Chapter 4

Sex-specific associations between person and environment-related childhood adverse events and levels of cortisol and DHEA in adolescence

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Abstract

Background: Person and environment-related childhood adverse events have been demonstrated to increase the risk of impaired mental health in later life differently for boys and girls. Altered hypothalamic pituitary adrenal (HPA)-axis functioning has been suggested as a key mechanism underlying this association. Cortisol and dehydroepiandrosterone (DHEA) are both output hormones of the HPA-axis. DHEA may have a protective function against long-term exposure to increased levels of cortisol, but has been little investigated in relation to childhood adversity.

Objective: We aimed to test the associations between person-, and environment-related childhood adversity and levels of cortisol, DHEA and cortisol/DHEA ratio in adolescent boys and girls.

Methods: A total of 215 Dutch adolescents participated in the study and filled out the 27-item Adverse Life Events Questionnaire for the assessment of childhood adversity, divided into a person-related and environment-related sub score. Cortisol and DHEA concentrations and cortisol/DHEA ratio were determined in proximal 3 cm long hair segments. Additionally, saliva samples were collected immediately and 30 minutes after waking up, at noon and at 8 pm. Multiple linear regression analyses were used to test associations between childhood adversity and cortisol and DHEA concentrations, for boys and girls separately, with age, BMI and pubertal development as covariates.

Results: Data were available for 74 boys and 116 girls with a mean age of 15.7 years (SD = 2.0). Higher levels of person-related childhood adversity were associated with higher hair DHEA levels in girls and with higher hair cortisol levels in boys. A trend towards a significant association was observed between higher levels of environment-related childhood adversity and higher DHEA levels in boys. Neither person- nor environment related childhood adversity was associated with cortisol/DHEA ratio. A trend was observed for environment-related childhood adversity and lower daily cortisol output in boys.

Conclusion: We found differential associations between childhood adversity and cortisol and DHEA levels in girls and boys, for respectively person-related and environment-related childhood adversity. Our findings suggest that different types of childhood adversity are not only linked to levels of cortisol, but also to DHEA concentrations, in a sex-specific manner, with possible future implications for mental health.

Introduction

Childhood adversity refers to a range of negative exposures during childhood, including abuse, neglect, violence, parental separation or incarceration, and is a common phenomenon (1). In high income countries, the prevalence of having experienced at least one adverse event during childhood has been estimated to be almost 40%. Childhood adversity can have detrimental effects on later mental health, leading to depression, substance abuse and suicide attempts in adulthood (1-3).

Childhood adversity can be divided into dependent or person-related events and independent or environment-related events (4, 5). Person-related events have a direct impact on the person himself or herself. Examples of such events are severe physical illness, a handicap, being the victim of abuse or sexual abuse. Environment-related events affect other individuals or the direct environment of an individual. Such events include parental separation, parental drug use or the death of a sibling or parent. It has been previously shown that these distinct categories of events seem to affect mental health in a different manner (5). In two studies, person-related childhood adverse events, but not environment-related childhood adverse events, were associated with more depressive symptoms among adolescents (6, 7). Environment-related childhood events including parental separation and sudden parental death, were associated with psychological maladjustment and conduct problems (8, 9). In a co-twin control study, person-related childhood adverse events were associated with the onset of depression among females more than environment related events (10). There is further evidence that the associations between person- and environment related childhood adverse events and development and subsequent mental health are different for boys and girls. Person-related childhood adversity, in the form of sexual abuse, has been associated with more depression, anxiety, post-traumatic stress disorder (PTSD), and suicidal ideation in girls, but not in boys (11, 12). Environment-related childhood adversity including family conflict has been associated with learning disability and decreased global functioning in adolescence to a greater extent in boys compared to girls (13).

The sex-specific associations between person and environment-related childhood adverse events and mental health outcomes might be (partly) explained by differential effects of childhood adverse events on the development of the hypothalamic pituitary adrenal (HPA)-axis in boys and girls. The main end product of the HPA-axis is cortisol, which is released after stress exposure and is involved in processes such as glucose release and immune responsivity. In addition to release after stress, cortisol follows a diurnal rhythm with peak levels shortly after awakening and decreasing levels during the day (14). Dysregulation of the HPA-axis is associated with depressive symptoms, anxiety disorders and PTSD in childhood and adulthood (15-17). Another important HPA-axis hormone is dehydroepiandrosterone (DHEA), a human steroid and androgen that is produced in the adrenal cortex and that has several effects on the neurological, endocrine- and immune system. Levels of DHEA typically increase when cortisol levels increase. DHEA has antiglucocorticoid properties and the co-release of DHEA with cortisol returns the stress system back to homeostasis, suggesting a protective function against long-term exposure to increased levels of cortisol (18, 19). Low, but also high DHEA concentrations have been associated with an increased prevalence of depression and PTSD in adults (20-23). An increased ratio of cortisol to DHEA concentration suggests higher stress hormone levels and lower 'protective' hormone levels, and has been related to poor mental health outcomes (20).

Associations between trauma exposure during childhood and altered HPA-axis functioning have frequently been observed in animal and human studies (24, 25). Adverse childhood events have been linked to blunted diurnal cortisol patterns in young children (26) and adolescents (27) and an increased cortisol awakening response in adult women (28). Childhood adverse events such as childhood maltreatment have also been linked to increased cortisol/DHEA ratio concentrations in adulthood (29) emphasizing the complexity of associations between childhood adversity and neuroendocrine variables.

There is evidence indicating that the association between non-specific childhood adversity and HPA-axis function might be different for boys and girls, such that boys who experienced pervasive maltreatment had higher DHEA and lower cortisol/DHEA ratio levels compared to girls with similar experiences (30). Other research showed that girls exposed to prenatal stressors (preterm birth, low birth weight and maternal stressors) had increased HPA-axis reactivity, compared to males. Among males, these stressors were associated with altered diurnal cortisol secretion (31). The sex-specific associations between person- and environment related adverse events during childhood and the development of mental health and

behavioral problems might be partially explained by differential associations with levels of cortisol and DHEA and with cortisol/DHEA ratio.

The association between childhood adversity and DHEA or cortisol/DHEA ratio has not been studied previously in adolescent boys and girls despite our understanding of the adolescent period as an important developmental period, including major changes in the HPA-axis and associated adaptability to stress. These changes have been linked to increased risk for the onset of internalizing and externalizing behavior problems among children and adolescents (26, 32). DHEA plays a role in the onset of puberty, the physical maturation as well as brain development during puberty (32). Also, to the best of our knowledge, associations of person- and environment-related childhood adverse events and HPA-axis measures have not yet been studied. Potential differential associations of person- and environment-related childhood adversity and HPA-axis dysregulation could possibly result in increased symptoms of depression and anxiety in girls, and learning and behavioral problems in boys, respectively. In order to further unravel the associations between different types of childhood adversity and cortisol, DHEA, cortisol/DHEA ratio and diurnal cortisol patterns in adolescence, the present study was carried out. We expected associations between environment-related childhood adversity and cortisol and DHEA levels in boys, and between person-related childhood adversity and cortisol and DHEA levels in girls.

Methods

Participants

Participants (age 12-18) were recruited from several high schools in the Netherlands. A total of 215 adolescents consented to participation in the study. The availability of questionnaire data, hair (part 1) and saliva (part 2) data is shown in Figure 1.

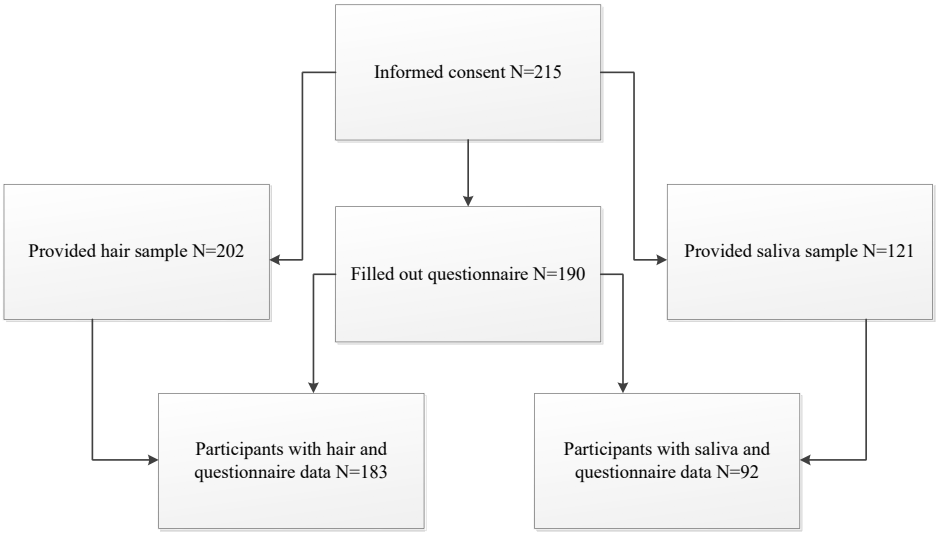

Figure 1. Flowchart of participants and data availability for questionnaires, hair (part 1) and saliva (part 2).

The study was approved by the ethical committee of the Faculty of Behavioral and Movement Sciences of the Vrije Universiteit Amsterdam. Participants, and in case of children under the age of 16 years the parents, gave written informed consent.

Procedure

Study assistants asked permission of high schools to invite students to participate in the study. When permission was obtained, youths and their parents received a written explanation of the study with an invitation to participate. The student assistants then visited schools to enroll adolescents willing to participate and who had filled out a written informed consent (by themselves or their parents). After explaining the study protocol, a piece of hair was cut as the first part of the study. In the second part of the study, within a week after the first part of the study, half of the

subjects received four tubes to collect saliva on a weekly day along with information on saliva collection. They were asked to collect the saliva in the tubes and return the tubes to the university by mail. The time of day each saliva sample had to be collected was explained in the information letter, and participants reported the exact time of collection of each saliva sample. After this, participants were emailed a link to a website where they were asked to fill out a questionnaire containing questions on demographic variables and seven different standardized questionnaires (see below for a description of the questionnaires used for the present study). Completing the questionnaires took about one hour.

Self-report measures

The questionnaire contained questions about height and weight, ethnic background, and if the participants had ever smoked. Postal code was also self-reported, upon which socio-economic status was based. The Pubertal Development Scale (PDS) was used to assess the stage of puberty for boys and girls. The PDS is a 5-item questionnaire regarding growth, bodily hair development, skin changes and sex-specific pubertal development questions about voice changes and facial hair (boys) and breast development and menarche (girls). A total score between 1 (no development) and 4 (development complete) was computed ($\alpha = 0.77$) (33, 34).

Childhood adversity can be studied by using a questionnaire assessing stressful life events, containing items about different types of events. In several studies these events have then been summed to a total number of events per person (29, 35). However, the death of a parent may have a much greater impact on the life of a child or adolescent, compared to the loss of a job by a parent. In order to take the potential impact of an event into account, questionnaires like the Holmes and Rahe Stress Scale use life change units (36). For the present study, we used the 27-item Adverse Life Events Questionnaire (ALEQ), a reliable and valid measure of childhood adversity, and also took the impact of events into account. For each adverse event, the participant filled out if he/she experienced the event, how many times, the age at the time of the adverse event, and the perceived severity (5, 37). If the event was not perceived as severe at all the event was not scored as a stressful event. Events were categorized and scored with the Holmes and Rahe stress scale, resulting in a total life change unit score, with higher scores indicating higher impact of stressful events (36). We split the total score in a person-related sub score and an environment-related sub score, based on a previous study in which these events were rated by trained psychologists in the field of life events (5). An overview of the reported person-related and environment-related is shown in table 3.

Neuroendocrine measures

Hair strands (about 3 mm diameter) were taken scalp-near from a posterior vertex region. Cortisol and DHEA concentrations were determined in the proximal 3 cm long hair segment which, based on an approximate hair growth rate of about 1 cm per month, is assumed to reflect hormone secretion over the three-month-period prior to hair sampling (38). The concentrations of cortisol and DHEA were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Participants were asked to passively drool saliva in the tubes at four different time points during a week day: immediately after waking up, 30 minutes after waking up, at noon (98% within one hour) and at 8 pm (94% within one hour). They were also asked to stay in bed before collecting the second sample, to not eat within the half hour preceding the saliva collection and to store the tubes with saliva in the freezer whenever possible. Cortisol concentrations were assessed by an ELISA assay. From the salivary cortisol values the day area under the curve (AUCg) with respect to ground cortisol levels was computed as a measure of daily cortisol output.

Statistical Analysis

Multiple linear regression analyses were used to test the hypotheses. Because the values for cortisol, DHEA and cortisol/DHEA ratio were not normally distributed, values were log transformed which resulted in normally distributed values. Outcomes included the hair variables DHEA from the last month, DHEA from the last 3 months, cortisol from the last month, cortisol from the last 3 months, cortisol/DHEA from the last month and cortisol/DHEA from the last 3 months. Childhood adversity measured by the ALEQ in life change units was used as independent variable. Person-related and environment-related sub scores were analyzed separately. Age, BMI and PDS score were entered as covariates in the models, and each outcome was analyzed separately for boys and girls. AUCg was analyzed as a dependent variable in a regression model with dichotomized person- and environment related childhood adversity as factors. All analyses were conducted with IBM SPSS version 24 (Armonk, NY, USA).

Results

Descriptive analyses

The responder analyses results are shown in Table 1. The characteristics of the group that participated in the hair collection and questionnaire part of the study were similar to the characteristics of the group that participated in the saliva collection and questionnaire part.

As shown in Table 2, the final sample consisted of 190 adolescents (n = 74; 39% boys) with a mean age of 15.7 years. DHEA levels in hair were comparable for girls and boys. Cortisol levels in hair from the last month were higher in girls (median = 3.6, inter quartile range (IQR) = 2.4-5.0) compared to boys (median = 2.4, IQR = 1.6-3.8, $p < 0.01$). Cortisol and DHEA from the last month were both positively associated with pubertal stage (r = 0.24 $p < 0.01$ and r = 0.22, $p < 0.01$ respectively). Cortisol and DHEA from the last month and last 3 months were not significantly correlated (r = 0.13, $p = 0.13$; r = 0.10, $p = 0.26$, respectively). Comparison of the most recent hair cortisol sample (last month) and the separate saliva cortisol samples, as a measure of cortisol output consistency, showed that hair cortisol from the last month was significantly correlated with the midday saliva cortisol sample (r = 0.21, $p < 0.05$), but not with the first (r = -0.01, $p = 0.92$), second (r = -0.06, $p = 0.59$) and last sample (r = 0.01, $p = 0.96$).

In Table 3, the report of life events is shown, divided into person-related events and environment-related events. For both girls and boys, the death of someone they cared about was the most common adverse event (37% and 35%, respectively), followed by severe illness or accident of a family member (21% and 24%, respectively) and having had a severe illness or accident themselves (12% and 19% respectively).

Childhood adversity and cortisol, DHEA and cortisol/DHEA ratio in hair

If adversity was analyzed as a total score, and not according to person- and environment related events, no significant associations were found (data not shown). However, if we split the analyses into person- and environment related events, significantly higher DHEA levels over the last month as well as over the last 3 months were observed in girls with more person-related childhood adversity, but not in girls with more environment-related childhood adversity, as reported in Table 4. In boys, no significant association was observed between person-related childhood adversity and DHEA levels, however DHEA levels from the last month showed a trend towards being significantly higher in boys with more environment-related childhood adversity. Hair cortisol levels in the last month were significantly higher in boys with more person-related childhood adversity. Environment-related childhood adversity was also associated with higher hair cortisol over the last 3 months in boys.

Daily cortisol output in saliva

The AUCg analyses showed no differences in daily cortisol output between boys or girls with and without person-related childhood adversity. There was a trend towards a significant lower daily cortisol output in boys with environment-related childhood adversity (b = -9.37; SE = 5.07; p = 0.08) compared to boys without environment-related childhood adversity (Figures 1-4).

Discussion

Our findings demonstrated that person- and environment related childhood adversity were differentially associated with HPA-axis functioning in adolescents in a sex-specific manner. Neither person- nor environment related childhood adversity was associated with an altered cortisol/DHEA ratio in hair. However, in girls, person-related childhood adversity was associated with higher hair DHEA levels in girls, while environment-related childhood adversity was not associated with HPA-axis measures. In boys, both person- and environment related childhood adversity were associated with higher hair cortisol levels. Also, for environment-related childhood adversity a trend was observed for lower daily cortisol output measured in saliva and higher DHEA levels in hair.

Based on previous studies suggesting that person- and environment-related adverse events during childhood could be associated with mental health in a sex-specific manner (4, 5, 11, 13), we studied person- and environment-related childhood adversity separately in boys and girls in relation to levels of cortisol, DHEA and cortisol/DHEA ratio. As we had expected, there were indeed sex-specific associations, which were also different depending on the type of adverse events. If the person- and environment-related events were combined in a total adverse event score, no associations were found with either cortisol or DHEA levels, which confirms the importance of studying person- and environment-related events separately. Our findings were generally in line with our hypotheses, showing that person-related childhood adversity was associated with altered HPA-axis functioning in girls, and environment-related childhood adversity was associated with altered HPA-axis functioning in boys. Contrary to our hypotheses though, we found that person-related childhood adversity was also associated with increased cortisol levels in boys, and that person- and environment related childhood adversity were not linked to an altered cortisol/DHEA ratio in girls or boys.

Previous research has suggested a sex-specific association between non-specific childhood adversity and altered HPA-axis functioning (30). A potential explanation for the sex-specific associations between childhood adversity and altered HPA-axis functioning might be the differences in puberty development between girls and boys. The functioning of the HPA-axis changes during puberty from relatively low to increasing levels of cortisol and DHEA, and these changes occur earlier in girls compared to boys, since puberty starts at an earlier age in girls (39, 40). Childhood adversity may lead to different HPA-axis functioning in girls and boys, such that the brain and endocrine system react differently to the

environment during pubertal development (30). Previous research has shown that early life adversity is associated with altered cortisol day output in boys, which is in line with our findings (31). Our results add that the type of event is also related to these sex-specific associations. In one study, boys experienced more behavioral problems after parental divorce compared to girls, which is consistent with the idea that environment-related childhood adversity affects boys to a greater extent than girls (41). These findings together suggest that the increased susceptibility for mental health and behavioral problems after childhood adverse events depend on both sex and type of event, although more research is necessary to confirm and further unravel these associations.

DHEA is a relatively new marker of mental health and recent studies have suggested a potential important role of DHEA in childhood adversity research, such that cortisol and DHEA levels could be used as predictors of mental health and behavioral problems after childhood adversity exposure (29, 30). The present study showed that there are sex-specific differential associations between different types of childhood adversity and levels of DHEA. Based on our results it seems that girls who have experienced person-related adverse events during childhood and boys who have experienced environment-related adverse childhood events have prolonged increased levels of DHEA, although the latter was not statistically significant. Increased DHEA levels have been shown in children with anxiety and obsessive-compulsive symptoms and in adult patients with major depressive disorder and addictive disorder (22, 42, 43). Taken together with the results from our study, childhood adversity may be linked to increased DHEA levels with potential long-term negative effects on mental wellbeing.

In a meta-analysis childhood adversity was associated with increased cortisol levels in hair in both girls and boys, which is in line with our findings, although in the meta-analysis cortisol levels were higher in boys compared to girls (44). Our finding of a trend towards an association between environment-related childhood adversity and lower daily cortisol output in boys is in line with previous research focusing on non-specific childhood adversity and childhood daily cortisol output (26, 31). These findings collectively indicate that different types of childhood adversity may be linked to blunted daily cortisol output, and prolonged elevated levels of cortisol, suggesting HPA-axis dysregulation, with possible future associations with impaired mental wellbeing.

Contrary to previous research, we did not find an association between person- and environment related childhood adversity and alterations of the cortisol/DHEA ratio. This null finding may be related to the small variation in cortisol/DHEA

ratio in our sample, or the greater severity of stress experienced in childhood in previous research describing effects on the cortisol/DHEA ratio (20, 30). In addition, cortisol/DHEA ratio was derived from plasma and saliva in these studies, whereas in our study cortisol/DHEA ratio was derived from hair, which may have resulted in different estimations. Based on previous research though, the cortisol/DHEA ratio is an important measure of neuroendocrine functioning (20), and additional research is needed to assess the sex-specific association between person- and environment related childhood adversity and this ratio.

Strengths of this study included the measurement of both cortisol and DHEA levels in hair, and daily salivary cortisol output, giving information about HPA-axis activity over a period of three months and over a one-day period. In line with previous studies showing a positive correlation between cortisol levels from hair and saliva (45-47), we also observed a positive correlation between the hair cortisol sample from the last month and the mid-day saliva sample, although measurements at other time points were not related. A limitation of this study is that we could not adjust for smoking, as reliable information was only available about having ever smoked. Since there are associations between smoking and DHEA and cortisol, adjustment for current smoking would have been preferred (48). Another limitation concerns the analysis of hair to determine cortisol and DHEA levels; in boys or girls with short hair it was not possible to take a hair sample or only a short piece of hair. Furthermore, the smaller number of adolescents participating in the saliva sample part of the study (48%) limited the statistical power for finding differences in the daily cortisol output. This particular group did not seem to be a selective group though as the responder analyses showed no differences.

Despite these limitations, the study revealed differential associations between person- and environment related childhood adversity and functioning of the HPA-axis in girls and boys. The results from this study contribute to the understanding of the associations between childhood adversity and neuroendocrine functioning and emphasize that different types of childhood adversity may not only be linked to cortisol but also DHEA levels in a sex-specific manner. This may partly explain the differences between boys and girls in the subsequent development of mental health and behavioral problems. Future research is recommended to assess sex-specific associations between different types of adverse events and later mental wellbeing.

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Table 1. Responder characteristics, presented as means (SD) unless stated otherwise.

	Hair & questionnaire data (n=183)	Saliva & questionnaire data (n=92)
Age in years	15.6 (2.0)	15.3 (2.1)
European ethnicity, n (%)	163 (89.1)	87 (94.6)
Height in cm	170.1 (10.7)	169.4 (10.9)
Weight in kg	59.1 (13.1)	58.0 (14.4)
BMI in kg/m²	20.3 (3.2)	20.0 (3.2)
High SES n (%)	20 (10.9)	11 (12.0)
Smokers (ever) n (%)	41 (22.4)	19 (20.7)
Puberty stage	2.9 (0.7)	2.8 (0.7)

Table 2. Clinical characteristics, presented as means (SD) unless stated otherwise.

	Total sample (n = 190)	Girls (n = 116)	Boys (n = 74)
Age in years	15.7 (2.0)	15.8 (1.9)	15.4 (2.1)
European ethnicity, n (%)	169 (88.9)	104 (89.7)	65 (87.8)
Height in cm	170.3 (10.6)	167.4 (7.4)	174.8 (13.0)
Weight in kg	59.3 (13.0)	57.8 (9.7)	61.7 (16.6)
BMI in kg/m²	20.3 (3.2)	20.6 (3.0)	20.0 (3.5)
High SES n (%)	22 (10.2)	15 (12.9)	7 (9.5)
Smokers (ever) n (%)	44 (23.2)	27 (23.3)	17 (23.0)
Life change units groups n (%)	70 (36.8)	41 (35.3)	29 (39.2)
- **None**	74 (38.9)	45 (38.8)	29 (39.2)
- **1-149**	39 (20.5)	25 (21.6)	14 (18.9)
- **150-299**	7 (3.7)	5 (4.3)	2 (2.7)
- **> 300**	2.9 (0.7)	3.1 (0.6)	2.6 (0.7)
Puberty stage			
DHEA pg/mg last month*	14.5 (9.0-25.0)	13.9 (8.5-22.9)	15.4 (9.4-29.5)
DHEA pg/mg last 3 months*	45.3 (29.9-72.7)	44.3 (29.2-67.4)	79.2 (34.5-111.3)
Cortisol pg/mg last month*	3.2 (2.1-4.3)	3.6 (2.4-5.0)	2.4 (1.6-3.8)
Cortisol pg/mg last 3 months*	8.6 (5.9-12.1)	8.6 (6.0-12.2)	8.6 (4.8-11.6)
Cortisol/DHEA last month*	0.21 (0.11-0.36)	0.26 (0.17-0.47)	0.15 (0.08-0.28)
Cortisol/DHEA last 3 months*	0.18 (0.12-0.31)	0.18 (0.12-0.30)	0.12 (0.05-0.35)

*presented as medians (inter quartile ranges).

Table 3. Description of adverse events and frequency of experiencing such an event for girls and boys, n (%), and mean total scores (SD).

	Girls (n = 115)	**Boys (n = 74)**
Person-related events		
Total score	12.9 (35.2)	14.4 (30.6)
Severe illness or accident	14 (12%)	14 (19%)
Physically handicapped	2 (2%)	1 (1%)
Moved to other family	2 (2%)	0
Victim of violence	3 (3%)	1 (1%)
Threatened with a weapon	1 (1%)	0
Environment-related events		
Total score	79.7 (92.2)	70.1 (80.9)
Severe illness or accident of family member	24 (21%)	18 (24%)
Severe illness or accident of good friend	8 (7%)	3 (4%)
Physically handicapped family member	1 (1%)	2 (3%)
Drugs addiction of family member	4 (4%)	1 (1%)
Death of a parent	3 (3%)	2 (3%)
Death of a sibling	1 (1%)	0
Death of someone you cared about	42 (37%)	25 (34%)
Parental unwanted unemployment	9 (8%)	11 (15%)
Parental divorce or separation	8 (7%)	5 (7%)
Family member moved	5 (4%)	1 (1%)
Family member taken into custody	1 (1%)	1 (1%)

Table 4. Regression models for the associations between person- and environment related childhood adversity and levels of DHEA, cortisol and cortisol/DHEA ratio in hair.

	Girls		Boys	
	Person-related childhood adversity	Environment-related childhood adversity	Person-related childhood adversity	Environment-related childhood adversity
DHEA pg/mg last month	0.079 (0.032)*	-0.007 (0.013)	-0.009 (0.134)	0.093 (0.050)†
DHEA pg/mg last 3 months	0.223 (0.093)*	-0.01 (0.038)	-0.085 (0.485)	0.035 (0.276)
Cortisol pg/mg last month	-0.001 (0.005)	-0.001 (0.002)	0.016 (0.007)*	-0.002 (0.003)
Cortisol pg/mg last 3 months	-0.01 (0.013)	-0.004 (0.005)	0.066 (0.05)	0.058 (0.022)*
Cortisol/DHEA last month	-0.0002 (0.001)	-0.000052 (0.0003)	-0.002 (0.003)	-0.001 (0.001)
Cortisol/DHEA last 3 months	-0.0005 (0.001)	-0.00004 (0.0002)	0.001 (0.002)	0.002 (0.001)

Results are displayed as B (standard error); *p value < 0.05; †p value < 0.1; all analyses were adjusted for age, BMI and PDS score.

Figure 1. Daily cortisol output from saliva for boys with and without environment-related adversity

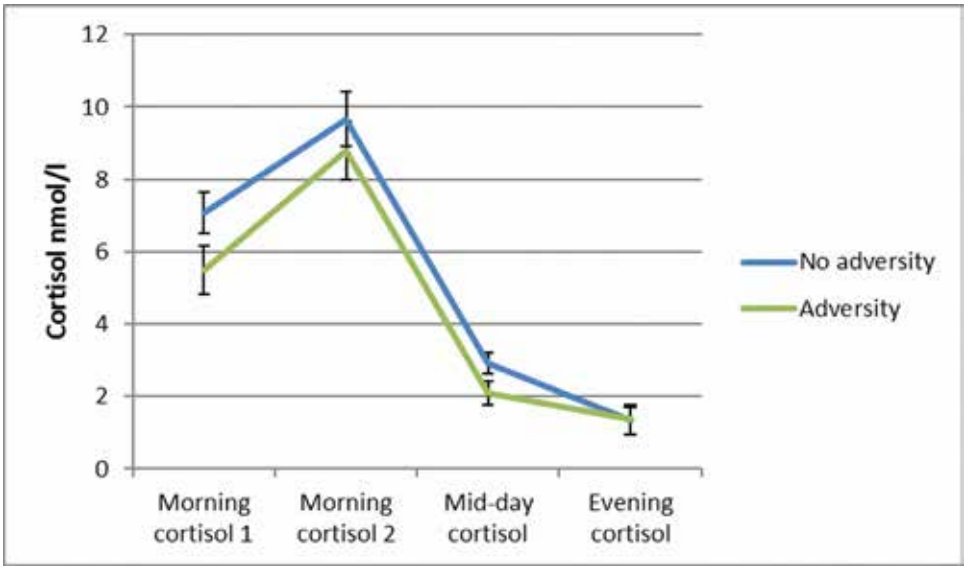

Figure 2. Daily cortisol output from saliva for boys with and without person-related adversity

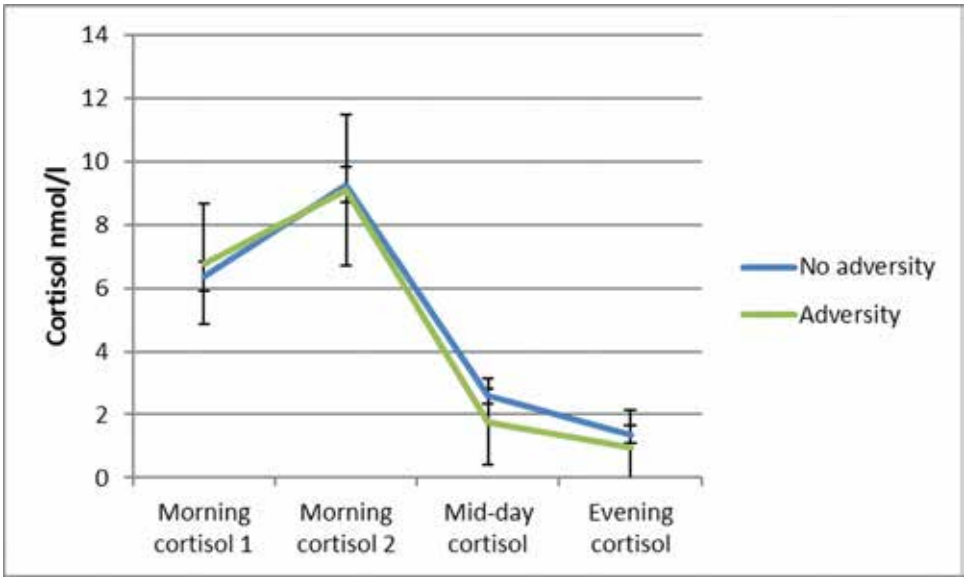

Figure 3. Daily cortisol output from saliva for girls with and without environment-related adversity

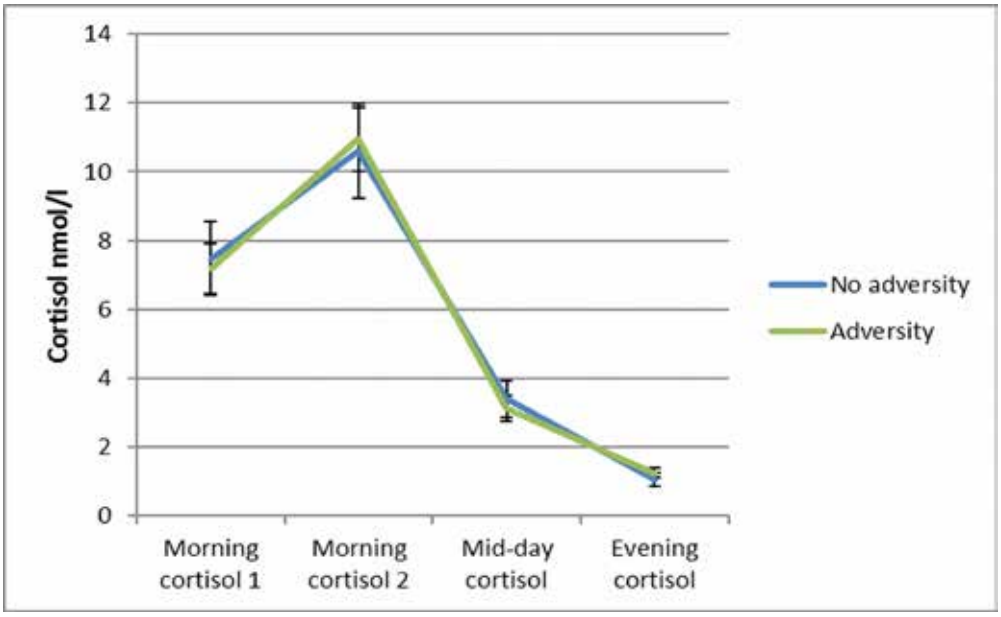

Figure 4. Daily cortisol output from saliva for girls with and without person-related adversity