

## Early psychosocial adversity and cortisol levels in children with attention-deficit/hyperactivity disorder

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**Abstract** Previous studies suggest a different regulation of the hypothalamus–pituitary–adrenal axis (HPA-axis) with lower diurnal cortisol levels, especially in the morning, in children with attention-deficit/hyperactivity disorder (ADHD) compared with controls. Since exposure to foetal and childhood psychosocial adversity has been associated with both ADHD and HPA-axis functioning, such exposures may explain these low cortisol levels in ADHD via early programming of the HPA-axis. Thus, our main aim was to retrospectively study foetal and early childhood exposures to psychosocial adversity in children with ADHD and to relate these exposures to cortisol levels. Saliva samples were collected during a regular weekday in children, 6–17 years old, with clinically confirmed ADHD ( $n = 197$ ) and non-affected comparisons ( $n = 221$ ) for radioimmunoassay analysis of cortisol. Parental rating scales were used for categorising subtypes of ADHD and degree of exposure to adversity. Children with ADHD had more reports of at least one rated foetal adversity ( $p = 0.041$ ) and childhood adversity ( $p < 0.001$ ) than comparisons. The association between low morning cortisol levels and ADHD-symptoms remained when analyses were adjusted for adversities, age, sex, sampling time and

symptoms of oppositional defiant disorder. No relation was found between exposures to foetal/childhood adversity and cortisol levels except for a positive relation between childhood adversity and cortisol morning increase in children with ADHD. The hypothesis that early adversity may influence the HPA-axis, leading to lower cortisol levels in children with ADHD, was not supported by our findings.

**Keywords** ADHD · Trauma · Adversity · HPA-axis · Cortisol · Endocrinology

### Introduction

Previous studies suggest a different regulation of the hypothalamus–pituitary–adrenal axis (HPA-axis) with lower diurnal cortisol levels in children with attention-deficit/hyperactivity disorder (ADHD) than in comparisons [1–3]. In a recent study, we found markedly lower cortisol levels in morning saliva samples in children with ADHD and slightly lower levels at bedtime [4]. The underlying mechanisms are not known. The general high heritability—especially in the morning—of cortisol levels [5] and the strong support for hereditary mechanisms underlying the development of ADHD [6, 7] suggest a genetic background. Since early adversity may constitute a risk factor for both ADHD [8] and dysregulation of the HPA-axis [9], a background of such exposures may be an alternative hypothesis to the HPA-dysfunction attributed to ADHD.

Foetal psychosocial adversity, often defined as maternal exposure to negative life events or maternal psychopathology, has in a number of studies been associated with subsequent emotional or cognitive problems in the child with ADHD-symptoms as a consistent adverse outcome [10–12]. Childhood psychosocial adversity—often defined

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as unfavourable family characteristics, socioeconomic disadvantage and parental psychopathology—has also been associated with ADHD-symptoms [6, 13]. Exposure to prenatal as well as early childhood psychosocial adversity has been associated mainly with HPA hyper-functioning in children [14–16]. However, different kinds of exposure to maltreatment seem to be associated with different cortisol regulation patterns [15, 17] and a down regulated HPA-axis with low diurnal cortisol levels has also been observed in association with early psychosocial adversity [17–21]. Furthermore, it has been argued that the observed alteration in cortisol levels observed early in life in the context of neglect and deprivation is not permanent [22].

The “programming theory” [12, 23, 24] is one of the most commonly proposed mechanisms that may explain how adverse exposures develop into behavioural symptoms. This theory means that exposure to a substantial amount and/or intensity of stressors during sensitive periods of development may lead to alterations in biological systems, which may in turn underlie deviating behaviour later in life. Particularly the brain regions involved in the regulation of the HPA-axis are susceptible to such environmental challenges [24, 25]. The HPA-axis is an important part of the neuroendocrine system. It has a number of effects, like mobilizing energy stores, potentiating the release of adrenaline, increasing cardiovascular tone, and inhibiting immune and inflammatory responses [23]. Animal studies have shown that maternal glucocorticoids cross the placenta, affecting foetal HPA development by modulations at the level of glucocorticoid receptors and mineralocorticoid receptors [26]. Such functional modulations may persist throughout life [26]. Also exposure to early childhood psychosocial adversity may affect the regulation of the HPA-axis. Animal studies have demonstrated that early exposure to substantial stressors may induce, e.g. attenuated development of glucocorticoid receptors and GABA-A receptors as well as changes in the structure of the brain [23], altered corticotrophin releasing factor (CRF) production and changed sensitivity of CRF receptors [27]. Teicher et al. [23] suggest that exposure to glucocorticoids exerts an organizing effect on the development, by facilitating subsequent adaptation to, e.g., deprivation or high levels of environmental stressors. Despite the quite extensive evidence from animal studies for HPA-axis programming theories, including behavioural consequences, human studies have not yet convincingly confirmed that similar processes are of importance also in humans [24, 25].

Thus, the main aim of this study was to retrospectively study foetal and early childhood exposures to psychosocial adversity in children with ADHD—also taking subgroup and sex into consideration—and to relate these exposures to cortisol levels. If exposure to psychosocial adversity

explains or changes the strength of the relationship between ADHD-symptoms and cortisol levels it may shed light on the early programming theory in humans.

## Methods

### Subjects and procedure

Children/adolescents (6–17 years of age) of the ADHD group were recruited from four child psychiatry outpatient units in three Swedish counties. The ADHD-diagnosis refers to the last assessment by the clinically responsible child psychiatrist before inclusion in the study. The ADHD-diagnoses had been based on a clinical interview, a previous physical/neurological assessment and neuropsychological testing by a clinical psychologist (choice of tests adapted for the individual needs of the child and the degree of uncertainty about the diagnosis), as well as parental and teacher ratings (SNAP-IV). Written project information was presented at a regular examination or—in one county—by mail to the parents and to the child (an age-adapted version). After written informed consent from parents (and child when  $\geq 15$  years of age) questionnaires about psychosocial adversity, tubes for saliva samplings and instructions were mailed to the family. Clarifications were given by phone. Information about diagnosis/es and symptom ratings (Swanson, Nolan and Pelham ADHD symptom rating scale [SNAP-IV], see below) were collected from the medical record. All data were re-coded and all identifying information was destroyed, thereby implementing total anonymity. After exclusion of eight children reporting saliva sampling during a weekend or a holiday and exclusion of four children that lacked questionnaires on adversities, the ADHD group amounted to 197 children (148 boys and 49 girls; mean age: 11.9 years). The response rate was 35 %.

For the comparison group, children of the same ages and from schools in the same areas as the study persons were invited by mail or parental meetings, depending on the decision of the principal. In the written information, we clarified that children with verified or suspected ADHD should not participate. When the informed consent from parents (and child when  $\geq 15$  years of age) was returned, the following material was mailed to the family: questionnaires about psychosocial adversity, SNAP-IV, tubes for saliva sampling and sampling instructions. Clarifications were given by phone. The data were made anonymous as described above. After exclusion of three children reporting saliva sampling during a weekend or a holiday and seven children with indications of ADHD (six parental SNAP-IV items for any symptom cluster scored as “quite a bit” or “very much”) the comparison group amounted to

221 children. (92 boys and 129 girls; mean age: 11.9 years). The response rate was 30.5 %.

The study was approved by the Regional Ethical Review Board in Uppsala, no. 2009/034 and performed in accordance with the latest version of the Declaration of Helsinki. Informed consent of all participants was obtained after the nature of the procedures had been fully explained.

### Questionnaires

Two questionnaires on adversities were administered to the parents, one concerning exposure to foetal psychosocial adversity (filled in by mothers) and one concerning psychosocial adversity during the first 6 years of life (filled in by any of the parents or both together). “Psychosocial Adversity” in this context means exposure to one or more negative life events.

The core of the items of the maternal questionnaire about foetal psychosocial adversity was the same as in a prospective study by Rosengren et al. [28]—reformulated to be used retrospectively—but the response alternatives (yes/no) were from a later adapted version of the same scale [29]. The 11 questions concern experiences during pregnancy of separation/divorce; relocation; change of employment; unemployment; insecurity at work; severe financial problems; legal sanctions; severe illness or accident (related to oneself or someone close); worries about someone close; death of someone close. The overall Cronbach’s Alpha in both groups was 0.62, specified in the ADHD group as 0.67 and in comparisons as 0.53.

The parental questionnaire about childhood psychosocial adversity during the first 6 years of life was based on general trauma items of The Early Trauma Inventory-Self Report (ETI-SR) Short Form [30]. This questionnaire has been confirmed to have good validity and reliability in another study on assessing childhood trauma in postpartum women [31]. The choice of using yes/no-items was made according to the author’s experiences with this scale [31]. Our original plan was to recruit patients during the—sometimes—critical referral phase (this approach was later changed due to practical obstacles). To avoid any negative influence on the referral process, we excluded items concerning potentially provocative issues (sexual/physical/emotional abuse), one item that may provoke feelings of guilt (separation of parents) and one item referring to an extremely unusual but emotionally repellent phenomenon (witness of murder). The number of items was, thus, reduced to nine concerning accidents; natural disasters; own, parental, siblings’ or friends’ severe injury/illness; parental psychiatric illness; substance abuse in the home environment; having witnessed violence. The overall Cronbach’s Alpha in both groups was 0.40, specified in the ADHD group as 0.44 and in comparisons as 0.23.

Parental ratings on SNAP-IV were used for estimating the severity of ADHD-symptoms and for excluding comparisons with high ADHD ratings [32]. Since the clinical diagnoses were ICD-based, these ratings were also used for identifying DSM-related subtypes of ADHD (predominantly inattention [ADHD-I], predominantly hyperactivity/impulsiveness [ADHD-HI], or combined [ADHD-C]). In the ADHD group 52 children were categorized as ADHD-I, 22 as ADHD-HI and 54 as ADHD-C. The version applied has 30 items [9 for inattention, 9 for hyperactivity/impulsivity, 8 for Oppositional Defiant Disorder (ODD), 4 control questions], scored by parents and teachers on a 4-point scale: 0 for “not at all”, 1 for “just a little”, 2 for “quite a bit” and 3 for “very much”. It has been psychometrically tested in a Northern American sample and has demonstrated “acceptable internal consistency and item selection and a factor structure consistent with the two-factor solution of ADHD-symptoms, and a third ODD factor” [33]. Normative data for Swedish children are not available. However, intercultural similarities and the firm basis in the DSM IV-criteria motivated this choice. The overall Cronbach’s Alpha was 0.97 in the ADHD group and comparison group together. Similar results were found in both groups. The alpha varied between 0.80 and 0.92 for the subscales. ODD was defined as at least four items scored as “quite a bit” or “very much”. In the ADHD group 84 children were categorized as having ODD.

### Cortisol analyses

Cortisol was analyzed in saliva and expressed in nmol/L. Sampling was performed at home during one ordinary weekday (a school day) when waking up, 30 min after waking up, at 4 PM or when coming home from school and when going to bed. Information was given that the sampling should be done before brushing teeth, at least 30 min after eating or drinking and at least 1 h after sport activity. The sampling times were filled in by the families immediately after completing saliva sampling. Samples were collected by swabs (Salivette; Sarstedt Inc., Rommelsdorf, Germany), which the participants kept in their mouth 1–2 min until soaked with saliva. The swabs were placed in plastic sampling tubes. The tubes were centrifuged and stored at  $-70^{\circ}\text{C}$  until analyzed with radioimmunoassay technique using the Spectria Cortisol (125I) kit from Orion Diagnostica, Espoo, Finland [34]. In 89 % of the saliva samples the volume was large enough for carrying out analyses. There were no statistically significant ( $\chi^2$ ) differences concerning the number of non-analyzable samples between the ADHD group and the comparison group for any of the sampling times. In addition to cortisol levels we analyzed “the morning increase” by calculating 30 min post awakening minus awakening value, i.e. the dynamic

change in post waking which is closely correlated to the area under the curve with reference to the first awakening [35]. Cortisol data for the comparison group and for a slightly different ADHD group (including four individuals lacking adversity data) has previously been published in Isaksson et al. [4].

### Statistical analyses

All analyses were performed with the statistical software program SPSS (version 19). As data on cortisol and adversity ratings showed a skewed distribution, we used non-parametric statistical methods (Mann–Whitney and  $\chi^2$ ) for calculating group differences and log-transformation of the saliva cortisol values. In the binary logistic model we used a reversed log scale since lower cortisol levels are associated with the outcome ADHD. The logistic regression was used to calculate the odds ratio of belonging to the ADHD group based on exposure to psychosocial adversity, sex, age (years) and cortisol levels. In this regression, we chose the cortisol awakening level since it was most clearly associated with ADHD-symptoms. As a consequence of the low internal consistency of the adversity questionnaires (especially childhood adversity) and with the intention of facilitating the interpretation of the results, exposures to adversity were transformed into categorical variables by severity: no reported adversity; one type of reported adversity; more than one type of reported adversity. Logistic regression does not have an equivalent to the  $R^2$  that is found in OLS regression. However, Nagelkerke  $R^2$  is a pseudo  $R^2$  statistics which gives an estimation of the model fit. Interaction effects between sex and group belongingness on exposures to adversity were calculated with ANOVA. Associations between cortisol levels and ADHD-symptoms—adjusted for exposures to adversity, sex, age, sampling time and ODD-symptoms—were calculated with a linear regression. Analyses of adversity exposures in relation to cortisol levels were performed using Pearson correlation. Two-tailed test with  $p$  values  $<0.05$  was considered significant.

### Results

Parents of children in the ADHD group presented more reports of at least one rated foetal psychosocial adversity (55 and 44 %, respectively;  $p = 0.041$ ), childhood psychosocial adversity (50 and 33 %, respectively;  $p < 0.001$ ) or both foetal and childhood adversity (53 and 30 %, respectively;  $p < 0.001$ ) than parents of comparisons. Frequencies of separate exposures and significant differences between both groups are presented in Table 1.

**Table 1** Psychosocial adversity and frequency of endorsement in both study groups

Adversity	ADHD group/comparison group
During pregnancy:	
Separation/divorce	9.4/0.9***
Relocation	28.6/20.6
Change of employment	6.9/7.4
Unemployment	19/8.3**
Insecurity at work	12.6/10.2
Severe financial problems	10.6/0.9***
Legal sanctions	3.1/0*
Severe illness or accident (self)	6.2/2.3
Severe illness or accident (other)	5.6/5.5
Worries about someone close	23.6/15.7
Death of someone close	6.2/6.9
0–6 years old	
Serious accidents	9.2/3.2**
Natural disasters	0/0.5
Serious personal injury/illness	12.3/9
Serious injury/illness of parent	16.3/6.8**
Serious injury/illness of sibling	7.7/7.7
Serious injury/illness of friend	4.1/5.9
Parental psychiatric illness	11.7/5*
Substance abuse in the home	11.8/3.2**
Having witnessed violence	11.7/2.7***

Differences marked ( $\chi^2$ : \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ )

Data on cortisol levels on the different sampling occasions are presented in Table 2. Children with ADHD had lower cortisol levels at awakening, 30 min later and before going to bed. In a logistic regression model—adjusted for age, cortisol level at awakening and sex—more than one exposure of foetal and/or childhood psychosocial adversity remained a predictor of belonging to the ADHD group. Also, cortisol levels (lower levels increased the risk of belonging to the ADHD group) and sex (male sex increased the risk of belonging to the ADHD group) were significant predictors in the model (Table 3). The model explained 32 % of the variation of belonging to the ADHD group.

Reports of at least one foetal or childhood psychosocial adversity did not differ between subgroups (ADHD-I, ADHD-HI and ADHD-C; concurrent ODD-symptoms or not). As to sex differences, girls in the ADHD group had higher reported rates than female comparisons of at least one psychosocial adversity during pregnancy (66.7 and 43.3 %, respectively;  $p = 0.011$ ) and childhood (49 and 32.6 %, respectively;  $p = 0.043$ ), whereas boys in the ADHD group only had higher rates of childhood psychosocial adversity than male comparisons (50.7 and 33.7 %, respectively;  $p = 0.043$ ).

**Table 2** Cortisol levels (nmol/L) on all sampling occasions and morning increase: 30 min after awakening minus the awakening sample (data for the comparison group have previously been published in Isaksson et al. [4])

Sampling times	ADHD group			<i>N</i>	Comparison group		<i>p</i> value
	<i>N</i>	Median (Q1–Q3)	Mean (SD)		Median (Q1–Q3)	Mean (SD)	
Awakening	157	8.7 (5.3–13.7)	10.5 (7.5)	168	12.7 (8.5–18.5)	15.5 (13.7)	<0.001*
+30 min	177	15.8 (10.3–20.2)	17.2 (14.5)	188	20.1 (14.4–27.2)	21.8 (10.9)	<0.001*
Afternoon	189	3.5 (2.1–5.0)	4.3 (5.0)	207	3.5 (2.1–5.2)	5.6 (16.5)	.746*
Bedtime	191	0.8 (0.3–1.5)	3.3 (14.0)	209	1.0 (0.7–1.5)	3.1 (13.8)	0.012*
Morning increase	150	5.8 (0.3–9.6)	6 (9.8)	158	7.2 (1.0–13.0)	6.9 (12.0)	0.064*

\* Mann–Whitney

**Table 3** Logistic regression of the association between foetal/childhood psychosocial adversity (no adversity constitutes reference), age, awakening cortisol level (reversed log scale) and sex (female constitutes reference) in relation to belongingness to the ADHD group, with odds ratios, *p* values and Nagelkerke  $R^2$ 

		OR (CI)	<i>p</i> value
Foetal adversity	Little (one)	0.9 (.46–1.77)	ns
	Several (>1)	2.03 (1.04–3.99)	0.039
Childhood adversity	Little (one)	0.99 (.52–1.86)	ns
	Several (>1)	3.77 (1.57–9.05)	0.003
Age		1.01 (.91–1.13)	ns
Cortisol		5.60 (2.30–13.64)	<0.001
Sex	Male	5.70 (3.24–10.06)	<0.001
Model $R^2$		0.32	

respectively;  $p = 0.010$ ). There was a significant interaction effect between sex and group belongingness for foetal adversity ( $f = 5.21$ ;  $p = 0.023$ ), but not for childhood adversity (not shown in tables).

There was a negative association between ADHD-symptoms and cortisol levels at awakening ( $b = -0.27$ ;  $p < 0.001$ ), 30 min after awakening ( $b = -0.24$ ;  $p < 0.001$ ) and at bedtime ( $b = -0.11$ ;  $p = 0.027$ ), as well as a trend in the afternoon sample ( $b = -0.092$ ;  $p = 0.073$ ). No association was found for the morning increase. The association between ADHD-symptoms and the two cortisol morning samples remained when adjusted for age, sex, foetal and childhood psychosocial adversity, sampling time and ODD-symptoms ( $b = -0.36$ ;  $p < 0.001$  and  $b = -0.25$ ;  $p = 0.008$ , respectively), whereas the association between ADHD-symptoms and cortisol levels at bedtime disappeared. ODD-symptoms were not associated with cortisol levels in the adjusted models. There was no relation between foetal/childhood psychosocial adversity and cortisol levels on the four sampling occasions in the two groups, separately. However, there was a positive correlation in the ADHD group (not in the comparison group) between childhood adversity and morning increase ( $r = .19$ ;  $p = 0.041$ ).

## Discussion

Children with clinically verified ADHD ( $n = 197$ ) had to a higher degree been exposed to foetal and childhood psychosocial adversity than comparisons ( $n = 221$ ). The previously reported association between low morning cortisol levels and ADHD-symptoms remained when adjusted for foetal and childhood psychosocial adversity as well as sex, age, sampling time and ODD-symptoms. No relation was found between exposures to psychosocial adversity and diurnal cortisol levels on any sampling point, but there was a positive correlation between childhood adversity and the cortisol morning increase in children with ADHD.

The high exposure of adversities in the ADHD group is in line with previous studies [6, 12]. This may hypothetically reflect a causal link. If so, an underlying mechanism could be the early programming of the HPA-axis [12, 23, 24]. However, the hypothesis that early psychosocial adversity (prenatal or postnatal) may have a programming effect on the HPA-axis, leading to a hyper- or hypocortisolism preceding psychiatric symptoms like ADHD, was not supported by our findings since there was no relation between adversity and diurnal cortisol levels in children with ADHD or in non-affected comparisons. However, there was a weak positive correlation between childhood adversity and the morning increase in children with ADHD. The morning increase is regarded as a distinct feature of the HPA-axis [36]. The function of this increase is not known, although it is hypothesized that a sharper increase is associated with chronic stress and/or anticipation of the upcoming day [36]. This is partly in line with our finding where the positive correlation in the ADHD group may reflect the greater extent of external stressors related to a higher degree of childhood adversity.

Although no previous studies have focused on the relation between early psychosocial adversity, cortisol and ADHD, there are some reports with a similar approach and a focus on “externalizing behaviour”, a concept that is overlapping ADHD. Externalizing behaviour has—just as ADHD—been associated with psychosocial adversity [37]



as well as with lower cortisol levels [38]. In a study on the relation between salivary cortisol, aggressive behaviour and psychosocial adversity, hypocortisolism was the characteristic of children displaying relational aggression. The authors conclude that the cortisol dysregulation was related to the aggression in itself rather than to the exposure to stressors [39]. This is in line with the conclusions from a review on antisocial behaviour and its relation to the HPA-axis, where the authors conclude that "... pathways to antisocial behaviour involving early environmental adversity may be less related to the HPA-axis than has often been assumed" [38]. It has been discussed that lower cortisol levels in ADHD are particularly associated with comorbid ODD [40]. However, in our study ADHD was associated with lowered cortisol levels even after adjusting for ODD-symptoms. Furthermore, there were no association between ODD and cortisol levels in the adjusted model.

In a human research program aimed at testing each step of the programming theory [41], only a weak correlation was found between maternal anxiety and maternal plasma cortisol levels and no correlation was found between maternal anxiety on one side and the amniotic cortisol levels and amniotic testosterone levels on the other. In a following study, the authors found that amniotic cortisol levels predicted cognitive development and infant cortisol levels at approximately 17 months. However, neither maternal anxiety ratings nor maternal plasma cortisol levels were associated with infant cortisol or the child's cognitive development [42, 43]. Accordingly, the findings so far from this research program also challenge the programming theory, i.e. in this case the hypothesis that cortisol mediates the effects of parental stress on the child's development.

Notably, the link between psychosocial adversity and ADHD may be built up by various mechanisms, which may not necessarily be psychological or psycho-physiological in nature. For instance, psychosocial adversity may be related to malnutrition which seems to have an independent effect on the development of externalising behaviour, mediated by cognitive ability [44]. The higher rates of exposures to foetal and childhood psychosocial adversity among children with ADHD may also reflect a common genetic background, where some of the adversities may be seen as direct or indirect expressions of parental psychological and psychiatric symptoms. HPA-axis functioning—expressed as cortisol levels—may act as an independent variable, thereby rather suggesting a genetic background. Previous findings from our group [4] that foremost the morning cortisol levels—which seem to be more strongly influenced by genetic factors than afternoon and evening levels [5]—are lower in the ADHD group, are in line with a genetic explanation of the hypocortisolism in children with ADHD. Furthermore, as seen in this study, psychosocial adversity

did not affect the relation between ADHD-symptoms and cortisol levels in the morning. Along the same line, a recent study showed that the link between prenatal stress and ADHD in the offspring was only present in biologically related mother–offspring pairs and, therefore, attributable to inherited factors [45].

### Limitations

There is a risk of recall bias in case–control studies where the disease has already occurred when exposure information is obtained. Recall bias may distort the measure of association between exposure and disease by under-reporting or over-reporting. Parents may be prone to over-report when seeking for an explanation of the disease or when they have an assumption about its underlying cause [46].

Possibly, other types of stressors like exposure to sexual, physical and emotional abuse would have had a measurable impact on the cortisol levels. The instruments focused on "classical" traumatic experiences like accidents, illness or violence, just as the LITE-questionnaire [47]. Taking potentially stressful situations from other sectors of life into consideration—like the Coddington questionnaire [48] with its broader and more extensive life event approach—may have brought up other results.

The Cronbach Alpha values of the adversity questionnaires were low, especially for the Early Trauma Inventory, considerably lower than what has been previously reported by Bremner et al. [30]. To a certain degree this probably reflects the lower prevalence of all types of childhood trauma in our study and the lower number of items. However, it also reflects that the scores of the scales concerning foetal and childhood exposures cannot be directly considered and utilised as indices. They should rather be looked upon as a tentative approach to the task of elucidating the relations between psychological trauma and ADHD. To minimize the problem of low internal consistency we chose to perform our analyses using categorised trauma data instead of using mean scores.

The saliva sampling was performed during one regular weekday, limited to four occasions. For optimal cortisol measurement, it is advisable to collect saliva for more than 1 day also assessing participant's adherence [35]. However, we wanted the sampling to be done on a regular school day since the day of cortisol assessment is crucial in psychoendocrinological stress studies [49]. We also assumed that the procedure with numerous collecting points would be too demanding and might increase attrition.

Subtypes of ADHD were defined by parental ratings and not diagnosis, thereby, reflecting symptoms rather than a psychiatric evaluation. However, all individuals in the ADHD group had undergone structured but individually

adapted investigations at specialized teams and received an ADHD-diagnosis.

It is a delicate matter to choose statistical methods in a study where the outcome measures are on a skewed ordinal or interval scale. We used non-parametrical methods as well as log-transformations, according to the design of analysis. The procedure with non-parametrical statistical approaches will, on the one hand, avoid false findings according to outliers in the dependent variables (type I errors), however; on the other hand, reduce the power to detect small, but significant differences between groups (type II errors). We considered  $p < 0.05$  significant, although several tests were done analyzing the data. An alternative would have been to use Bonferroni adjustments. However, this method has been criticized since the interpretations depend on the number of tests that are performed and the likelihood of type II errors is also increased [50]. Instead it is argued that the best approach is to describe what has been done and why [50]. However, the relation in our models was high, pointing at robust results.

In conclusion, children with ADHD had been exposed to foetal and childhood psychosocial adversity to a higher degree than comparisons. No relation was found between such exposures to foetal/childhood adversity and cortisol levels, but there was a positive correlation between childhood adversity and the morning increase in children with ADHD. The low cortisol levels in children with ADHD still stand out as a mystery.

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**Conflict of interest** The authors declare that they have no conflicts of interest.

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