Excessive hair cortisol concentration as an indicator of psychological disorders in children

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ABSTRACT

Cortisol in hair is a new biomarker assessing long-term hypothalamic-pituitary-adrenal (HPA) axis activity, which is related to emotion regulation. We compare hair cortisol concentrations (HCC), in clinically referred children with disruptive mood dysregulation disorder (DMDD) (n = 19), children with other types of psychological disorders (n = 48), and healthy subjects (n = 36). We also investigate the association between HCC and irritability, age, and sex. Our results show that children with DMDD or other types of psychological disorders have higher HCC than healthy subjects, p < .001, ηp2 = .39. No difference between children with DMDD and those with other types of psychological disorders was found, p = .91, nor an association between HCC and irritability in the clinical sample, p = .32. We found a significant negative correlation between HCC and age in those with DMDD, r = −0.54, p < .05, but not in the normative sample, r = −0.20, p = .25. No differences in HCC between girls and boys were found in the normative sample, p = .49. Children in need of psychological treatment, including those with DMDD, seem to have dysregulated HPA-axis activity over time. Excessive accumulated cortisol concentrations in hair could be an indicator of a psychological disorder in children.

1. Introduction

The Research Domain Criteria initiative (NIMH, 2009) has prompted more focus on biological components of psychological disorders, including cortisol’s role in emotion regulation (Jentsch et al., 2019). Cortisol, often referred to as the stress hormone, is released through the limbic-hypothalamic-pituitary-adrenal (HPA) axis when our body experiences a demand or challenge, for example in situations requiring effortful emotion regulation (Denson et al., 2014; Jentsch et al., 2019). Increases in cortisol levels are, therefore, a natural response, with consequent normalization of these levels as one adapts to the situation or regulates one’s emotions. However, frequent and excessive cortisol secretion can lead to dysregulation of the HPA-axis. Over time, this could potentially influence the development of psychological disorders or vice versa (Kessel et al., 2021; Koss and Gunnar, 2018). The present study aims to explore the potential association between disruptive mood dysregulation disorder (DMDD) and HPA-axis dysregulation.

1.1. Disruptive mood dysregulation disorder

Emotion regulation and mood dysregulation are superordinate terms that can include any dysregulated emotion of negative or positive valence (Stringaris et al., 2018). They refer to difficulties in controlling or managing one’s emotions. If children show severe problems with emotion regulation, characterized by chronic irritability with frequent temper outbursts for at least 12 months, they may fulfill the criteria for DMDD which is a new affective disorder in the DSM-5 (APA, 2013). Irritability is defined as having a propensity for experiencing and expressing anger (Brotman et al., 2017) and is related to reactivity to stimulus (Stringaris and Taylor, 2015). Irritability is among the most
common reasons families seek child mental health services (Evans et al., 2022). Children with DMDD have high levels of psychosocial impairments (Coldevin et al., 2023; Copeland et al., 2013) and challenges in daily functioning, self-harm, and suicidal thoughts (Althoff et al., 2016). Research also reveals that these children have a higher risk for adult depression or anxiety (Copeland et al., 2014; Vidal-Ribas et al., 2016).

Recently, our research group found that children with DMDD are characterized by difficulties with emotion control and cognitive flexibility (Branden et al., 2023a,b). Regardless of diagnoses, higher levels of irritability were also associated with less emotion control and cognitive flexibility in a clinical sample. Interestingly, meta-analytic evidence suggest that stress impairs cognitive flexibility (Shields et al., 2016) which is critical to efficient emotion regulation. Furthermore, the study concluded that stress may contribute to “a reactive cognitive state” that is optimized to quickly process highly salient information. This description fits well with the reactive nature of irritable mood. Moreover, Hommer et al. (2014) found that children with severe mood dysregulation, referred to as the precursor of DMDD used in research, exhibit an attention bias towards threatening (i.e., salient) stimuli. From a clinician’s perspective, children with DMDD or highly irritable tendencies also appear stressed and emotionally reactive. Thus, although stress may influence executive functions and emotion regulation through more complex biological processes than cortisol alone (Shields et al., 2016), it may be worth testing if children with DMDD show indications of abnormal HPA-axis activity.

1.2. Hair samples to measure chronic HPA-axis function

Because of the long-term nature of the irritable mood in children with DMDD, one might expect evidence of dysregulated HPA-axis activity in measures of long-term cortisol concentrations. Compared to saliva or blood samples, which have been frequently used to test cortisol levels, hair samples are less sensitive to daily fluctuations and environmental stimuli. Hair collection is also less invasive. Thus, hair samples can effectively measure long-term cortisol levels as an index of chronic HPA-axis activity (Faresajo et al., 2020; Greff et al., 2019). The first centimeter of hair closest to the scalp reflects the accumulated hair cortisol concentrations (HCC) from the preceding month. Although hair sampling is a new method in clinical child populations, initial studies suggest effects of age and sex. One cohort study found that the cortisol values generally decreased and stabilized over time within children and that the variability in cortisol values across the children decreased with increased ages (Karlén et al., 2013). No differences between girls and boys were observed. However, a meta-analytic study, including various age samples although most were adults, has found that men have significantly higher cortisol levels than women (Stalder et al., 2017). Sex differences might also vary within clinical and healthy populations (Kudielka and Kirschbaum, 2005). Given the scarce knowledge on HCC, age, and sex, exploring these effects within new samples could be useful.

1.3. Cortisol levels and irritability

To our knowledge, no research has investigated cortisol levels in children with DMDD. However, in a population-based sample of boys, higher saliva cortisol levels were found in those with increased tendencies to overreact with anger and get angry easily when feeling teased or threatened (van Bokhoven et al., 2005). Since aberrant threat processing is observed in children with DMDD (for a review, see Brotman et al., 2017), this raises the question of whether these children could have elevated cortisol levels. Furthermore, increased saliva cortisol levels were found in angry and aggressive preschoolers (Rout et al., 1998) and young adults displaying irritability (Gerra et al., 1997). Among preschoolers born premature, saliva cortisol levels rise following stressors and correlate with emotional reactivity (Bagnier et al., 2010).

Interest has been given to the multifinality of irritability by trying to identify factors that might moderate the developmental trajectories of early irritability. One hypothesis is that patterns of cortisol secretion are one of these factors as excessive cortisol levels are associated with internalizing disorders whereas externalizing disorders are often characterized by blunted cortisol secretion (Klein et al., 2021). A recent study investigating diurnal saliva cortisol slopes and irritability in a community sample supports this hypothesis; the results show that among children with higher levels of irritability at age 3, excessive cortisol at age 9 predicted the development of more internalizing symptoms and increases in irritability at age 12, whereas blunted cortisol levels at age 9 predicted more externalizing symptoms at age 12 (Kessel et al., 2021). This aligns with evidence indicating that higher cortisol levels pose a risk for subsequent depression (for a review, see Herbert, 2013). Given that children with DMDD have an increased long-term risk for internalizing disorders (Copeland et al., 2014; Vidal-Ribas et al., 2016), it is plausible that they may exhibit excessive cortisol levels. Building on the studies described in the previous paragraph, one might also expect a correlation between higher levels of irritability and increased cortisol levels. However, the aforementioned studies did not incorporate measures of accumulated cortisol levels over time, nor did they investigate this in clinical samples where irritability has, by definition, persisted over an extended period. Consequently, there is a lack of knowledge regarding the association between chronic irritability and long-term cortisol secretion.

1.4. Cortisol levels in associated disorders

Although the present sample size is insufficient for comparing HCC in different groups of co-occurring disorders, it is noteworthy that children with DMDD exhibit high rates of comorbidity. For example, 58% and 21% of children with DMDD also have attention deficit hyperactivity disorder (ADHD) or anxiety disorders, respectively (Coldevin et al., 2023). Moreover, prior to the inclusion of DMDD in the DSM-5, children with mood dysregulation may have been mistakenly diagnosed with disruptive behavior disorders (DBD) like oppositional defiant disorder (ODD) or conduct disorder (CD). Thus, research on cortisol secretion in relation to ADHD, anxiety disorders, or DBD may be worth considering.

Through a meta-analysis Scassellati et al. (2012) identified that reduced baseline salivary cortisol levels could indicate ADHD. Similarly Bernhard et al. (2021) discovered in a systematic review that diminished cortisol response to stress was frequently observed in children and adolescents with DBD. Earlier reviews highlighted that particularly those with CD presents with stress hypo-reactivity and that CD/ODD primarily, not ADHD, is associated with cortisol hypo-reactivity (Fairchild et al., 2018). Recently, Pauli-Pott et al. (2023) investigated HCC in a clinical sample of children with ADHD, with or without CD/ODD (cf. DSM-5 criteria), compared to healthy controls. They found that girls with ADHD+ODD/CD showed higher HCC. However, no significant differences were observed after adjusting for adverse childhood experiences. Notably, no sex differences in HCC were found.

Berens et al. (2023) studied the relation of ADHD symptoms to diurnal cortisol, observing correlations between ADHD symptoms and higher cortisol, and ODD symptoms with lower cortisol secretion. No associations were found between cortisol and symptoms of CD, anxiety, or depression. In a community sample, Ursache et al. (2017) found similar results for depression and anxiety symptoms and HCC, after controlling for socioeconomic factors and parental stress. Ma et al. (2019) studied anxiety symptoms correlation with salivary cortisol in children from economically disadvantaged neighborhoods and found that elevated cortisol levels coincided with increased physiological symptoms, but long-term cortisol blunting was predicted by worry. Physiological symptoms did not predict long-term cortisol effects.

Taken together, the studies discussed here show mixed results using various approaches to investigate cortisol secretion and its association with disorders or symptoms that overlap with DMDD in both clinical and nonclinical groups. Few studies have employed HCC to examine long-term cortisol secretion. Notably, Pauli-Pott et al. (2023) investigated
HCC in a clinical sample, yet all participants had ADHD, and cortisol levels varied based on coexisting symptoms. Given the DSM-5 diagnostic criteria, children with DMDD will exhibit substantially more irritability and mood dysregulation than those with ADHD or DBD (without DMDD). Furthermore, much like children with anxiety, those with DMDD or high irritability levels are noted for a high-arousal negative affect (Cardinale et al., 2019). Given this similar physiological state (despite differences in behavioral symptoms, such as approach vs. avoidance behavior), and considering the severe and chronic symptoms experienced by those with DMDD, it could be hypothesized that these children may demonstrate chronic overproduction of cortisol.

While children with DMDD may show higher HCC compared to a non-clinical sample of children, it’s plausible that all children referred to mental health services would have endured substantial stress over recent time. Consequently, it remains to be determined, necessitating further investigation, whether children with DMDD will present with significantly higher HCC than other children in need of clinical assessment or treatment.

1.5. The present study

This study aims to compare chronic HPA-axis function among children with DMDD, children with other types of psychological disorders, and those without any psychological disorders. Additionally, it will investigate the association between long-term HPA-axis function and irritability in a clinical sample, encompassing those with DMDD and other types of psychological disorders. A secondary objective involves exploring HCC in relation to age and sex in children with DMDD and in a sample of children without psychological disorders.

Our hypothesis is that children with DMDD will demonstrate higher HCC compared to healthy controls, but not in comparison to those with other types of psychological disorders. Furthermore, we anticipate that higher levels of irritability will correlate with higher HCC.

2. Materials and methods

The participants were recruited from a Child- and Adolescent Mental Health (CAMH) clinic in Oslo during 2019–2021, to take part in a larger study of clinical characteristics and underlying mechanisms in children with severe emotional dysregulation. Both the main study and the present project were approved by the Regional Committee for Medical Research Ethics (#2017/135) and are part of the registered study protocol (NCT05049356). The study included a treatment-seeking sample, that is, these children had not received psychiatric treatment or medication at the time of inclusion and study participation. Informed oral and written consent was attained from parents. Inclusion criteria were 6 – 12 years of age and IQ ≥ 70, and for parents; language skills good enough to read and respond to questionnaire and semi-structured clinical interview.

A subsample (n = 109) from the main study was invited to provide hair samples for cortisol analyses (see Fig. 1). To evaluate potential selection biases, we compared the group of children who provided hair samples (n = 67) with those who did not (n = 36), based on several descriptive variables: age, sex, income level, living situation, and irritability.

Analyses showed differences regarding the income level only; a relatively higher percentage of the non-hair samples provides reported irritability in a clinical sample, yet all participants had ADHD, and cortisol levels varied based on coexisting symptoms. Given the DSM-5 diagnostic criteria, children with DMDD will exhibit substantially more irritability and mood dysregulation than those with ADHD or DBD (without DMDD). Furthermore, much like children with anxiety, those with DMDD or high irritability levels are noted for a high-arousal negative affect (Cardinale et al., 2019). Given this similar physiological state (despite differences in behavioral symptoms, such as approach vs. avoidance behavior), and considering the severe and chronic symptoms experienced by those with DMDD, it could be hypothesized that these children may demonstrate chronic overproduction of cortisol.

While children with DMDD may show higher HCC compared to a non-clinical sample of children, it’s plausible that all children referred to mental health services would have endured substantial stress over recent time. Consequently, it remains to be determined, necessitating further investigation, whether children with DMDD will present with significantly higher HCC than other children in need of clinical assessment or treatment.

Fig. 1. Sample recruitment and inclusion process. Note. *For example, unable to meet at the clinic due to anxiety, or their behavior during the test situation rendered it infeasible to collect hair samples. ** Two participants were not asked to provide hair samples after testing due to a lack of clarity regarding permissions concerning human distancing because of COVID-19 restrictions.

2.1. Materials

2.1.1. Psychological diagnostic testing

The Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version (K-SADS-PL-5; Kaufman et al., 2016) was used to find the psychiatric diagnoses. K-SADS-PL-5 is an internationally validated semi-structured diagnostic interview corresponding with DSM-5 diagnoses frequently applied in research and clinical practice (Kornør and Skarphedinsson, 2016). The Norwegian version of K-SADS-PL-5 was administered with parents. Reliability was established in three ways: First, interviewers were trained in the administration of K-SADS-PL-5 before they contributed to the data collection. Secondly, cases were discussed in conference with other clinicians. Thirdly, 9% (N = 19 of the 218 in NCT05049356) of the interviews were scored independently by two clinicians, demonstrating a substantial agreement between the interviewers’ diagnostic evaluations (Cohen’s k = 0.80, for DMDD specifically, 0.90).

2.1.2. Irritability

The Achenbach System of Empirically Based Assessment (ASEBA; Achenbach and Rescorla, 2001) was used to measure irritability. Parents completed the Child Behavior Check List (CBCL) with items rated on a 3-point Likert scale (not true, sometimes true, often true). The CBCL Irritability scale was used to measure irritability and consists of three items: (I) temper tantrums or hot temper, (II) stubborn, sullen, or irritable, and (III) sudden changes in mood or feelings. The CBCL Irritability scale has a range from 0 (none) to 6 (high) and its validity and reliability have been supported in previous studies (Evans et al., 2020; Tseng et al.,...
2017). In the present study, internal consistency reliability analysis indicated a high level of reliability for the CBCL Irritability, with a Cronbach’s alpha of 0.85.

2.1.3. Hair sampling and cortisol concentration analysis

Hair was cut from the vertex posterior of the scalp close to the skin by trained clinical staff in compliance with the recommendations of the Society of Hair Testing (Wennig and Kintz, 2000), and because this area has a consistent growth rate (Cooper et al., 2012; Kintz, 2017; Thomson et al., 2010). The caregivers reported whether the children used steroid products. Each hair sample had a minimum length of 3.0 cm, representing exposure to stress around 12 weeks in the past. The method applied for extraction and analysis of cortisol levels in hair was a competitive radioimmunoassay (RIA), that enables analyzing small samples (Karlen et al., 2011; Pragt and Balikova, 2006). The hair samples were cut into smaller pieces, frozen for 2 min in liquid nitrogen, and minced together with a 5 mm steel ball using a Cry mill for 10 min. Methanol (1 ml) was added to each tube and the samples were extracted overnight on a moving board. Then, 0.8 ml of the methanol supernatant was pipetted of and lyophilized using a Savant Speed Vac Plus SC210A. The lyophilized extracts of hair samples were dissolved in 300 μl, 0.1 mol/L phosphate buffer, pH 7.4, containing 0.02% bovine serum albumin and 0.01% triton X-100, and cortisol concentrations were analyzed as described by Morelius et al. (2004). Hair samples of 5 mg or more were needed to maintain a total inter-assay coefficient of variation below 8% for hair extraction and measurement of cortisol by the radioimmunoassay. The intra-assay coefficient of variation for the radioimmunoassay itself was 7% at 10 nmol/L (Karlen et al., 2011). Furthermore, the gamma radiation used for detection in the RIA-method is not influenced by colored hair which is extracted to the methanol together with the cortisol. The research protocol and all methods in the study were performed in compliance with relevant guidelines and regulations.

2.1.4. Income level and living situation

Data regarding living situation and annual income were provided by parents, aligning with the 2019 income level index from the Norwegian Central Statistical Bureau (SSB, 2019). Families with children falling below 60% of a country’s median income are considered impoverished. In 2019, a family with at least one child in Norway was designated as impoverished if the annual income was below 314,500 NOK. The term ‘living situation’ refers to the arrangement of a child’s residence, whether it be a consistent setting with both parents or caregivers, or a shared arrangement, such as alternating weeks between two different parents.

2.2. Statistical analyses

Analyses were performed using IBM SPSS Statistics Version 28.0 and R version 4.2. A p-value ≤ .05 was considered statistically significant. Effect sizes using partial eta squared with 0.01, 0.06, and 1.4, and Cohen’s d with ≥ 0.5, 0.8, and 0.8 were interpreted as small, medium, and large effects, respectively (Cohen, 1969; Richardson, 2011). Differences were analyzed using one-way analysis-of-variance (ANOVA) or independent sample t-test. The effects of sex and age on the measured variables were also examined using Kruskal-Wallis test and Pearson correlational analysis. Raw hair cortisol concentrations (pg/mg) were logarithm-transformed due to the skewed distribution. Analyses-of-covariance were conducted to examine differences between diagnostic groups with Tukey HSD for planned contrasts. Two-sided Pearson’s correlation analysis was used to test the association between cortisol concentrations and irritability in the total clinical sample including both children with DMDD and those with other psychological disorders. Supplemental analyses were done controlling for the potential effects of steroid product usage in the total sample and the presence of ADHD in the clinical samples. Furthermore, additional analyses were done to control for outliers in the analyses exploring the association between HCC, age, and sex in the normative sample. Potential outliers (based on the natural pg/mg values) were removed using a threshold based on the upper limit of 1.5 times the interquartile range above the third quartile.

3. Results

Participant characteristics are presented in Table 1, showing that 19 children fulfilled the diagnostic criteria for DMDD. No differences were found between groups in terms of sex, χ² = 3.57, p = .17, or income level, χ² = 2.65, p = .27. Although living situations differed between diagnostic groups (see Table 1), no HCC differences based on living situations were observed, t(24.64) = 0.88, p = .39. Homogeneity of variances in HCC could be assumed across groups, as indicated by Levene’s test, p = .21. Kruskal-Wallis tests revealed differences in age between groups, p < .05. Of the total clinical sample, nine children used products (i.e., salve or inhalers) that included corticosteroids. None of the children in the healthy control group used any corticosteroids.

3.1. Sex, age, and cortisol concentrations

In the clinical sample of children with DMDD, a negative correlation was found between age and HCC (r = −0.54, p < .05). A similar but weaker and non-significant trend was observed in the normative sample (r = −0.20, p = .25). After excluding outliers (n = 4) from the normative sample, similar results were obtained (r = −0.19, p = .31). When examining sex differences within the DMDD group, the mean HCC in pg/mg were M = 151, SD = 162 (MDlog = 1.91, SDlog = 0.57) for girls, and M = 193, SD = 227 (MDlog = 2.08, SDlog = 0.42) for boys. However, due to the small sample of girls (n = 5) compared to boys (n = 14) with DMDD, we did not perform a statistical comparison of their HCC. In the normative sample, there was no significant difference in HCC between girls (M = 49, SD = 135, and MDlog = 1.29, SDlog = 0.42) and boys (M = 36, SD = 37, and MDlog = 1.38, SDlog = 0.37), t(34) = 0.70, p = .49. These findings remained consistent even after removing outliers from the normative sample, t(27.53) = 0.45, p = .65.

3.2. Group differences in cortisol concentrations

Group-wise cortisol concentrations are presented in Table 2. As age varied significantly between groups, it was included as a covariate in the Table 1: Participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n = 36)</th>
<th>DMDD (n = 19)</th>
<th>Other psychological disorders (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>8.6 (1.9)</td>
<td>8.6 (1.8)</td>
<td>10.1 (1.6)</td>
</tr>
<tr>
<td>Range (n)</td>
<td>6–12</td>
<td>6–11</td>
<td>6–12</td>
</tr>
<tr>
<td>Girls (%)</td>
<td>19 (49)</td>
<td>5 (26)</td>
<td>22 (46)</td>
</tr>
<tr>
<td>Income level (NOK) N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>300,000</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt; 300,000</td>
<td>2 (11)</td>
<td>5 (10)</td>
<td></td>
</tr>
<tr>
<td>&gt; 700,000</td>
<td>14 (74)</td>
<td>39 (81)</td>
<td></td>
</tr>
<tr>
<td>Living situation (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consistent (%)</td>
<td>11 (58)</td>
<td>40 (83)</td>
<td></td>
</tr>
<tr>
<td>Shared arrangement (%)</td>
<td>8 (42)</td>
<td>7 (15)</td>
<td></td>
</tr>
<tr>
<td>Diagnoses (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression (%)</td>
<td>1 (5)</td>
<td>3 (6)</td>
<td></td>
</tr>
<tr>
<td>Anxiety (%)</td>
<td>13 (68)</td>
<td>29 (60)</td>
<td></td>
</tr>
<tr>
<td>ADHD (%)</td>
<td>14 (74)</td>
<td>19 (40)</td>
<td></td>
</tr>
<tr>
<td>CD (%)</td>
<td>2 (11)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>ODD (%)</td>
<td>n/a</td>
<td>11 (23)</td>
<td></td>
</tr>
<tr>
<td>Tic (%)</td>
<td>1 (5)</td>
<td>5 (10)</td>
<td></td>
</tr>
<tr>
<td>Trauma (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Note. NOK = Norwegian krone. n/a = DMDD excludes the possibility of having ODD cf. DSM-5. *p < .05. **p < .001.
analyses of variance. A significant difference in HCC was found between children with DMDD, healthy controls, and those with other psychological disorders, $F(2,99) = 31.8, p < .001, \eta^2_{p} = .39$. Moreover, additional analysis controlling for the use of corticosteroids also revealed similar results, $F(2,98) = 31.7, p < .001, \eta^2_{p} = .39$. Tukey HSD test showed that children with DMDD had higher HCC than the healthy controls, $p < .001$, but not compared to those with other psychological disorders, $p = .96$. Children with other psychological disorders also showed higher HCC than healthy controls, $p < .001$. Follow-up analyses of covariance demonstrated large effect sizes for the differences between DMDD and healthy controls, $d = 1.67$, and healthy controls vs. those with other psychological disorders, $d = 1.32$. The results are illustrated in Fig. 2. Nonsignificant differences in HCC between children with DMDD and those with other psychological disorders also retained after controlling for the use of corticosteroids or the presence of ADHD. Furthermore, in these analyses, corticosteroids ($\eta^2_{p} = .01, p = .38$) or ADHD ($\eta^2_{p} = .04, p = .11$) did not significantly explain variance in HCC.

### Table 2

<table>
<thead>
<tr>
<th>Group-wise hair cortisol concentrations</th>
<th>pg/mg Log-transformed</th>
<th>Log-transformed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M SD Range</td>
<td>M SD Range</td>
</tr>
<tr>
<td>Healthy</td>
<td>43 101 6.4–607.0</td>
<td>1.34 0.40 0.81–</td>
</tr>
<tr>
<td>DMDD</td>
<td>182 209 21.2–820.0</td>
<td>2.03 0.45 1.33–</td>
</tr>
<tr>
<td>Other psychological disorders</td>
<td>164 262 9.8–1610.0</td>
<td>1.93 0.48 0.99–</td>
</tr>
</tbody>
</table>

3.3. Irritability and cortisol concentrations

Irritability correlated significantly with age ($r = -0.28, p < .05$).

Irritability did not show a linear relationship with HCC in the clinical sample ($r = 0.13, p = .32$), nor when controlling for age ($r = -0.01, p = .96$).

### 4. Discussion

The purpose of the present study was to investigate if children with DMDD have a dysregulated HPA-axis functioning. The study also examined the correlation between irritability and accumulated cortisol secretion in a clinical sample, and the association of HCC with age and sex in children with DMDD and in a normative sample. It was hypothesized that relative to children with no psychological disorder, children with DMDD would show higher HCC. It was also expected that higher levels of irritability would be associated with higher levels of HCC. The results provide no evidence that DMDD or irritability is uniquely related to dysregulation of the HPA axis. Compared to healthy controls, however, children with a variety of psychological disorders, including DMDD, display higher hair cortisol. Due to the cross-sectional design of the present study, it is important to recognize that causality cannot be inferred in any of the present results. However, they may provide new perspectives which can be used in further hypothesis generation.

4.1. Long-term cortisol concentrations in DMDD and other psychological disorders

The results show that children with DMDD have excessive HCC as compared to healthy controls. This corresponds with findings of children with higher irritability, anger, or emotional dysregulation and increased cortisol levels (Bagner et al., 2010; Gerra et al., 1997; Tout et al., 1998; van Bokhoven et al., 2005). This might suggest that if a child over time experience overwhelming emotions without successful control of these, as those with DMDD, their HPA-axis response becomes dysregulated through the continued release of cortisol. Indeed, a study measuring
control-related beliefs and saliva cortisol before and after a parent-child conflict task suggests that children (7–17 years old) with increased cortisol concentrations are more likely to perceive themselves as having little personal control (Granger et al., 1994). Furthermore, because children with DMDD experience cognitive inflexibility (Brænden et al., 2023a; 2023b), and stress impairs cognitive flexibility (Shields et al., 2016), the present findings could suggest that these mechanisms are relevant for understanding DMDD. Hypercortisolism could be reported in the heightened reactivity and irritability experienced by these children. However, in the present study, excessive cortisol concentrations were not specific to DMDD and causality cannot be established. The results show that children with other psychological disorders also have excessive cortisol concentrations as compared to healthy controls and that there were no statistical differences in cortisol concentrations between children with DMDD and those with other psychological disorders. Abnormal cortisol concentrations over time could be a general indicator of psychological strain (Selye, 1976) which does not correspond to distinct diagnostic categories. The symptom expression (e.g., irritable mood, anxiety, and/or BD) could depend on the HPA-axis complex interaction with other cognitive and neurobiological systems and the environment.

Interestingly, a great amount of variability in HCC was found within each group, including in those without a psychological disorder. Regardless of whether they meet the criteria for a psychological disorder, children with long-term excessive cortisol concentrations could potentially have an increased vulnerability for developing such disorders. This is suggested by the stress-vulnerability model, originally proposed by Zubin and Spring (1977), which highlights the interaction between environmental stress and an individual’s biological vulnerability (i.e., likelihood) of developing a disorder. Compared to their peers with both a psychological disorder and high HCC, it could be that the “healthy” children with high HCC in the present sample live under circumstances better suited to their vulnerabilities (i.e., there is a more appropriate match between the child and their environment).

A recent study demonstrated that maltreatment in childhood coincided with reductions in HCC (White et al., 2017). Furthermore, HCC reduction mediated the effect of maltreatment on externalizing symptoms. In the present study, none of the children were identified as having a trauma-related disorder. Pauli-Pott et al. (2023) found that in children with ADHD, but not in healthy controls, lower HCC correlated significantly with mothers’ lower educational level and higher depressive symptoms. However, Ursache et al. (2017) found that socioeconomic disadvantages are associated with higher HCC in parents and children. In a study where our research group explored parental and child HCC, we found a strong correlation between parents and their children’s HCC (Melinder et al., 2023). As the design of this study was not causative, one cannot conclude whether it is shared environmental and/or mutually influential factors that cause this association (e.g., the child has a disorder that is highly demanding for the parent causing excessive parental cortisol concentrations), or if a dysregulated HPA-response could be an innate/inherited vulnerability. Indeed, HPA response to stress is suggested to be a product of both individual and environmental variables (Sauer et al., 2002). Presumably, prolonged cortisol secretion and psychological functioning mutually influence and reinforce each other. Furthermore, the families participating in the present study have likely reached a critical juncture with their children’s difficulties, prompting them to seek child mental health services. This situation most likely comes after enduring substantial familial stress over several weeks or even months. If such heightened stress results in an acute increase in cortisol levels, it could suggest that the HPA system is functioning appropriately. Nevertheless, prolonged elevation in hair cortisol levels detected in these children could signify that they are indeed in need of intervention and support.

4.2. Long-term cortisol concentrations and irritability

In the present clinical sample, and in contrast to this study’s hypothesis, an association between cortisol concentrations over time and irritability was not found. This contrasts with findings of a positive association between irritability and higher saliva, i.e., short-term, cortisol levels (Gerra et al., 1997; van Bokhoven et al., 2005). The caregiver’s view of the children’s irritable mood could bear too little resemblance with the children’s experienced levels of irritability and thus with their HCC. Furthermore, in the analyses concerning the association between irritability and cortisol concentrations, healthy controls were not included. This study may therefore lack the lower (normal) range of irritability, affecting the results on the cortisol-irritability association.

4.3. Long-term cortisol concentrations, age, and sex

In the clinical groups, age correlated significantly with cortisol concentrations. Specifically, lower ages were associated with higher long-term cortisol concentrations which corresponds with findings of decreased cortisol levels through development in healthy children (Karlen et al., 2013). In the healthy control group, no significant correlation was found between age and HCC. Given the excessive HCC in children with psychological disorders, it raises the question of whether hypercortisolism could indicate a delay in the development of biological processes related to HPA-axis functioning and associated systems. Regrettably, we were not able to compare HCC between girls and boys in those with DMDD. However, in the present sample of children without psychological disorders, no difference in mean HCC across boys and girls was found which corresponds with previous findings (Karlen et al., 2013). In 7-years-old born premature, boys had significantly higher HCC than girls, but there was no sex difference in HCC among those born full-term (Grunau et al., 2013). However, in a meta-analysis, which included the latter mentioned study, Stalder et al. (2017) found that men had a 21%-higher estimated HCC compared to women. Altogether, these findings may suggest that sex differences in cortisol secretion could be linked to later developmental processes (e.g., puberty) or to gender socialization, which carries different associated stressors and expectations for handling them.

4.4. Strengths and limitations

A strength of our study is that it uses a non-invasive method, i.e., hair samples, to investigate a potential biomarker of psychological disorders. Furthermore, it explores long-term cortisol levels in both clinical and healthy groups and dimensional measures of psychological symptoms. There are, however, several limitations within our study. First, our study design is not causative. Second, the clinical sample sizes are indicative of reduced power, i.e., Type-II error, in the clinical group comparison. Third, the study lacks irritability scores from the healthy control group. Further studies are needed to conclude about the relationship between cortisol and irritability. Fourth, a potential selection effect could exist, as lower-income families appear to be less likely to provide hair samples compared to those from higher-income families. Furthermore, we lack data on the reasons why some children declined to provide hair samples; the children could say no without any consequences and without needing to explain themselves. Lastly, we were not able to compare different groups of co-occurring disorders or control for adverse childhood experiences. However, none fulfilled the criteria for trauma related disorders. Despite these limitations and the fact that our results need replication, our findings offer some interesting suggestions.

4.5. Conclusion

The present study suggests that excessive cortisol concentrations accumulated in hair might serve as a potential indicator for psychological disorders in children, yet they do not provide the necessary...
specificity to distinguish between DMDD and other types of psychological disorders. Still, awareness should be raised about the potential biological manifestations or associations of children’s psychological disorders. If these findings are replicated, understanding HPA-axis activity through HCC may provide markers for mental disorders in children, their severity, and their treatment response. Future research should investigate whether psychological treatment affects HPA-axis regulation, and whether it can help not only to improve symptoms but also to reduce long-term cortisol levels. There is also a necessity to investigate the causal relationship between psychological disorders and HPA-axis activity, which might demand new or improved methodology.

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CRediT authorship contribution statement

Astrid Brænden: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. Andrea Lebena: Data curation, Methodology, Writing – review & editing. Åshild Faresjö: Writing – review & editing. Elvar Theodorsson: Project administration. Marit Coldevin: Data curation, Writing – review & editing. Jan Stubberud: Writing – review & editing, Project administration. Pål Zeiener: Funding acquisition, Supervision, Project administration, Writing – review & editing. Annika Melinder: Conceptualization, Funding acquisition, Supervision, Project administration, Writing – review & editing.

Author statement

The manuscript is not under consideration for publication elsewhere. The work has not been previously published.

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During the revision of this work, after receiving feedback from reviewers, the author(s) used ChatGPT-4 in order to improve readability and language. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

None.

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