questionnaire to complete. Testing was done in a quiet room at the child's school and was split into two 2-h sessions on different days for a total of four hours. The first 2-h session was dedicated to cognitive assessments. Children were given breaks in between tasks as necessary and earned small toys and stickers for their participation. Parents and schools received comprehensive reports of cognitive testing results. Height and weight measurements, and children's hair samples were collected immediately after completing cognitive assessments, in the first testing session.

2.3. Measures: demographic information

2.3.1. Age, gestational age, sex, SES

Age, gestational age, sex, and SES were collected as part of a demographic questionnaire completed by parents. SES was indexed by parental education, (the primary caregivers'), in years. Parents also reported on how frequently children washed their hair (times per week), whether their child used hair products with chemicals, and whether their child used steroids.

2.3.2. Body mass index, height, weight

Children were asked to remove their socks and shoes and any bulky clothing before height and weight were measured. Weight was measured using the Nokia Body Cardio scale. Height and weight measurements were collected 3 times to ensure reliability and these data points were then averaged. Body mass index (BMI) was calculated using the following formula: BMI = weight (kg) / height $(m)^2$.

2.4. Measures: executive function

2.4.1. Working memory test battery for children, 2nd Ed (WMTB-C-2), Block Recall

WMTB-C-2 is a standardized test battery measuring children's WM (Pickering & Gathercole, 2001). In this current study, we used the Block Recall subtest to measure participants' visuospatial WM. In the block recall task, the participant is presented a board with 9 raised wooden cubes on top. The test administrator taps a sequence of blocks and asks the child to repeat the sequence. The task begins with a single block and increases in difficulty to multi-block sequences. The test-retest reliability coefficient for children aged 5–8 is .63.

2.4.2. NIH Toolbox dimensional change card sort test (age 3–7) (DCCS)

DCCS is a measure of cognitive flexibility – the ability to shift attention between tasks. This test has been widely used to measure EF in children (Zelazo et al., 2013). During the task, participants are presented two targeted cards on an iPad, and then assessed on their ability to distinguish the dimensional differences (shape or color) by selecting a series of test cards. This test has shown high test-retest reliability (ICCs = .86–.95, Zelazo et al., 2013).

2.5. Measures: hair cortisol and DHEA

2.5.1. Scalp hair collection

Hair was collected from the posterior vertex region on the head. Each sample contained 30–50 strands of hair. Hair was collected by a trained research assistant using stylists' scissors and was cut as close to the scalp as possible (recommended by the Society of Hair Testing, 1997). Hair was not collected in cases when hair was too short or when the participant opposed collection. Previous research has shown that hair wash frequency and hair products do not affect hair cortisol analyses if proximal segments of hair are used (Flom, St John, Meyer, & Tarullo, 2017; Groeneveld et al., 2013). The hair samples were wrapped in aluminum foil and placed in individually sealed envelopes for protection and storage (Wennig, 2000).

2.5.2. Hair processing

Samples were mailed to Behavioral Immunology and Endocrinology Lab at the University of Colorado Anschutz Medical Campus. Analysis was performed on the proximal 3 cm of hair, representing the average cortisol accumulation over the previous 3 months (Russell et al., 2012). Hair sample processing was identical to the method used by Hoffman, D'Anna-Hernandez, Benitez, Ross, & Laudenslager, 2017 and D'Anna--Hernandez, Ross, Natvig, & Laudenslager, 2011. In short, hair samples were washed 3 times with 2.5 ml 100 % isopropanol, then dried. Hair samples were then submerged in liquid nitrogen for freezing before being ground for 4–5 min using a ball mill. The team then took the powdered hair (2–15 mg based on after-wash weights) and extracted it in 1000 μl high performance liquid chromatography (HPLC) grade methanol at room temperature for 24 h on a side to side shaker platform. Following this process, samples were then spun in their cryovials for 3 min in a centrifuge at 1700*g*. 133 ml of the supernatant was removed and dried with nitrogen in a drying rack. This extract was then reconstituted with 400 μl assay diluent. Results are in fluid units (micrograms/deciliter) that were converted to pg cortisol or DHEA per mg hair.

All assays were run in duplicate. Cortisol and DHEA levels were determined through an enzyme immunoassay (EIA) kit (Salimetrics), and a control of previously ground hair was extracted and processed as above. The control was included on each EIA plate in duplicate to determine the coefficients of variation (CV). For cortisol, the intra-assay CV was 1.7 %. The inter-assay CVs were 9.9 and 6.4 % for low and high controls, respectively. For DHEA, the intra- and inter-assay CVs were 1.7 % and 9 %, respectively.

2.6. Data analysis

Per participant, a separate measure of each hair biomarker (HCC, DHEA, and their ratio) in pg/mg was obtained after processing and used in further analyses. A Kolmogorov-Smirnov test indicated HCC and DHEA were not normally distributed ($p<0.001$). The skewness and kurtosis for HCC were 3.52 and 17.24, respectively, and for DHEA were 3.01 and 14.58 respectively. Therefore we log-transformed both HCC and DHEA values (Bland & Altman, 1996). After log transformation of both hormones and their ratio and removal of outliers (mean +/- 3 SD) using log transformed values, (see section 2.1 above) the skewness and kurtosis for HCC were reduced to 0.84 and 1.06, respectively. For DHEA, skewness was reduced to 0.51 and kurtosis to 1.14. For the HCC/DHEA ratio, skewness was reduced to 0.85 and kurtosis to 1.32. Although a second Kolmogorov-Smirnov test run post-transformation was significant, the skewness and kurtosis of HCC and DHEA were reduced to within acceptable limits and indicate no severe violations of normality (Kim, 2013), and regression analyses do not assume a normal distribution of predictor variables (Ernst & Albers, 2017). Therefore, log-transformed values of HCC, DHEA, and their ratio were used in all analyses conducted to test hypothesis 1. However, because of the effect of log transformation, we used untransformed values for HCC in testing for curvilinear effects in hypothesis 2.

Six individuals' demographic information was incomplete. Two participants were missing data for parental education; in these cases, they were substituted with the series mean (Rubin, Witkiewitz, Andreest., & Reilly, 2007). Four participants were missing data for race. In 2 of these cases, we were able to determine the child's race through demographic information on the child's biological parents. In the other 2 cases where information on the child's biological parents was not available, child race was coded as 'unknown.'

Data analyses were conducted to test for competing hypotheses 1 and 2. To test for hypothesis 1, we examined linear associations between hair biomarkers and measures of EF using log-transformed values for HCC, DHEA, and their ratio. To test hypothesis 2, we examined quadratic associations between hair cortisol and measures of EF using untransformed values for HCC. Multivariate regression analyses controlling for potential covariates were used (see sections 3.2 and 3.3 below). We used age-corrected standard scores for the EF assessements in all analyses. All statistical analyses were run in R (Version 3.5.1; R Core Team, 2018).

3. Results

3.1. Descriptive statistics and relations between variables of interest

The descriptive statistics of the variables of interest are reported in Table 1. Mean HCC of the sample was M=6.84 pg/mg (SD=6.94, range: 1.52–45.12; log transformed M=0.72, SD=0.29). These values are within the reference range of HCC values for children of this age (Noppe et al., 2014). Mean DHEA of the sample was M=26.87 pg/mg (range: 6.72–134; log transformed M=1.36, SD=0.24).

Bartlett Tests revealed homogeneity of variance in hair biomarkers based on race and sex. No significant group differences existed in HCC, DHEA, or their ratio based on sex or between those who reported using a hair conditioner and those who did not (both $ps>.05$). There was a significant group difference in DHEA (and therefore also the ratio) based on race as determined by one-way ANOVA ($F_{(4,95)}$=3.10, p=.012). A Tukey post-hoc test revealed this difference existed between children identified as White and those identified as Asian (p=.008) such that White children had significantly higher levels of DHEA than Asian children. Further analyses revealed one data point with a DHEA value greater than 2 standard deviations from the mean. To determine whether this data point was causing the effect, we removed it from the analysis and conducted a second one-way ANOVA. This second test showed that this difference was attenuated ($F_{(4,94)}$=2.78, p=.031) but still significant. Another Tukey test confirmed this result (p=.014), so we included this data point in all further analyses.

A one-way ANOVA also showed similar group differences in race for the HCC/DHEA ratio ($F_{(4,95)}$=3.97, p=.005). A Tukey test revealed that the difference was also between children identified as White and those identified as Asian (p=.002).

3.2. Zero-order correlations

Several factors such as age, gestational age, sex, race, SES, and BMI have been previously shown to covary with these hair biomarkers (Gray et al., 2018). Thus, in the current study we added these variables as covariates. Only two participants reported use of steroids, one participant reported use of hair color or bleach, and no participants reported use of chemical hair straighteners or having recently had a perm. Therefore, these variables were not investigated further nor included as nuisance variables. Results on zero-order correlations between hair biomarkers and other variables mentioned here are reported in Table 2.

In terms of the main variables of interest, as expected, HCC and DHEA were significantly positively correlated (r=.22, p=.031). Also as expected, measures of EF were related; cognitive flexibility and WM had a significant positive relation (r=.25, p=.012). Other significant findings include that DHEA significantly negatively correlated with SES (r=-.22, p=0.03). The HCC/DHEA ratio positively correlated with SES (r=.28, p=.005). Furthermore, DHEA was significantly positively correlated with cognitive flexibility (r=.23, p=.02), and WM was significantly

Table 2
Zero-order correlations between variables of interest.

Variables	1	2	3	4	5	6	7	8
1. HCC	–	–	–	–	–	–	–	–
2. DHEA	.22*	–	–	–	–	–	–	–
3. Ratio	.72***	−.52***	–	–	–	–	–	–
4. Age	−.11	.05	−.13	–	–	–		
5. Gestational age	.03	.03	.03	.02	–	–	–	–
6. SES	.14	−.22*	.28**	.03	.06	–	–	
7. BMI	.07	.00	.06	.17	−.06	.05	–	–
8. Cognitive flexibility (DCCS)	.06	.23*	−.11	−.07	.00	−.01	.08	–
9. Working memory (WMTB-C)	−.18	−.02	−.15	.20*	.22*	.16	.05	.25*

* $p < .05$.
** $p < .01$.
*** $p<.001$.

positively correlated with gestational age (r=.22, p=.04). Due to established relations with the variables of interest (see Gray et al., 2018) and the results of our correlations, age, race, sex, and gestational age were entered as nuisance variables in all subsequent analyses.

3.3. Associations between hair biomarkers and measures of EF

3.3.1. Linear regression models (Hypothesis 1)

Multivariate linear regression models were run to examine the relations between hair biomarkers and cognitive flexibility. To identify the model with the best fit, we used the MASS package in R. First, we created a model including all potential predictors of cognitive flexibility (HCC, DHEA, HCC/DHEA ratio, age, gestational age, sex, SES, and race) using complete observations (N=91). Next, we ran a backwards stepwise regression procedure. The model identified as the best fit as determined by Akaike's Information Criterion (AIC) is presented in Table 3 and Fig. 2. In this model, DHEA was included as a predictor. DHEA significantly and positively predicted standard scores of cognitive flexibility (β=12.123, $t_{(89)}$=2.323, p=.023). This model explained 4.66 % of the variance in cognitive flexibility (R^2=.0466, $F_{(1,89)}$=5.40, p=.022).

We followed a similar procedure to examine relations between hair biomarkers and WM. The model identified as the best fit as determined by the AIC is presented in Table 4 and Fig. 3. This model included HCC, age, gestational age, and SES as predictors. Gestational age significantly predicted standard scores of WM positively (β=1.915, $t_{(86)}$=2.089, p=.04). Furthermore, both HCC and SES showed effects approaching significance where there was a trend for HCC negatively predicting WM and SES positively predicting WM (β=-11.127, $t_{(86)}$=-1.909, p=.055, and β=1.59, $t_{(86)}$=1.74, p=.085, respectively). This model explained 10.8 % of the variance in WM (R^2=.1081, $F_{(4,86)}$=3.73, p=.008).

3.3.2. Quadratic regression models (Hypothesis 2)

To test Hypothesis 2, we first ran linear and quadratic regression models on the untransformed data to examine a potential relation between HCC and cognitive flexibility. The results of these models are reported in Table 5. To determine the significance of model predictors, we ran permutation tests (exhaustive) using the lmPerm function in R because the residuals of the models were not normally distributed. Neither the linear model nor the quadratic model was significant. Neither model resulted in a good fit (linear: R^2=-.005, $F_{(1,98)}$ =.546, p=.462; quadratic: R^2=-.015, $F_{(2,97)}$ =.271, p=.763). To compare the linear and quadratic models and determine which was a better fit for our data, we ran an ANOVA. The ANOVA revealed that there was no significant difference between the linear model and the quadratic model (p > .05).

We followed a similar procedure to examine the relation between HCC and WM. The results of the initial linear models are also reported in Table 5. The permutation tests revealed that HCC was a significant predictor in the linear model (p=.0328), but neither HCC or HCC^2 was a significant predictor in the quadratic model. The linear model was significant and explained 3.59 % of the variance (R^2=.0359, $F_{(1,98)}$=4.69, p=.033), whereas the quadratic model was not significant and explained only 2.65 % of the variance (R^2=.0265, $F_{(2,97)}$=2.35, p=.101). To determine which model was a better fit, we ran an ANOVA, which again revealed no significant differences between the linear model and the quadratic model (p > .05).

Table 3
Regression table of DHEA predicting cognitive flexibility.

	β	SE β	t	p
DHEA (pg/mg)	12.123	5.22	2.323	.023*
Constant	80.24	7.21	11.13	<.001***

**p < .01.
* p < .05.
*** p<.001.

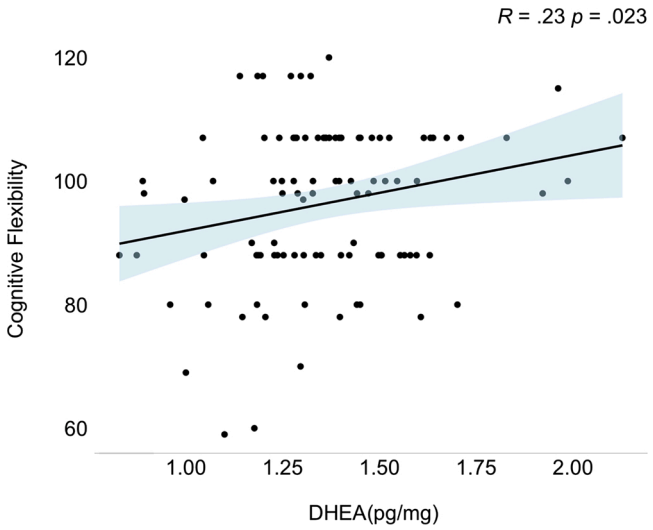

Fig. 2. Scatterplot of DHEA values predicting cognitive flexibility scores.

Table 4
Regression table of age, SES, HCC, and gestational age predicting WM.

	β	SE β	t	p
HCC (pg/mg)	−11.13	5.83	−1.909	.06
Age (wks)	.831	.470	1.77	.08
Gestational age (wks)	1.915	.917	2.089	.04*
SES	1.59	.914	1.74	.09
Constant	−49.68	49.02	−1.013	.314

**p < .01, ***p<.001.
* p < .05.

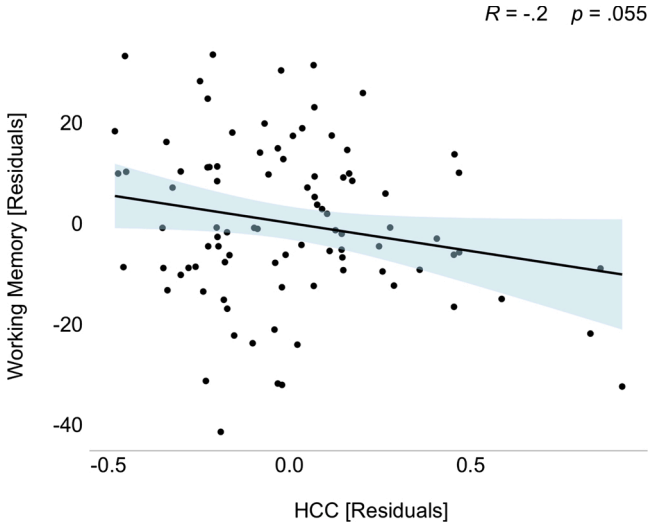

Fig. 3. Scatterplot of HCC predicting Working Memory.

Table 5
Linear and quadratic associations of HCC and EF.

	Cognitive Flexibility (DCCS)		Working Memory (WMTB-C)	
	Linear Model	Quadratic Model	Linear Model	Quadratic Model
HCC	.136 (.739)	.149 (.269)	−.511* (-2.166)	−.354 (-.501)
HCC^2		−.0003 (-.024)		−.004 (-.235)

t statistics in parentheses.
**p < .01, ***p<.001.
* p < .05.

4. Discussion

In this study, we aimed to examine the relations between hair cortisol and DHEA and childhood cognitive outcomes. We generated 2 competing hypotheses based on past research: (1) If chronic stress detrimentally affects cognitive performance, there would be significant negative correlations between stress biomarkers (particularly HCC) and measures of EF (and possibly a positive correlation between DHEA and EF); or (2) If cortisol patterns are similar between acute and chronic cortisol exposure, there would be a curvilinear association between HCC and measures of EF. The present findings provided greater support for the first hypothesis. DHEA did show significant positive correlation with cognitive flexibility, and while the results only approached significance, HCC showed a trend for a negative correlation with WM. There was no evidence supporting the second hypothesis, as no significant curvilinear associations between HCC and measures of EF were found.

4.1. DHEA and measures of EF

Our study found that DHEA predicted cognitive flexibility such that increased DHEA predicted increased cognitive flexibility scores. Further research is necessary to replicate this finding and examine the significance of this relation. Hirshman et al. (2004) have suggested that in adults, the effects of DHEA on cognition may arise from its metabolism into other hormones (estrogens and testosterone) that have differing effects on cognition; however, this theory has not yet been applied to children. Another possibility is that, in this middle to high SES sample (as evidenced by the average parental education being higher than a four-year undergraduate degree [M=16.43 years]), DHEA's effect is extended from merely protective against cortisol to beneficial for EF in general. This speculation is in line with current research investigating the effects of DHEA on cognition in aging populations (Maggio et al., 2015). Given that there have been few studies completed so far that have attempted to link EF with DHEA in children, our findings are limited in scope and exploratory in nature. However, as DHEA has been implicated in neural development during the aging process, future research should examine the effects of DHEA on EF and begin to characterize its role in early childhood and across development.

4.2. HCC and measures of EF

Based on past research characterizing the detrimental effects of both acute and chronic stress on EF, we expected to find negative correlations between stress biomarkers and measures of EF; however, our results showed no relation between hair cortisol and cognitive flexibility. Furthermore, we found that the relation between HCC and WM was negative, as expected, but was not statistically significant. Although the regression model that best fit our data did include HCC as a predictor of WM, many other covariates of hair biomarkers were also included in the model, and in fact, the only significant predictor of WM was gestational age.

There are a few possible explanations for these results. First, there is limited research on the relation between EF and HCC, and to our knowledge, our study is the first to examine this link in children. Thus, it is possible that the potential association between EF and hair concentrations of cortisol is qualitatively different from the associations between EF and other measures of cortisol (e.g. saliva and serum). HCC is a relatively new measure of chronic stress and is still not yet fully validated, therefore further studies should examine the potentially differential effects of HCC and other measures of cortisol on EF. Furthermore, our sample contains children largely from middle to high socioeconomic backgrounds. Being of a middle to high SES and benefitting from certain environmental factors such as better nutrition (Pechey & Monsivais, 2016) and a lower level of family stress may act as a protective factor against the detrimental effects of cortisol on EF. However, further research on these associations is necessary to continue to characterize these relations and the possible role of HCC in predicting EF performance.

We also hypothesized that if acute and chronic cortisol patterns are comparable, there should be curvilinear relations between HCC and measures of EF. Our results did not lend support to this hypothesis. We tested a linear model and a quadratic model for each measure of EF. Our data did not show any relation between HCC and cognitive flexibility, and the linear model for the relation between HCC and WM proved to be a better fit than the quadratic model. To the authors' knowledge, all of the research that shows a quadratic relation between stress and EF has been done with salivary and serum measures of cortisol (e.g. Arnsten, 2009). However, because HCC reflects chronic exposure, our findings are consistent with the allostatic load hypothesis, with chronic elevations of HCC showing a trend toward an association with poorer performance on EF. As this relationship was not statistically significant (p=.055), it is important for further research to continue characterizing the potential association between chronic exposure to stress hormones and EF.

4.3. HCC/DHEA ratio

We found that the HCC/DHEA ratio did not relate to the majority of the variables we examined. The only significant correlation we found was a positive association between the ratio and SES, however this result is likely due to how the ratio is calculated. As there are no other papers to our knowledge that examined the HCC/DHEA ratio in children, interpretation of this result remains problematic. Theoretically, the ratio should show the net effect of cortisol after the protective effects of DHEA, so we would expect it to negatively correlate with measures of EF. It is possible that the ratio is not as sensitive in populations of mid-high SES. However, due to the lack of relations between the ratio and the majority of the variables we examined, our results suggest that the ratio may be unsuitable for use in children. Further investigation of the ratio is necessary to determine whether it may be used reliably in children.

4.4. Limitations

It is worth noting that this study has several limitations. First, the surveys conducted did not request information about childhood trauma or family stress, and the data we collected on SES was limited to parental education. Therefore, although chronic stress responsive hormones were measured and certain demographic factors that might affect hormone concentrations were covaried in analyses, other potential determinants of hair concentrations of cortisol and DHEA in this sample remain unexamined and thus we cannot determine the relation between exposure to chronic stressors and cortisol and DHEA in this sample. It is important for future studies to further characterize potential causes of stress biomarker concentrations and ultimately connect causes of chronic stress such as poverty, adverse childhood experiences, and family stress to stress biomarkers and measures of EF. A second, related limitation is that our population was of a mid-high SES. Therefore, we cannot generalize the results from this study to low-income populations.

As noted in previous literature (Anderson, 2002), accurately measuring EF in young children can be challenging due to limitations of the assessments themselves (Anderson & Reidy, 2012). In the current study, we used WMTB-C Block Recall to measure WM. However, for this age group, the task does not progress to repeating patterns in reverse, making it difficult to gauge whether and to what degree WM is being assessed. Furthermore, many of the children in our sample did not progress to the switching phase of DCCS. Another difficulty with measuring EF in this age group concerns the distractibility and potentially shorter attention spans commonly encountered with young children (Anderson & Reidy, 2012). In the current study, EF assessments were administered at the end of the first testing block, so the possibility of participant fatigue was likely higher than if the assessments had been administered earlier in the testing battery. However, the mean

age-corrected standard score was 96.37 for DCCS and 99.99 for WMTB-C, which are within the average range.

Another limitation of this study is the nature of measuring cortisol and DHEA concentrations in hair. Hair concentrations of cortisol and DHEA are believed to represent cumulative HPA activity over long time periods, and as such, cover both basic functioning and reactivity (Russell et al., 2012).Therefore, it is difficult to disentangle the various potential influences on cortisol and DHEA concentrations found in hair samples. Finally, the study is cross-sectional, so we are unable to relate stress biomarkers to future performance on measures of EF – however, we recognize that this is an important area of research.

5. Conclusion

This exploratory study is a notable contribution to further characterizing the use of hair cortisol and DHEA as chronic stress biomarkers in children using a non-clinical sample. Covariates such as SES, age, race, sex, and gestational age should be examined in detail in future research for a better understanding of the relations we found. Our results also found that EF, and in particular cognitive flexibility, was significantly positively correlated with DHEA. Future research is necessary to explore this relation. Other areas for future research include investigating the relation between HCC, DHEA, and their ratio and health outcomes (e.g. blood pressure, heart rate), as chronic stress is known to affect health as well as EF. Finally, a better understanding of the relation between these hormones and their ratio to other potential chronic stressors (exposure to trauma, family conflict, etc.) is needed.

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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