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Hair steroid before and after COVID-19 in preschoolers: the moderation of family characteristics

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A R T I C L E I N F O

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A B S T R A C T

Background: Frequent or prolonged exposure to stressors may jeopardize young children's health. The onset of the COVID-19 pandemic, coupled with disruptions in daily routines and social isolation resulting from public health preventive measures, have raised concerns about its potential impact on children' experienced stress, particularly for young children and vulnerable families. However, whether the pandemic was accompanied by changes in physiological stress remains unknown as perceived stress is not a good proxy of physiological stress. This study examined if preschoolers showed increasing hair steroid concentrations following the onset of the COVID-19 pandemic and whether family characteristics may have exacerbated or buffered these changes.
Methods: 136 preschoolers (2–4 years) provided hair for steroid measurement (cortisol, dehydroepiandrosterone (DHEA), cortisone, cortisol-to-DHEA ratio, cortisol-to-cortisone ratio) in October-November 2019 (T0) and in July-August 2020 (T1). A 2-centimeter hair segment was analyzed, reflecting steroid production over the two months leading up to collection. Family income, conflict resolution and lack of cohesion, as well as parents' COVID-19 stress were reported by parents. Linear mixed models for repeated measures and Bayes factors were used.
Results: No significant changes were noted from before to after the onset of the COVID-19 pandemic for most hair steroids. However, a moderating role of family conflict resolution was noted. Children living with parents with a better ability to resolve conflicts had lower levels of DHEA compared to those who had more difficulty managing conflicts. Additionally, lower levels of family cohesion and income were linked to some steroids, especially DHEA, suggesting that these factors may relate to children's physiological stress. Finally, boys had higher DHEA levels than girls.
Conclusion: Our findings suggest that stress biomarkers were comparable from before to during the COVID-19 pandemic. This observation holds true despite the pandemic being perceived by many as a novel,

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unpredictable, and potentially threatening event. Findings further suggest that family characteristics are associated with hair steroid, especially DHEA, which deserves further investigation.

1. Introduction

The quick spread of a new coronavirus SARS-CoV-2 at the end of 2019 has led the World Health Organization to declare COVID-19 a pandemic disease in March of 2020. Accordingly, most countries took important public health measures, e.g., strict national confinements, travel bans, and school/childcare closures to stem the propagation of the virus and protect their populations. In addition to facing the fear of contamination, families encountered unparalleled disruptions at work and in health and education services. Changes in daily routine and work-family balance due to COVID-19 has led to novel, uncontrollable, and unpredictable situations. Furthermore, some parents perceived the pandemic and related public health measures as a threat to their physical, psychological, and economic well-being. The pandemic thus possessed the hallmarks of situations capable of inducing prolonged stress response, with potential cost for health (Koss and Gunnar, 2018; Lupien et al., 2009). A meta-analysis comprising the information from 22 countries between January to June, 2020 estimated that a third of the population (36.5 %) reported being under significant stress during the pandemic (Nochaiwong et al., 2021). Youth also suddenly lost many daily activities that provided structure and interactions with peers (Courtney et al., 2020). A recent scoping review based on articles published between December 2019 and December 2021 indicated increasing levels of perceived stress among children and adolescents in longitudinal studies including pre- and post- pandemic measures (Wolf and Schmitz, 2023). However, whether the pandemic was accompanied by changes in stress biomarkers is unclear, since measures of perceived and biological stress poorly correlate (Crosswell and Lockwood, 2020; Lupien et al., 2022).

Determining whether persistent changes in stress biomarkers, such as the glucocorticoid hormone cortisol, occurred in the first months of the COVID-19 pandemic matters, because dysregulation of stress hormones may jeopardize health and social behaviors (Shonkoff et al., 2012). It is also an experiment of nature for stress researchers. While nobody is immune to the effects of enduring stressors, young children are thought to be particularly vulnerable to it, because many brain structures targeted by cortisol are still developing (Lupien et al., 2009; Shonkoff, 2010). This could further tax the child's immature capacity to regulate their stress, emotions, and behaviors. Since 2020, we identified six studies that have investigated whether cortisol levels changed from before to after the beginning of the COVID-19 pandemic among participants under 20 years of age. While Andelic et al. (2022) relied on salivary cortisol measured among university students (N = 152, 18–20 years old) in response to arithmetic challenges before (March 2020) and six months after the lockdown, Vasquez-Salgado et al. (2022) asked two separate cohorts (N = 136) to collect saliva samples at awakening and 30 min later, on two consecutive weekdays in falls 2019 and 2020. The first study showed that participants had elevated cortisol responses to the stress (Andelic et al., 2022), which concurs with a flatter cortisol awakening response found in the second study, also suggestive of a prolonged stress exposure (Vasquez-Salgado et al., 2022). Three other studies (Fung et al., 2022; Raymond et al., 2022; Taylor et al., 2022) measured hair cortisol concentration (HCC) among children and adolescents (N_s = 49, 69, 44, respectively) aged between nine to 18 years and noted HCC increases from pre- (2019 and early 2020) to post-lockdown (May-June 2020) for the entire sample (Fung et al., 2022; Taylor et al., 2022), or only among girls (Raymond et al., 2022). To our knowledge, only one study examined this question among preschoolers (4–5 years) and found no evidence of HCC changes between 2019 and June 2020 (Hastings et al., 2021). The relatively small size (52 mother-child dyads) might explain this non-significant finding. However, the study comprised predominantly low-income families for which social and economic characteristics could be too homogeneous to uncover changes emerging as a function of these factors (Hastings et al., 2021). Recognizing the hypothesized heightened harmful effect of stress on young children and considering the limited research conducted on this population, there is a need for studies that rely on larger samples of preschoolers drawn from diverse socioeconomic backgrounds. These studies will be ideally positioned to help determine whether the initial months of the pandemic co-occurred with increasing levels of stress biomarkers.

Other biomarkers can help to examine whether the pandemic was accompanied by changes in stress systems beyond cortisol. Jia et al. (2023) have reported a 23 % average increase in adults' (mean age=49.6 years) hair cortisone concentrations during the United Kingdom's first national lockdown (Jia et al., 2023). Cortisone represents the inactive metabolite of cortisol. In a stressful context, cortisol is secreted and transformed into cortisone through the enzymatic action of 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), though it can be converted back into cortisol by 11β-HSD1 (Jia et al., 2023; Vanaelst et al., 2013). Another steroid, the dehydroepiandrosterone (DHEA) may also be of interest as it has antagonist's effects on the glucocorticoid receptor (Farooqi et al., 2018) and has been linked to perceived stress (Maninger et al., 2010). However, little is known about hair cortisone and stressful events (Davison et al., 2019) and mixed findings have been reported between prolonged exposure to stress and DHEA concentrations (Kamin and Kertes, 2017). Furthermore, these biomarkers do not work in isolation. The hormones' secretion may, for instance, shift in favor of corticosteroid pathways to the detriment of androgens, resulting in attenuated production of DHEA (Kamin and Kertes, 2017). At a physiological level, the ratios between cortisol-DHEA, and cortisol-cortisone, may better reflect their joint influence. The cortisol-to-cortisone ratio reflects the enzymatic activity of 11β-HSB (Shimanoe et al., 2021). Under acute stress, both cortisol and cortisone concentrations may increase rapidly, facilitated by the enzymatic action of 11β-HSD2. However, during chronic stress, sustained elevation of cortisol levels may overwhelm the capacity of 11β-HSD2, leading to cortisol accumulation and cortisol-to-cortisone ratio imbalance, which could be detected in hair. For its part, the cortisol-to-DHEA ratio indicates the amount of DHEA available to counteract cortisol (Farooqi et al., 2018; Kamin and Kertes, 2017). Studying steroid levels accumulated over months in hair may help to test whether the beginning of the COVID-19 pandemic and the resulting disruption to daily life were followed by increasing levels of stress biomarkers. No study has yet investigated this possibility in young children.

Moreover, it is unlikely that the negative effect of stress equally impacts all children. Children rely on their parents' care to satisfy their physical, emotional, social, and cognitive needs (Gunnar, 2020). This is particularly true for preschoolers who depend nearly exclusively on their parents and close relatives for their day-to-day interactions. Some parents may be more apt to buffer their children's stress, or to reduce their exposure to stress, thus preventing repeated or sustained secretion of stress hormones or facilitating a swift return to baseline levels. Furthermore, during prolonged home confinement, the parents' own fear of contamination or strain of juggling work and family duties during school or childcare closures may have affected families differently, and hence their capacity to buffer their children' stress (Courtney et al., 2020). Previous studies using hair cortisol have shown that parents tended to report higher levels of perceived (Brown et al., 2020; Miller et al., 2022) and physiological stress, notably linked to pandemic-related job loss and social isolation (Perry et al., 2022). That is, while higher levels of family functioning (i.e., how family members achieve common goals, help, and support one another) may protect children against pandemic disruptions, other factors such as family stress and lower family income may intensify their impacts (Wolf and Schmitz, 2023). Furthermore, parents who reported teaching their children emotional regulate and successfully maintained home routines at the onset of the pandemic reported fewer emotional and behavioral problems for their children in April 2020 (Cohodes et al., 2021). Altogether, these findings suggest that family characteristics may modify the putative impact of the COVID-19 stressors on children's steroid hormones. However, few studies have tested whether family characteristics exacerbated or buffered changes in stress hormones following the COVID-19 pandemic. Perry et al. (2022) showed that mothers' and children's (mean age = 8.9 years; n=180) HCC correlated during the COVID-19 pandemic especially for younger children. Furthermore, while Hastings et al. (2021) did not find an overall increase in HCC from prior to following the beginning of the pandemic (June 2020), they noted that more negative changes in social interactions, daily activities, routines, and psychological well-being in family predicted larger increases in preschoolers' HCC. These findings underline the importance of considering the family environment when investigating changes in stress systems ensuing from putative stressors among young children.

In this study, we used data prospectively and repeatedly collected in 2–4 years-old children several months before (October-November 2019) and after the onset of the pandemic (and childcare centers closure) (July-August 2020) to assess whether we could detect changes in hair steroid concentrations (cortisol, cortisone, DHEA, cortisol-to-DHEA ratio, cortisol-to-cortisone ratio). We further examined whether the changes in these hormones and ratios could have been affected by family functioning (i.e. conflict resolution and lack of family cohesion) as well as COVID-19 parental stress and family income. The study's original aim was to test whether interventions promoting positive relationships at home (targeting parents) or in childcare centers (targeting children through training childcare educators) reduce chronic stress. However, these interventions did not take place as planned. As such, we first examined whether the interventions induced changes to the children's hair steroids before conducting the main analyses.

2. Methods

2.1. Study setting and participants

Participants were part of the I-CARE trial, a cluster randomized controlled trial aimed to test the efficacy of a bimodal intervention targeting childcare educators and/or parents on the reduction of disruptive behaviors and stress among children from heterogeneous socioeconomic neighborhoods. Eligibility criteria were for the childcare centers (1) to be publicly funded, (2) not to have used other socioemotional training programs on a regular basis in the last 3 years and (3) including a part of the children coming from low-income families. Data collection for this trial unfolded in three waves, for which the process was repeated at each wave (i.e. selection of centers, pre- and post-intervention evaluation). The current study, corresponding to data from the first wave, took place between October 2019 and August 2020 (for most children). Of 119 childcare centers initially contacted, 26 showed interest and 22 accepted to participate, but 3 dropped out right after randomization (Fig. 1). The I-CARE trial was approved by the Research Ethics Boards of the CHU Ste-Justine Research Center and of the University of Montreal. The Clinical Trial Registry number is ISRCTN12620982.

2.2. Randomization

Randomization of childcare centers to intervention conditions was completed by the Applied Clinical Research Unit (URCA, CHU Ste-Justine Research Center, Montreal, Canada) before pre-intervention evaluation. The I-CARE trial is an open-label design, as it is not possible to blind educators and parents to intervention conditions. Childcare centers were randomly assigned to one of four conditions: 1) childcare educator training only; 2) parental training only; 3) childcare educator and parental training; 4) waitlist/control group, who received information on healthy lifestyle habits for children.

2.3. Interventions

Because interventions could not be implemented as planned, two groups were examined: *Control condition* including childcare centers

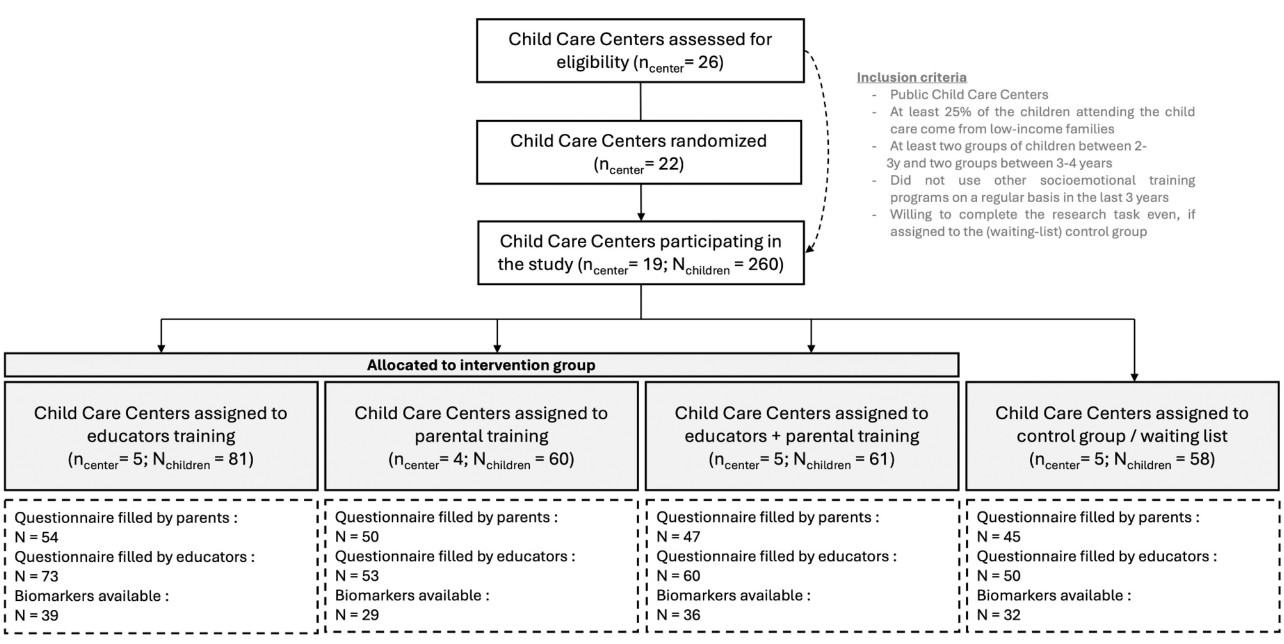

Fig. 1. Trial Flow Diagram. Notes. For biological data, number of children for whom information is available at T0 or T1.

assigned to only the childcare educator training combined with the initial control group, as children in the educators' training only condition have virtually not been exposed to the intervention (one or two sessions). *Parental training condition* which regrouped the conditions of parental training, which was also severely disrupted; about 7–8 sessions out of 14 took place with the parents before being resumed online, which may have led to substantial loss in content. Higher-than-expected attrition and absence rates were also noted.

To account for potential imbalances at baseline, despite randomization of centers, we first performed preliminary balance checks relative to the control condition. Next, we tested if the intervention impacted hair steroid concentrations using multilevel analyses. The fixed effect included intervention status. We also statistically controlled the number of sessions the parent participated (ranging from 0 to 14) and the baseline (T0) steroid levels. Given that no effect of intervention was observed for any biomarker, the status of the intervention will not be considered further in this article (see details in Supplemental Table 1 and 2).

2.4. Measures

2.4.1. Steroids measures

The collection of hair samples was planned both prior (October to November 2019, T0; i.e., at least four months before childcare closure) and at the end of the planned intervention (July to August 2020 (for 72.9 % of the children), T1; i.e., at least three months after childcare closure). A 2-cm hair segment was cut, reflecting cortisol production in the two months prior. Children with hair less than 1 cm long were excluded, whereas those between 1- and 2-cm long were included. The collection of the hair sample at T0 was performed, as planned, by trained research assistants who visited the childcare centers. The second (T1) was completed by the parents, relying on a previous study showing a strong correlation between samples collected at home and in the lab by trained research assistants ($r=0.91$, $p<0.001$; Ouellet-Morin et al., 2016). Thus, this deviation from the original protocol presumably did not affect the results of this study. However, the time (in weeks) taken to complete the sampling may have been extended for a few participants (mean (SD) = 4.64 (4.96)). We therefore sent by regular mail materials (curved scissors, hair clamps, collection cardboard, etc.) and instructions (with photos) detailing how to collect a 1 cm wide (and at least 2 cm long) sample of children's hair from the posterior vertex area of the scalp at the family's home. The participants sent the hair sample in a Ziploc bag back to our lab using a prepaid and pre-addressed envelope. Hair samples were stored at room temperature until the assay. Only the samples of children from whom hair was collected at T1 were sent out for analysis, for a total of 136 children, including 26 (19.1 %) who had missing samples at T0. Only one of the key variables used in this study significantly correlated with missing hair sample at T1: lower income families ($W = 5464$, $p=0.003$) and, at a trend level, girls ($\chi2\ (1) = 3.70$, $p=0.055$). These two potential confounders were controlled for in the main analyses (Supplemental Table 3).

The hair samples were assayed at the Biopsychological Department at the Technical University Dresden, Germany, to determine hair steroid concentration using liquid chromatography-tandem mass assay (LC-MS; (Gao et al., 2016, 2015)). The samples were washed twice with 2.5 ml of isopropanol, and the extraction process was conducted with 1.5 ml of methanol. Hair steroid concentrations were determined via a commercially available immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Germany). The intra and inter-assay variance was 12 % or less for all steroid hormones. Outliers that were beyond five standard deviations (SD) were excluded from analyses (N = from two to nine data points per biomarker). Next, to ensure that extreme values did not exert a disproportionate influence on analysis, values beyond 3 SD of the sample mean were winsorised (N = from one to seven data points per biomarker). A log10 transformation was applied to minimize skewness of the distribution. Missing data on hair steroids ranged from 8.1 % for cortisol and DHEA at T1 to 25.7 % for cortisol at T0 (after excluding outliers). A correlation matrix of hormones at T0 and T1 is presented in Supplemental Table 4.

2.4.2. Hair treatment and medication

Because uncertainty remains regarding which confounders affect hair steroid concentrations (Russell et al., 2012; Stalder et al., 2017), a wide range of information about the child's natural state of the hair (e.g., color, curvature), usual care (e.g., frequency of washing, treatments), and health (e.g., medications use, physical activity, height, weight, exposure to cigarette smoke, health problems) were reported by the parents. These variables were tested for unique association with each hormone and time point. To mitigate overfitting and preserve statistical power, we statistically controlled for the factors that were correlated to a specific hormone, at either or both time points (Supplemental Table 5 A). Cortisol-to-DHEA and cortisol-to-cortisone ratios were derived using the standardized residuals of hormones and winsorized at 3 SD of the sample mean, as described above.

2.4.3. Potential moderators

Questionnaires were sent to parents at T0 and T1 enquiring about their health, family functioning, and children's characteristics. In this study, we considered factors that can be related to or moderate hormonal changes that took place since the start of the COVID-19 pandemic. Among them, we selected family income measured at T0 and COVID-19 related stress, parental conflict resolution and family cohesion, all three measured exclusively at T1 (see items in Supplemental Table 5B).

COVID-19 related stress was based on five items (internal consistency alpha or $\alpha=0.82$) asking to what extent parents were worried about their own health or the health of their child, loved ones, and about keeping their job (or financial means), as well as their ability to stock up properly (e.g., food, hygienic products, etc.). For each item, seven response options were proposed, ranging from "*Not at all*" to "*A lot*". A score of *conflict resolution* was built using nine items from the revised conflict tactics scales (Straus et al., 1996), which primarily aims to measure individual engagement in a relationship or experience of physical or psychological violence with an intimate partner. We focused on items relating to the subscales "*negotiation*" and "*psychological aggression*" (9 items; $\alpha=0.87$) (Straus et al., 1996) with eight response options ranging from "*This has never happened*" to "*More than 20 times in the past three months*". The *lack of family cohesion* was derived from items from the McMaster Family Assessment Device that describe structural and organizational features of the family and transactions among family members (Epstein et al., 1983). We used five items of the subscale "General functioning" ($\alpha=0.71$), with response options ranging from "*Strongly agree*" to "*Strongly disagree*". The items of each family factor were separately summed up and rescaled to range from 0 to 10, with a score of 10 representing the highest level of COVID-19 related stress, lack of family cohesion, and the ability to resolve conflict.

Finally, one of the parents reported *family income* before taxes and deductions of all household members from all sources in the past 12 months, ranging from "*less than Can$5000*" to "*more than Can$190,000*".

2.4.4. Other confounders

Although sex differences in hair steroid concentration are inconsistently found in the literature (Lu et al., 2018; Rippe et al., 2016), their potential confounding effect was controlled in the analyses, along with family income, which correlated with parental conflict and family cohesion (Zhang, 2012).

2.5. Statistical analyses

2.5.1. Main analyses

We tested whether the onset of the COVID-19 pandemic was accompanied by changes in steroid hormones and whether this association was moderated by family characteristics (i.e., COVID-19 related stress, conflict resolution, lack of family cohesion, and family income) using linear mixed models for repeated measures. This method allows to model multiple data points nested within individuals to investigate between-subject differences. We first estimated a model considering the time influence on the steroids, controlling for child's age, sex, and family income. In subsequent models, we tested the main and the moderating associations (i.e., interaction term) of each family characteristic on steroid changes over time. In all models, we used full information maximum likelihood (FIML) to incorporate all available information. In this case, missing cells are not treated as a single value but as a distribution of possible values, which provides an unbiased statistic of the maximum likelihood estimate (Schminkey et al., 2016).

2.5.2. Bayes factors

We complemented our inferential approach by a Bayesian approach considering the relatively small size of our sample (Schmalz et al., 2021), which quantifies the evidence for the alternative (H1) relative to the null (H0) hypotheses. Specifically, we tested whether the evidence supporting changes in the notion that steroid levels following the beginning of the pandemic were moderated by family characteristics (H1) over the evidence supporting an absence of moderation (H0; yielding a BF10 statistic). Bayes factors greater than 1 indicate relative evidence supporting H1, whereas those smaller than 1 indicate relative evidence in favor of H0. Bayes factors ranging between 1 and 3, 3–10 or greater than 10 further suggest anecdotal, moderate, and strong evidence for H1, respectively (Aczel et al., 2017). Statistical analyses were performed using R. version 4.1.0 within R studio version 2022.02.2 and Mplus version 8.7.

3. Results

3.1. Participants characteristics

Children's descriptive statistics for the total sample are provided in Table 1. The sample included 60.3 % of boys (N=82). Children's mean age was 28.3 months and they have spent around 13.4 months in childcare at the time of the study. Families came from various socioeconomic backgrounds, as indicated by the highest level of schooling reported which ranged from primary school or less to Ph.D. completed, and family income ranging from less than 5000$ to more than 190,000$.

Table 1
Children's descriptive statistics for the total sample.

	Total sample with at least one biomarker		
	N (%)	mean (SD)	min-max
Socioeconomic variables			
Child age (months)	112 (82.4)	28.3 (5.3)	18–42
Child sex (%)			
Girl	54 (39.7)	-	-
Boy	82 (60.3)	-	
Number of months in childcare	110 (80.9)	13.4 (8.9)	0–36
Parent's education level	114 (83.8)	7.5 (2.7)	1–12
Steroid variables			
Cortisol at T0 (pg/mg)	101 (74.3)	13.6 (17.7)	0.7–97.7
DHEA at T0 (pg/mg)	104 (76.5)	7.5 (7.5)	0.3–36.9
Cortisone at T0 (pg/mg)	110 (80.9)	27 (18.8)	3.6–100.2
Cortisol at T1 (pg/mg)	125 (91.9)	18.4 (29.6)	0.7–137.7
DHEA at T1 (pg/mg)	125 (91.9)	10.6 (9.1)	0.7–43.8
Cortisone at T1 (pg/mg)	136 (100)	26.1 (24.7)	1.4–182.6
Family characteristic variables			
COVID19 pandemic stress	126 (92.6)	3.4 (2.4)	0–9
Conflict resolution	120 (88.2)	4.8 (2.3)	0.5–8.7
Lack of family cohesion	124 (91.2)	1.7 (1.5)	0–5.3
Family income	112 (82.4)	9.8 (4.7)	1–21

Notes. SD = standard deviation

3.2. Effect of time and family characteristics on steroid hormonal change

Among all steroid hormones (and ratio), only the cortisol-to-DHEA ratio significantly changed, indicating increased ratio with time (β95 %CI=0.73 [0.03; 1.43]; Table 2). Besides conflict resolution modifying the magnitude of steroid change over time in DHEA concentration (β95 %CI=-0.11 [-0.20; −0.01]), no other interaction was observed (Table 2). Fig. 2 depicts this interaction whereby no significant changes in DHEA levels were noted after the onset of the pandemic in children of parents reporting lower or average levels of conflict resolution (β=0.19, p=0.217 and β=-0.05, p=0.659, respectively). Contrastingly, children whose parents reported more abilities to deal with conflicts had a trend towards lower DHEA levels (β=-0.28, p=0.060). However, this interaction was not supported by the Bayes factors, indicating anecdotal evidence in favor of H0 (Table 3). Anecdotal-to-strong evidence in favor of H0 was noted for all other family characteristics, supporting the previous evidence for non-significant interaction effects.

We detected a few association of family characteristics with hair steroids (Table 2). Specifically, children living with parents who reported being more apt to resolve their conflicts had lower cortisol-to-cortisone ratio (β95 %CI=-0.16 [-0.28; −0.04]), whereas children living in a household with a lack of family cohesion had higher levels of DHEA (β95 %CI= 0.13 [0.03; 0.22]). Finally, children living in a higher income family had lower levels of DHEA during the reference period (β95 %CI=-0.05 [-0.08; −0.02]). Similarly, the Bayes factors generally yielded anecdotal-to-strong evidence in favor of H0, except for the association between the lack of family cohesion or family income and DHEA, as well as for the association between conflict resolution on cortisol-to-cortisone ratio, for which anecdotal-to-moderate evidence in favor of H1 was noted (Table 3). Notably, sex was associated with DHEA levels, with higher levels found for boys (β95 %CI = 0.52 [0.23; 0.80]). The findings remained largely unchanged when we statistically accounted for the variance in sampling (see Supplemental Table 6).

4. Discussion

This study tested if preschoolers had higher hair steroid concentrations following the onset of the COVID-19 pandemic and the coupled disruption to daily life. The evidence for such an association was weak; only the cortisol-to-DHEA ratio increased following the onset of the pandemic. Additionally, while parents' abilities for conflict resolution seemed to buffer changes in DHEA in the same period, the robustness of this association was not supported by the Bayes factor, and no other family characteristics mitigated (or exacerbated) the changes in the stress biomarkers. More generally, children of parents who reported more family cohesion or higher income had overall lower DHEA levels, whereas children of families with higher ability to resolve conflicts had lower cortisol-to-cortisone ratio.

The overall limited evidence for changes in hair steroids accompanying the onset of the pandemic may be, at first glance, somewhat surprising. This contrasts with the recent scoping review based on longitudinal studies, which suggested increasing levels of perceived stress among school-aged children (i.e., ≥6 years) (Wolf and Schmitz, 2023). Mixed results have nevertheless been reported regarding cortisol secretion, possibly in part due to differences in cortisol measurement. Interestingly, most studies that have reported increased HCC over time have been conducted among samples composed of children and adolescents aged between nine and 20 years (Andelic et al., 2022; Fung et al., 2022; Raymond et al., 2022; Taylor et al., 2022; Vasquez-Salgado et al., 2022). In contrast, the sole study examining the impact of the COVID-19 pandemic on children under 5 years (Hastings et al., 2021) revealed no association, similarly to our findings among preschoolers. We speculate that the distinct findings between younger vs. older children may be anchored in a developmentally normative difference in their primary sources of socialization. Younger children generally rely

Table 2
Linear mixed models testing pre- and post- pandemic changes in steroid hormones and their associations with the family characteristics.

		Cortisol (N = 125)	DHEA (N = 125)	Cortisone (N = 136)	Cortisol-to-DHEA ratio (N = 116)	Cortisol-to-cortisone ratio (N = 123)
		Estimate (95 %CI)	Estimate (95 %CI)	Estimate (95 %CI)	Estimate (95 %CI)	Estimate (95 %CI)
Model 1	Time	0.03 (-0.20; 0.26)	-0.05 (-0.26; 0.16)	-0.01 (-0.21; 0.19)	**0.73 (0.03; 1.43)***	-0.12 (-0.64; 0.40)
	Age	-0.003 (-0.04; 0.03)	-0.02 (-0.05; 0.01)	-0.01 (-0.04; 0.02)	0.04 (-0.05; 0.13)	-0.03 (-0.09; 0.02)
	Sex	-0.18 (-0.47; 0.11)	**0.52 (0.23; 0.80)****	0.04 (-0.25; 0.34)	-0.22 (-1.08; 0.64)	0.33 (-0.22; 0.87)
	Income	-0.03 (-0.06; 0.01)	**-0.05 (-0.08; −0.02)****	-0.03 (-0.07; 0.002)	-0.03 (-0.12; 0.07)	0.06 (-0.01; 0.12)
A. COVID-19 related stress						
Model 2$	Time	0.03 (-0.20; 0.27)	-0.05 (-0.26; 0.16)	-0.01 (-0.21; 0.19)	**0.74 (0.04; 1.44)***	-0.12 (-0.64; 0.41)
	Family Charact.	-0.04 (-0.11; 0.03)	0.001 (-0.07; 0.07)	-0.02 (-0.09; 0.05)	-0.13 (-0.33; 0.08)	-0.06 (-0.17; 0.06)
Model 3$	Time	0.16 (-0.25; 0.57)	0.07 (-0.31; 0.44)	0.04 (-0.31; 0.39)	1.17 (-0.12; 2.47)	-0.75 (-1.69; 0.18)
	Family Charact.	-0.02 (-0.11; 0.07)	0.02 (-0.07; 0.11)	-0.01 (-0.10; 0.07)	-0.04 (-0.33; 0.25)	-0.16 (-0.34; 0.02)
	Time x Family Charact.	-0.04 (-0.14; 0.07)	-0.03 (-0.13; 0.06)	-0.02 (-0.10; 0.07)	-0.13 (-0.47; 0.20)	0.19 (-0.04; 0.43)
B. Conflict resolution						
Model 2$	Time	0.03 (-0.20; 0.26)	-0.05 (-0.27; 0.16)	-0.01 (-0.21; 0.19)	**0.73 (0.03; 1.43)***	-0.15 (-0.66; 0.38)
	Family Charact.	-0.01 (-0.08; 0.06)	-0.06 (-0.12; 0.01)	-0.05 (-0.12; 0.02)	0.02 (-0.18; 0.23)	**-0.16 (-0.28; −0.04)****
Model 3$	Time	0.52 (-0.06; 1.09)	0.48 (-0.04; 1.00)	-0.04 (-0.51; 0.43)	0.63 (-1.39; 2.66)	-0.01 (-1.34; 1.31)
	Family Charact.	0.04 (-0.05; 0.13)	0.002 (-0.08; 0.09)	-0.05 (-0.14; 0.03)	0.01 (-0.30; 0.32)	-0.14 (-0.32; 0.04)
	Time x Family Charact.	-0.10 (-0.21; 0.01)	**-0.11 (-0.20; −0.01)***	0.01 (-0.08; 0.10)	0.02 (-0.35; 0.39)	-0.03 (-0.27, 0.22)
C. Lack of family cohesion						
Model 2$	Time	0.03 (-0.20; 0.27)	-0.05 (-0.26; 0.16)	-0.01 (-0.20; 0.19)	**0.72 (0.02; 1.42)***	-0.12 (-0.64; 0.40)
	Family Charact.	0.07 (-0.03; 0.17)	**0.13 (0.03; 0.22)****	0.07 (-0.03; 0.17)	-0.08 (-0.37; 0.22)	0.04 (-0.15; 0.23)
Model 3$	Time	`0.24 (-0.13; 0.62)	0.02 (-0.31; 0.36)	0.17 (-0.15; 0.48)	0.66 (-0.49; 1.80)	-0.47 (-1.33; 0.39)
	Family Charact.	0.14 (-0.001; 0.27)*	**0.15 (0.02; 0.28)***	0.13 (-0.004; 0.25)	-0.10 (-0.53; 0.33)	-0.07 (-0.35; 0.22)
	Time x Family Charact.	-0.12 (-0.28; 0.05)	-0.04 (-0.19; 0.11)	-0.10 (-0.23; 0.04)	0.04 (-0.48; 0.56)	0.19 (-0.19; 0.58)
D. Family income						
Model 3$	Time	0.36 (-0.20; 0.92)	-0.04 (-0.57; 0.50)	0.33 (-0.16; 0.81)	-0.21 (-1.99; 1.56)	-1.09 (-2.32; 0.13)
	Family Charact.	-0.01 (-0.05; 0.04)	**-0.05 (-0.09; −0.004)***	-0.01 (-0.06; 0.03)	-0.08 (-0.21; 0.06)	0.002 (-0.09; 0.09)
	Time x Family Charact.	-0.03 (-0.09; 0.02)	-0.002 (-0.05; 0.05)	-0.03 (-0.08; 0.01)	0.09 (-0.07; 0.26)	0.10 (-0.02; 0.21)

Notes. * = p<0.05; ** = p<0.01; *** = p<0.001; $ models additionally controlled for age, sex and income; estimates for these covariates change only slightly compared with model 1 (not shown)

Fig. 2. Moderating role of conflict resolution on DHEA concentration following the onset of COVID-19 pandemic. Notes. SD = standard deviation.

more on their parents and immediate family for interactions and upbringing, relationships that were arguably less affected by childcare and school closures. In contrast, older children and adolescents may have experienced more disruptions in their relationships, activities, and contacts with institutions (e.g., school) that may be more central to their socialization and key in providing a sense of belonging (Masten and Motti-Stefanidi, 2020). Moreover, while we did not detect a significant change in cortisol alone, we uncovered increasing cortisol-to-DHEA ratio after the COVID-19 pandemic onset, suggesting a possible excessive production of cortisol in comparison to DHEA levels. This possible imbalance between cortisol and DHEA may reflect HPA disruption associated with chronic stress (Kimonis et al., 2019). A higher cortisol-to-DHEA ratio has also been associated with childhood maltreatment (Kimonis et al., 2019; Van Voorhees et al., 2014) and anxiety and negative mood (van Niekerk et al., 2001). To our knowledge, no other studies have reported the impact of the COVID-19 pandemic on hair steroids other than cortisol and we should consider this finding as preliminary.

Akin to other stress-inducing circumstances, the advent of the COVID-19 pandemic may have induced heterogeneous stress responses among children. This may be especially the case for preschoolers who depend more on their closest relatives to protect them from stress exposure. Not only have parents experienced distinct levels of objective and subjective stressors resulting from the pandemic (e.g., fear of contamination and loss of income; (Courtney et al., 2020)), but differences may have also emerged in the family members' competences to deal with the pandemic (Courtney et al., 2020; Wolf and Schmitz, 2023). We had thus hypothesized that family characteristics (COVID-19-related stress pandemic, conflict resolution, lack of family cohesion, family income) had the potential to exacerbate (or buffer) the impact of the onset

Table 3
Bayes factors calculated for the potential association and moderating effect of family characteristics and family income on biological change over time.

	Cortisol (N = 125)			
	BF10 (m1-m2)	**Interpretation for the main effect**	**BF10** (m2-m3)	**Interpretation for the interaction**
COVID-19 related stress	0174	Moderate evidence in favor of H0	0115	Moderate evidence in favor of H0
Conflict resolution	0094	Strong evidence in favor of H0	0443	Anecdotal evidence in favor of H0
Lack of family cohesion	0236	Moderate evidence in favor of H0	0225	Moderate evidence in favor of H0
Family income	0288[ù]	Moderate evidence in favor of H0	0202	Moderate evidence in favor of H0
	DHEA (N = 125)			
	BF10 (m1-m2)		**BF10** (m2-m3)	
COVID-19 related stress	0090	Strong evidence in favor of H0	0116	Moderate evidence in favor of H0
Conflict resolution	0437	Anecdotal evidence in favor of H0	0872	Anecdotal evidence in favor of H0
Lack of family cohesion	2070	Anecdotal evidence in favor of H1	0104	Moderate evidence in favor of H0
Family income	5942[ù]	Moderate evidence in favor of H1	0090	Strong evidence in favor of H0
	Cortisone (N = 136)			
	BF10 (m1-m2)		**BF10** (m2-m3)	
COVID-19 related stress	0101	Moderate evidence in favor of H0	0090	Strong evidence in favor of H0
Conflict resolution	0243	Moderate evidence in favor of H0	0087	Strong evidence in favor of H0
Lack of family cohesion	0207	Moderate evidence in favor of H0	0218	Moderate evidence in favor of H0
Family income	0463[ù]	Anecdotal evidence in favor of H0	0262	Moderate evidence in favor of H0
	Cortisol-to-DHEA ratio (N = 116)			
	BF10 (m1-m2)		**BF10** (m2-m3)	
COVID-19 related stress	0196	Moderate evidence in favor of H0	0125	Moderate evidence in favor of H0
Conflict resolution	0095	Strong evidence in favor of H0	0093	Strong evidence in favor of H0
Lack of family cohesion	0106	Moderate evidence in favor of H0	0094	Strong evidence in favor of H0
Family income	0106[ù]	Moderate evidence in favor of H0	0173	Moderate evidence in favor of H0
	Cortisol-to-cortisone ratio (N = 123)			
	BF10 (m1-m2)		**BF10** (m2-m3)	
COVID-19 related stress	0135	Moderate evidence in favor of H0	0319	Moderate evidence in favor of H0
Conflict resolution	2572	Anecdotal evidence in favor of H1	0092	Strong evidence in favor of H0
Lack of family cohesion	0100	Moderate evidence in favor of H0	0148	Moderate evidence in favor of H0
Family income	0430[ù]	Anecdotal evidence in favor of H0	0368	Anecdotal evidence in favor of H0

Notes. m1 is adjusted for time, age, sex and income; m2 is adjusted for time, age, sex, income and family characteristic; m3 is adjusted for time, age, sex, income, family characteristic and time x family characteristic interaction. [ù]Compared to an adapted model 1, i.e. including only time, age and sex.

of the pandemic on children's hair steroids, during which most childcare closures and other confinement measures occurred. While a handful of studies have tested the association between family characteristics and HCC after the beginning of the pandemic, none have formally tested whether these characteristics modified the change in stress biomarkers over time. For example, Hastings et al. (2021) showed that, although HCC did not increase from 2019 to June 2020, preschoolers from families confronted to more COVID-19 related negative changes in the family life had higher HCC. It means that this group of children (i.e. from families experiencing more disruption) are more likely to express physiological stress than others, suggesting a moderation even though it was not formally tested (Hastings et al., 2021). The role of family characteristics in modifying the impact of the pandemic has been more widely studied regarding mental health. For example, the impact of COVID-19-related stress on mental health (anxiety, depression, behavioral and emotional control, positive affect and emotional ties) was shown to be exacerbated in couples with lower levels of family cohesion and flexibility (Hassan-Abbas, 2023). In our study, we did not detect changes in DHEA concentration following the onset of the pandemic for children of parents reporting less ability to resolve conflicts. However, a trend for decreasing DHEA levels was noted for those with more abilities for conflict resolution. These results must be interpreted with caution, as the Bayes factor suggested anecdotal evidence for a null finding in this relatively small study sample.

Beyond the changes in stress biomarkers following the onset of the pandemic, we showed that preschoolers from low-income families and from less cohesive families had higher overall DHEA levels. Living in an environment with higher family cohesion may be particularly helpful for young children who rely heavily on their parents to provide a sense of security (Gunnar, 2020), help them to cope with potential stressors, thus preventing the activation of the HPA axis or facilitating a swift return to baseline levels. In addition, children living in a family with lower income had higher DHEA levels and a trend for higher cortisol and cortisone. This finding is somewhat consistent with another study examining child and family characteristics influencing cortisol and cortisone levels in 6-year-old children, which found that lower family income was associated with higher cortisol and cortisone levels (Rippe et al., 2016). These results were interpreted as indicating elevated stress that these children experience. Our study adds to previous work highlighting how the family environment may affect children's capacity to cope with everyday life stressful events.

Notably, DHEA was the most sensitive to preschoolers' family environment, with concentrations increasing in families lacking cohesion and with lower income. Interestingly, these associations were observed in preschoolers whose DHEA concentrations are, during this developmental period, very low. As described by Sulcová et al. (1997), DHEA concentration decreases from birth until approximately the age of 5–9 years, before increasing to reach maximum levels at the age of 20 years. In young children, girls reach their minimum levels earlier than boys (Sulcová et al., 1997). Although there is no consensus in the literature on sex/gender differences in DHEA concentration between boys/men and girls/women (Chen et al., 2018; Goldman and Glei, 2007; Johannsen et al., 2018; Sulcová et al., 1997), this developmental normative change in DHEA in early childhood may explain why boys had higher levels than girls in this sample of preschoolers. More generally, our findings calls for a closer consideration of DHEA in future studies aiming to better understand how exposure to early-life stress relates to subsequent behavioral or socioemotional difficulties.

4.1. Strengths and limits

This study had many strengths, including the multi-method approach with a combination of self-reported and physiological data collected twice in a longitudinal design—in this case, before and after the beginning of the COVID-19 pandemic. The sample included children from socioeconomically diverse families, which is generally viewed as a more sensitive strategy to test the impact of early-life stress on children's hair steroid levels. Five biomarkers of stress were measured or derived rather than focusing solely on cortisol secretion. Several limitations should be acknowledged, however. First, while no other COVID-related studies have relied on samples comprising as many preschoolers, our study may have still been underpowered to detect the mitigating (or exacerbating) effects of family characteristics unfolding from before to after the onset of the pandemic. Second, many tests have been conducted, leading to the risk of false positive findings. As such, the results should be interpreted with caution. Thirdly, family characteristics were collected only after the start of the pandemic (T1 – July-August 2020), which did not allow testing pre-existing and early-onset difficulties due to the pandemic, which were shown to predict HCC in previous studies (Hastings et al., 2021). Fourthly, our results are limited to biomarkers measured in months following the onset of the pandemic. Accordingly, we cannot rule out that changes could have emerged at a later point in time (Jia et al., 2023). Finally, stress can slow down hair growth, which can modify the conversion and accumulation of cortisol and cortisone in hair. This variability in hair growth may have led to measurement errors and reduced our capacity to detect significant temporal changes in our sample. As such, it can be a factor to control in order to test more reliably the changes in hair steroids following the exposure to prolonged stress.

5. Conclusions

Our findings do not substantiate the impact of the COVID-19 pandemic on stress biomarkers measured in hair among young children. Additionally, there is no indication that family characteristics, such as difficulties in resolving conflict or stress related COVID-19, may have amplified or concealed these associations. Nevertheless, our findings encourage future studies to broaden their focus to include markers other than cortisol, such as DHEA, which can also be reliably measured in hair. This is crucial to understand the complex and multisystemic changes family stressors may induce over time.

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CRediT authorship contribution statement

Michel Boivin: Writing – review & editing. **Mara Brendgen:** Writing – review & editing. **Sonia Lupien:** Writing – review & editing. **Marie-Claude Geoffroy:** Writing – review & editing. **Sylvana Côté:** Writing – review & editing, Conceptualization. **Eloise Berger:** Writing – review & editing, Writing – original draft, Formal analysis. **Benoit Masse:** Writing – review & editing, Conceptualization. **Richard Tremblay:** Writing – review & editing, Conceptualization. **Frank Vitaro:** Writing – review & editing. **France Capuano:** Writing – review & editing, Conceptualization. **Isabelle Ouellet-Morin:** Writing – review & editing, Writing – original draft, Conceptualization. **Marie-Pier Larose:** Writing – review & editing, Conceptualization. **Marie-Josée Letarte:** Writing – review & editing, Conceptualization.

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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Ethics approval and consent to participate

Consents to participate in the study were obtained from parents and educators. The I-CARE trial was approved by the Research Ethics Boards of the CHU Ste-Justine Research Center and of University of Montreal (31/05/2019) ref: 2019–2218. (https://doi.org/10.1186/ISRCTN12620982).

Declaration of competing interest

All authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107072.

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