Negative life events, emotions and psychological difficulties as determinants of salivary cortisol in Belgian primary school children.

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ABSTRACT

PURPOSE: This paper describes whether children's life events, emotions and psychological difficulties are related to their salivary cortisol patterns and whether this is different between sexes.

METHODS: In 385 children (5 to 10 years old) participating in the ChiBS study, salivary cortisol samples were collected when waking up, 30 minutes and 60 minutes after wake up and in the evening on two consecutive weekdays. Moreover, data on children's life events, emotions and difficulties were collected. Statistical analysis was done separately for boys and girls by multilevel growth curve modelling with adjustments for age, body mass index, socio-economic status and wake up time.

RESULTS: In boys and girls with more negative life events during the last three months, the diurnal cortisol slope was steeper (more decline). Boys with higher self-reported happiness showed lower overall, morning and evening cortisol levels. In contrast, the diurnal slope was steeper (more decline) in boys with emotional problems due to higher morning values. In girls, peer problems were associated with lower overall and morning cortisol levels.

CONCLUSIONS: Children's salivary cortisol patterns were related to some negative life events, emotions and difficulties, although differently in boys and girls. As such, sex-differences in HPA functioning are already present in young children. Most findings support the upregulation of the cortisol response to stress, although lower morning values were found in the presence of peer problems in girls. Future studies should focus on sex differences, positive emotions and the diurnal cortisol slope.

KEYWORDS: salivary cortisol; children; emotions; strengths and difficulties; life events; stress; sex differences; diurnal slope

1. Introduction

Problem behaviour and stress are frequent phenomena in children, with prevalence estimates between 5 and 26% (Brauner and Stephens, 2006). Stress is linked not only to psychological but also physiological health complaints through behavioural responses and, more importantly, changes in the neuroendocrine system (Cohen et al., 2007). One of the major neuroendocrine systems adapting the organisms to stress situations, is the hypothalamus-pituitary-adrenal (HPA) axis with cortisol as hormonal end product.

Cortisol secretion has a circadian rhythm with lowest levels in the first half of the night and a peak in the early morning. Apart from this circadian rhythm, there is also a cortisol awakening response (CAR) showing a quick cortisol increase within 30 minutes after wake up (Fries et al., 2009). Salivary cortisol is a reliable reflection of serum free cortisol and due to its non-invasive character, it is routinely used as a stress biomarker in children (Tornhage, 2009). These cortisol patterns can be influenced by stress exposure, showing the psycho-physiological pathway through which psychosocial factors could influence health (Cohen et al., 2007). As such, research on the associations between psychometric data and cortisol patterns in children can help in disentangling the HPA functioning, in identifying psychobiological developmental pathways and in the prevention of stress-induced pathology.

Negative life events have been associated with both emotional and behavioural symptoms in children (Grant et al., 2004; Timmermans et al., 2010) and children exposed to a moderate degree of this psychosocial load, differed in their cortisol levels (Gustafsson et al., 2006). Stress has often been associated with higher cortisol values (Selye, 1956; Michaud et al., 2008). Nevertheless, the associations are quite complex, giving conflicting results and a hyper-/hypo-cortisolism hypothesis was published suggesting that recent exposure to a stressor may initially elevate cortisol levels, while the HPA axis may develop a counter-regulatory response of cortisol lowering after extended exposure to stress (Heim et al., 2000). This hypocortisolism is an allostatic situation characterised by lower morning cortisol, higher evening cortisol and a flatter diurnal slope and may also be a common phenomenon in children (Gunnar and Vazquez, 2001). Apart from this, variability in cortisol response is attributable to the nature of the stressor (social or physical) and also to the person facing it (emotional response and psychiatric sequelae) (Miller et al., 2007).

Furthermore, age and sex differences are observed in the HPA development. In adolescents, nonlinear age related increases were seen for both basal cortisol and cortisol reactivity and higher basal cortisol and cortisol reactivity were seen in pubertal girls compared to boys (Netherton et al., 2004; Gunnar et al., 2009). Most of the available research has been done on pubertal children and only few data are available in young children. Nevertheless, puberty-related increases in stress response and emotional reactivity have been reported (Spear, 2009). It is still unclear whether sex differences also exist in young children in which pubertal hormonal pathways are not yet activated. Possibly, the demonstrated sex differences in psychological functioning and development (Crick and Zahn-Waxler, 2003) could explain sex differences in cortisol reactivity.

To fill this research gap, we investigated the response of cortisol patterns on challenges during children's development, in a non-clinical, healthy population of 5-10 year old children. This was done by studying the relationship of healthy children's negative life events, emotions and difficulties with their salivary cortisol patterns (overall elevation, diurnal slope and CAR). Special focus is dedicated to sex differences in these preadolescent children. An advanced multilevel model enabled a detailed analysis of cortisol patterns allowing to simultaneously assess all parameters related to overall concentrations as well as CAR and diurnal slope (Hruschka et al., 2005; Adam, 2006). Moreover, apart from several stress-inducing psychometric data (negative events, emotions and difficulties), also the role of positive emotions is considered, which is new for this young age group.

2. Methods

2.1. Participants and general procedures

Participating children were part of the Belgian control region (i.e. Aalter, a city in Flanders, the northern, Dutch-speaking part of Belgium) of the IDEFICS study, funded within the European Sixth Framework Programme. The aim of IDEFICS was to identify and prevent dietary- and lifestyle-induced health effects in infants and children (Ahrens et al., 2011). In Belgium, children were selected for IDEFICS by random cluster sampling (all children derived from a selection of schools in the control city) and they could only participate after written informed consent of the parents.

The data used in this paper were collected from February 2010 to June 2010. The participating children were at that time between 5 and 10 years old. The IDEFICS children in the Belgian control region also participated in a sub-study (ChiBS, i.e. 'Children's Body Composition and Stress') to measure stress and to examine the relationship between stress and development of body composition. Stress was measured biologically by assessing salivary cortisol, as well as psychosocially via questionnaires. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital.

Although 750 children participated in the control region of Belgium, only 444 children provided voluntary salivary cortisol samples and 492 parent-child couples completed the necessary questionnaires. Eventually, 385 children were included for statistical analysis. No differences in psychometric, salivary cortisol or socio-demographic data were observed between the included and excluded children.

2.2. Questionnaires on life events, emotions and difficulties

<u>Parental questionnaire</u>

The parents were asked to complete the ‘Strengths and Difficulties Questionnaire’ (SDQ), a self-administered questionnaire on their children’s difficulties over the last six months (Goodman, 1997). For each statement, parents could give three possible answers: ‘not true’, ‘somewhat true’ and ‘certainly true’. The original SDQ consists of 5 subscales: emotional symptoms, conduct problems, hyperactivity, peer problems and a prosocial scale. The SDQ was part of the IDEFICS study, except for the hyperactivity scale which was not conducted. A *‘difficulties’* score was calculated by summing up the subscales for emotional symptoms, conduct problems and peer problems, in accordance to the questionnaire manual. The prosocial scale was considered as a separate concept namely as a strength.

<u>Child questionnaire</u>

The children were individually interviewed by field workers at school to assess life events and emotions.

Life events. The ‘Coddington Life Events Scale’ for children (CLES-C) (Coddington, 1972), a tool for clinicians, was used to identify potential stress-inducing events. The English questionnaire was professionally translated into Dutch and translated back into English. This validated 36-item questionnaire assesses the frequency and timing of events relevant for this age group during the last year and it results in a *‘life change units’* score for different time spans (0-3 months ago, 0- 6 months ago, 0-9 months ago and 0-12 months ago). Apart from total events (negative and neutral/positive events), separate scores for negative events were calculated following the questionnaire guidelines.

Emotions. Children were inquired for their recent feelings. As in the study of Zimmer-Gembeck, the feelings anger, anxiety, sadness and happiness were rated on a 0 to 10 Likert-scale (Zimmer-Gembeck et al., 2009). To help the children to understand the feelings, pictures from a social skills training game for very young children (Dupondt, 1992) were displayed simultaneously.

2.3. Salivary cortisol

Saliva was collected into Salivette synthetic swabs especially designed for cortisol analysis (Sarstedt, Germany). The participants were asked to collect saliva during two consecutive weekdays at four time points: immediately after wake up, 30 minutes after wake up, 60 minutes after wake up and in the evening between 1900h and 2000h. To standardize sample collection, sampling instructions for the children and their parents were provided in a manual: the children were asked to sample when healthy and on a normal day; to strictly respect the time points; not to eat, drink or brush their teeth in the hour before collection; to avoid physical activity two hours before sampling; and to avoid caffeine-rich drinks and minimize medication on the sampling days. The parents were also asked to fill in a checklist aboutinstruction compliance.

Adherence to the sampling points was strictly requested as this may partially invalidate cortisol results (Kudielka et al., 2003). We found that cortisol concentrations differed between parental reported ‘time compliers’ and ‘time non-compliers’ (Michels et al., 2011). Based on these results, morning samples collected more than 5 minutes different from the requested time point and evening samples not collected between 7 and 9 PM, were excluded. Furthermore, samples of corticosteroid-users were also excluded (N=5).

The Salivettes were centrifuged for 5 minutes at 3000 RPM (Jouan CR412 centrifuge) and the filtrates were stored at -20 °C. Salivary cortisol was assayed within the first month after collection in the routine laboratory of the Ghent University Hospital on a Modular E 170 immunoanalyser system (Roche Diagnostics, Mannheim, Germany) using the Roche Cobas Cortisol assay. Details of this analysis technique are described elsewhere (van Aken et al., 2003). This competitive electrochemiluminescence immunoassay (ECLIA) with a measuring range of 0.018 - 63.4 µg/dl (0.49-1748.95 nmol/L) had an inter-assay coefficient of variation of 3.9% and an intra-assay coefficient of variation of 1.9% while for samples near the lower detection limit the coefficients of variation were 12.7% and 10.2%, respectively (based on laboratory's internal quality assessment). The cortisol concentrations from the analysis (µg/dL) were converted into SI units (nmol/L) by multiplying the values with a conversion factor of 27.586 (Young and Huth, 1998). More details and reference ranges on the cortisol values in this population were published elsewhere (Michels et al., 2011).

2.4. Confounding variables

Based on previous research on cortisol values, age, socioeconomic status, wake up time and body mass index (BMI) z-score were considered as potential confounding variables (Adam and Kumari, 2009). The parents completed a general questionnaire on demographic characteristics. The highest educational qualification of both parents was assessed according to the International Standard Classification of Education (ISCED, (Unesco, 2010)). ISCED levels 5 and 6 were collapsed to level 5. The highest ISCED level of both parents was considered as a proxy for socioeconomic status. Day-specific wake up time was reported in the parental checklist. Weight was recorded with an electronic scale (TANITA BC 420 SMA) to the nearest 0.1 kg and height was measured with a stadiometer (SECA 225) to the nearest 0.1 cm. For the weight and height measurement, the children wore only underwear and Tshirts. The BMI z-score was obtained by adjusting the children's BMI (BMI=weight(kg)/height(m)^2) for age and sex (Cole et al., 2000).

2.5. Statistical analyses

Due to a skewed distribution, cortisol concentrations were transformed by the natural logarithm. Mann-Whitney U test showed sex differences in psychometric data and sex differences were also observed in the exploratory correlation analyses. Therefore, the statistical model was built for girls and boys separately. Hierarchical linear modelling (HLM) was used to analyse the relation between the children's cortisol pattern and questionnaire data (life events, emotions and difficulties), adjusted for potential confounders (age, BMI, parental education and wake up time). HLM is a variant of multiple linear regression useful for data with a nested design, which is the case in this study as repeated cortisol measurements were obtained for each participating child. Therefore, a two-level model on the dependent variable 'cortisol' was used with the intra-individual parameters modelled at level 1 (cortisol pattern created by time of day) and the inter-individual parameters (questionnaire data and the personal characteristics as possible confounders) at level 2. HLM is increasingly used and has its advantages when analysing cortisol data: a high tolerance for within- and between-subject variation in sampling time, simultaneous modelling of multiple cortisol parameters (elevation, diurnal slope and cortisol awakening response) and added statistical power because of the within-person repeated-measures design (Hruschka et al., 2005). The HLM was performed in the HLM/2L program (version 7.0), using an approach similar to the one published by Adam (Adam, 2006). The significance level was set to $p<0.05$.

At level 1, the child's cortisol values were predicted by the time of day, to estimate the shape of each child's cortisol curve during the day. Time of day values were expressed as 'number of hours since wake up' for each participant each day and centred to midday as six hours post-awakening. A curvilinear model did not fit better than the linear model. To characterise the CAR, a design variable was created assigning the value 1 to the sample taken 30 minutes after wake up, and the value 0 to all other samples. By representing the CAR as a separate variable in the model, the CAR became a separate coefficient that could be predicted independently of the other parameters, such as the diurnal slope. A dummy variable for day of measurement was included to account for possible systematic cortisol differences across days. The reported day-specific wake up time, considered as a cortisol-confounder, was also included as a level 1 parameter because of its day-dependence.

In an exploratory analysis, each relevant parameter resulting from the questionnaires (total and negative events 0-3, 0-6, 0-9 and 0-12 months ago; the emotions happy, anger, anxiety, and sadness; and the SDQ-scores) was entered as a level 2 predictor of each of the relevant level 1 predictors (intercept, slope and CAR) and these models were adjusted for potential confounders (age, BMI, parental education and wake up time). The significant level 2 predictors were then entered simultaneously (all remained significant) to result in one model although also the other level 2 predictors were retested on their significance in this model. No multicollinearity was detected in the final model using the tau-matrix. Post-hoc HLM analyses were performed to examine the effect of the significant level 2 predictors on morning and evening cortisol by entering a noncentered 'time since wake up' or by centering at 12 hours post-awakening, respectively.

Maximal effects of the psychosocial factors on cortisol concentrations in our population can be calculated as follows: (% difference/scale point, as displayed in Table 2 or 3) * (range of this scale in the sample, as displayed in Table 1). Nevertheless, these predictions should be interpreted with caution as they may be influenced by outliers.

3. Results

In total, 183 boys and 202 girls were included in this study. Descriptive data resulting from the questionnaires, salivary cortisol samples and possible confounding variables are provided in Table 1. No significant differences between boys and girls were observed for salivary cortisol and the sociodemographic characteristics, while anxiety (p=0.015), sadness (p=0.001) and prosocial behaviour (p=0.004) were higher in girls and conduct problems (p<0.001) were higher in boys. When using the multilevel model not stratified for sex, no sex difference was seen in overall mean cortisol, although girls showed somewhat steeper diurnal slope (p=0.30, 0.9% higher).

(Insert Table 1)

Based on the intra-class correlation, the between-subject variance in boys determined 32.8% of the total cortisol variance, while their within-subject variance across time was 67.2%. In girls, the between- and within-subject variance were 38.7% and 61.3%, respectively. Boys and girls showed the expected diurnal slope, with higher levels in the morning and an average decline of 15.8% per hour for boys and 15.6% per hour for girls. A CAR increase of 21.3% and 26.1% was seen, for boys and girls respectively. Wake up time was significant in both boys and girls, with a maximal 28.56% and 48.95% cortisol change, respectively. BMI was associated with CAR in both boys (maximum 9.45%) and girls (maximum 26.4%) and also with the diurnal slope in boys (maximum 0.2%). Higher cortisol with increasing age was only found in girls (maximum 44.2%).

Table 2 and 3 provide the final hierarchical linear model results for boys and girls, respectively. For level 2, only variables with significant effects were retained.

In boys, overall cortisol values were lower among those with higher levels of self-reported happiness, even after adjusting for negative emotions. Diurnal cortisol slope was steeper in boys having a high negative event score in the last three months and having a high emotional symptom score or total score based on the SDQ. Levels of anger, anxiety, sadness, other SDQ subscales, total event scores and negative event scores on longer time-period were not significantly associated with any of the cortisol pattern parameters. Remarkably, also the total SDQ difficulties score was significant, although to a lesser extent than the emotional subscale. Post-hoc analyses in Table 4 showed lower morning and evening cortisol levels in boys with a high score on happiness and a higher morning cortisol levels in boys with a high emotional symptoms score.

In girls, overall cortisol values were negatively associated with peer problems and the diurnal cortisol slope was steeper when having a high negative event score in the last three months. Levels of happiness, anger, anxiety, sadness, other SDQ subscales, total event scores and negative event scores on longer time-period were not significantly associated with any of the cortisol pattern parameters. Post-hoc analyses in Table 4 showed lower cortisol morning levels in girls with peer problems.

The maximal cortisol difference between lowest and highest psychometric score was 2.2% in boys and 1.7% in girls for negative life events during the last three months, 3.6% for emotional symptoms in boys, 25.2% for happiness in boys and 26.6% for peer problems in girls.

(Insert Table 2, 3 and 4)

4. Discussion

The salivary cortisol concentrations from this child population showed a clearly significant diurnal pattern with higher morning and lower evening values and a less clear, although significant, CAR. The diurnal slope was comparable to a similar child study (Adam, 2006), but the CAR was quite low as not all children showed a morning increase. This was recently described in detail (Michels et al., 2011). The higher within-person compared to between-person variability clearly illustrated the impact of collection time resulting in a broad diurnal pattern. Age was a significant and powerful determinant for cortisol in girls, while BMI and wake up time were significant in both boys and girls. Several associations of negative events, emotions and difficulties were observed, with the strongest for happiness in boys (21.6% cortisol difference between lowest and highest happiness) and for peer problems in girls (22.4% cortisol difference between lowest and highest peer problems) (see methods for formula).

4.1. Overall sex differences

The association of life events, difficulties and emotions with salivary cortisol found in this study was different in boys and girls. Indeed, sex-specific stress-cortisol relations have been previously published but are conflicting as e.g. a newly validated questionnaire on symptoms, emotions, events and coping found positive correlations with cortisol only in girls (Osika et al., 2007), whereas in another study cortisol values were positively correlated with distress only in boys (Vigil et al., 2010). Almost all of these sex-specific associations were done in children starting from 10 years old or older and as such covering adolescence. Still, a study on 5-year olds showed sex differences in the association with psychometric data giving significance of hyperactivity and emotional problems in boys and of positive emotions in girls (Hatzinger et al., 2007).

Several aspects could explain these sex differences in cortisol associations. First of all, sex differences on the HPA-axis itself have been published (Netherton et al., 2004; Gunnar et al., 2009). The literature overall indicates higher basal and stress-challenged cortisol in girls (Netherton et al., 2004; Rosmalen et al., 2005; Hatzinger et al., 2007; Gunnar et al., 2009), although this was not always the case in preadolescent children (Netherton et al., 2004; Gunnar et al., 2009). In our study, no overall cortisol sex differences and only a slightly steeper diurnal slope in girls were detected. Secondly, differences in boys and girls have regularly been shown in the prevalence, developmental pathways and manifestation of psychopathology (Crick and Zahn-Waxler, 2003) and in handling stressful situations (Hampel and Petermann, 2005). After all, girls are considered more mature, have more advanced language skills and show more empathic and prosocial responses than boys during childhood (Crick and Zahn-Waxler, 2003). In addition, gender could be a moderator in the relation between symptoms and subjective well-being (Derdikman-Eiron et al., 2011). So, the sex differences present in the psychometric data of this study are in line with general literature: more sadness, anxiety and prosocial behaviour in girls, while more conduct problems in boys (Crick and Zahn-Waxler, 2003). In the next paragraphs, these sex differences are examined further in our group of young children and in relation to the specific psychosocial factors.

4.2. Associations between life events (CLES) and cortisol patterns

Concerning life events, no associations with cortisol were observed for total event scores but the diurnal slope was steeper for boys and girls when more negative events occurred during the last three months. This indicates that stress researchers should also consider the negative event score separately and not only the total event score that includes also some neutral/positive events. A recent study using the CLES in 9 to 16 year old children also found no association with wake up and evening cortisol, although the steepness of the diurnal slope was depending on the amount of lifetime adversities with a steeper slope in children having a moderate amount of adversities and a less steeper slope in children with a high amount of adversities(Gustafsson et al., 2010). A possible explanation of this different result, is the time frame in which the events took place. Our observed steeper slope for children with more negative events might be explained by only considering events that took place during the last year. In contrast, the study of Gustafsson used lifetime adversities and suggested that the accumulation of adversities over time might be a trigger to develop the counter-regulatory hypocortisolism. Overall, the calculated cortisol difference in the presence of negative life events was only minimal. Furthermore, the questionnaire only measured the occurrence of events and not the subjective stress perception or coping. So, it is not known whether the reported events were perceived as stressful and how the children dealt with it (Miller et al., 2007). Consequently, this could have disturbed the ability to observe stronger associations.

4.3. Associations between behavioural difficulties (SDQ) and cortisol patterns

The impact of SDQ difficulties has already been related with increased evening cortisol (Gustaffson 2006). Considering the behavioural difficulties, total score, the emotional scale and the peer problem scale were related to cortisol. No significance of conduct problems was seen, which was in line with previous research in 10 to 12 year olds (Sondeijker et al., 2007) and in 5 year olds using the same questionnaire (Hatzinger et al., 2007).

For boys, emotional symptoms and total SDQ score were associated with a higher slope and higher morning cortisol. This is quite in line with the study of Hatzinger et al. (2007) demonstrating a positive association between emotional symptoms and mean morning cortisol in boys , although they did not examine the diurnal slope. Indeed, a positive association between children’s internalizing/emotional problems and morning cortisol was also seen in another cross-sectional analysis, while the longitudinal analyses showed negative associations

(Ruttle et al., 2011), possibly reflecting the hyper/hypo-cortisolism hypothesis. In our study and in line with a previous publication (Crick and Zahn-Waxler, 2003), parental underestimation of emotional problems was higher for girls, which could possibly be explained by the sometimes undisclosed feelings in girls and by the differential treatment of sons and daughters by the parents. This underreporting might impede significance in the associations of emotional problems reported by the parents with cortisol in girls.

For peer problems we detected an opposite direction: this was associated with lower cortisol in girls. As far as we know, no other studies have demonstrated this sex difference in our age group. Previously, lower cortisol was observed to be related to lower peer status at school in 15-year olds (West et al., 2010), although a study in preschoolers showed highest median cortisol in the least liked/most disliked children (Gunnar et al., 2003). Sex differences in children's peer context has been reviewed with girls being more sensitive to the status of their friendships, more exposed to a wide variety of peer stressors and receiving higher levels of emotional provisions in their friendships (Rose and Rudolph, 2006). As such, peer problems may create a strong chronic stress situation in children leading to disturbed cortisol patterns.

4.4. Associations between children self-reported emotions and cortisol patterns

Although parental reported emotional problems were significant in boys, none of the scores for self-reported negative emotions (anger, sadness and anxiety) were related to cortisol. Nevertheless, self-reported happiness (positive emotion) was negatively associated with morning and evening cortisol in boys. This suggests that also positive emotions can have an influence on cortisol concentrations. Indeed, a longitudinal study showed that positive emotions could have a protective effect on well-being (Harker and Keltner, 2001). As far as we know, associations between positive affect and cortisol have not been reported in children, except for an indirect study demonstrating higher mean morning cortisol in 5-year olds that completed a story, of which only the beginning was fixed, with positive emotions, but only in girls (Hatzinger et al., 2007). In adults, Lai et al. found a negative association of evening cortisol with positive emotions and positive affect was more potent to influence cortisol secretion than its negative counterpart (Lai et al., 2005). Sex differences have been shown as positive affect was negatively associated with morning cortisol in women and with afternoon cortisol in men (Polk et al., 2005) and positive psychosocial resources could attenuate the cortisol response to an acute stressor only in men (Kirschbaum et al., 1995). In our study, we found only small negative correlations for self-reported happiness in boys, and no significant correlations in girls (data not shown). Since positive and negative affect are quite independent (Ryff and Singer, 1998), we cannot directly infer from the significant happiness finding that elevated cortisol can be interpreted as a sign of unhappiness. Consequently, further research should elucidate these sex differences and their presence in childhood.

4.5. Associations with the CAR

Psychometric data were not associated with the CAR. A recent meta-analysis found a global positive relation of the CAR with life stress, but not with negative emotions and only a limited with positive emotions (Chida and Steptoe, 2009). Nevertheless, the CAR phenomenon in our study population was difficult to examine as there was a high variability in the CAR with only half of the children having a morning increase.

4.6. Overall patterns in stress-cortisol associations

Overall, the stress-cortisol relations found in this study were more in line with the stress hypercortisolism hypothesis. Nevertheless, lower cortisol values were found in girls with peer problems. This might indicate (1) that the subjective stress assessment in this study mostly focused on recent stressors, or (2) that hypocortisolism in children is less frequent as it is mostly a consequence of chronic stress and thus time is needed to be induced. The latter might explain why there are more published hypocortisolism patterns in adolescents than in children (Bevans et al., 2008). Overall, both hypo- and hypercortisolism have been demonstrated as deviations from the normal functioning and much of the variability is attributable to the stressor and person characteristics (Miller et al., 2007). As a result, inconsistency in literature could be caused by the population characteristics (sex, age and clinical sequelae), the psychometric data (time perspective, psychological concept and reporter) and the cortisol methodology (high/low cortisol may have different implications at different times of the day; used statistics).

4.7. Strengths and limitations

Important strengths of this study were the standardized procedures, the use of different questionnaires including positive affect, the use of different reporters (child and parent) and the sophisticated statistics stratified by sex. Some limitations should also be mentioned. First of all, we only took saliva samples in the morning and evening, leaving noon and afternoon un-sampled. Nevertheless, morning and evening cortisol samples should be sufficient to study the diurnal slope (Kraemer et al., 2006). Secondly, more than two days of sampling could increase the reliability of our results, especially for the CAR (Hellhammer et al., 2007). Thirdly, we only used a subjective measure of time compliance in the salivary cortisol sampling since objective measurement of compliance was not feasible in this large population. We stressed the importance of timing and the exclusion of self-reported non-compliers can already improve the accuracy (DeSantis et al., 2010). Nevertheless, it is probable that non-compliant people are the most likely to report their timing incorrectly, which could lead to missing a part of the morning increase and as such result in the observed low CAR (Kudielka et al., 2003). Other sampling related factors could also have an influence on the cortisol pattern, although we tried to restrict them by using an elaborated manual

(Adam and Kumari, 2009) and excluding the non-compliant samples (Michels et al., 2011). Furthermore, we need to consider that the used psychometric questionnaires cannot describe the whole psychological experience of these children and that over- or underestimation is possible. Nevertheless, we tried to standardize the interviewing conditions in the absence of parents and schoolteachers. Moreover, this study was cross-sectional and questionnaires covered only a short time period (last year's events, six month's difficulties, recent emotions). Analysing rather trauma of years ago might be more appropriate to unravel the hypocortisolism hypothesis (Bevans et al., 2008). Nevertheless, it is planned to follow up these participating children during two years within the ChiBS study to further examine this aspect.

5. Conclusion

The relation between psychometric data and cortisol levels predominantly supported a possible cortisol stimulating effect of stressors in children, although lower cortisol values were detected for peer problems in girls. The strongest associations with salivary cortisol were found for happiness and peer problems. Apart from these, we have shown a relationship for stressors and emotional symptoms with the diurnal slope, emphasizing the need for slope examination. Furthermore, different associations were observed for boys and girls, suggesting that the psychometric influences on the stress-system are already sex-specific in preadolescent children. Especially, we discussed new findings on children's sex-differences in associations with peer problems and happiness. Consequently, the prevention of stress-induced pathology should focus on sex differences and also on the aspect of positive affect apart from the negative affect.

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Tables

Table 1: The children's personal and sociodemographic characteristics and descriptive data from the cortisol values and questionnaires on life events, emotions and difficulties

Table 2: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and difficulties corrected for relevant confounders in boys

Table 3: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and diffictulties corrected for relevant confounders in girls

Table 4: Post-hoc analyses for the significant level 2 predictors stratified by sex: effect on morning (wake up) and evening cortisol

Table 1: The children's personal and sociodemographic characteristics and descriptive data from the cortisol values and questionnaires on life events, emotions and difficulties

	Boys (n=183)				Girls (n=202)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Salivary cortisol (nmol/L)								
Immediately after wake up	12.66	6.12	1.66	56.00	13.84	8.96	1.49	76.41
30 minutes after wake up	13.28	8.17	2.07	77.79	14.61	6.96	2.01	55.72
60 minutes after wake up	8.97	5.00	0.74	40.55	9.15	5.98	1.27	44.14
Evening	2.17	1.97	0.52	17.93	2.11	1.82	0.50	14.65
Personal and sociodemographic characteristics								
Age (years)	8.44	1.18	6.21	10.99	8.39	1.20	5.23	10.93
Average wake up time (h; decimal)	6.97	0.45	5.95	8.50	6.95	0.52	5.83	8.28
Body mass index (z-score)	-0.36	1.74	-2.57	2.68	-0.18	1.23	-3.66	4.10
Highest parental occupation (ISCED-level between 0-5)	4.31	0.82	3.00	5.00	4.14	1.00	1.00	5.00
Child psychometric report								
Coddington Life Events Scale								
Total score last 3 months	50.07	61.63	0	357	44.67	55.66	0	265
Total score last 6 months	61.52	67.04	0	428	55.33	64.59	0	291
Total score last 9 months	68.01	69.52	0	447	65.39	68.04	0	343
Total score last 12 months	81.83	74.04	0	460	81.08	68.46	0	369
Negative event score last 3 months	27.14	43.76	0	220	28.64	41.00	0	172
Negative event score last 6 months	34.01	47.93	0	284	36.07	48.54	0	276
Negative event score last 9 months	37.63	50.36	0	303	42.11	52.29	0	328
Negative event score last 12 months	47.68	53.66	0	303	53.26	54.40	0	354
Emotions								
Happy	7.91	1.94	1	10	7.92	1.84	2	10

Angry	2.93	2.45	0	10	2.88	2.40	0	10
Sad	2.36	2.51	0	10	3.06	2.74	0	10
Anxious	1.80	2.64	0	10	2.36	2.85	0	10
Parental psychometric report of child								
Child's Strengths and Difficulties								
Total difficulties	5.08	3.55	0	18	4.81	3.51	0	19
Conduct problems scale	1.49	1.24	0	5	1.02	1.06	0	5
Emotional symptoms scale	2.28	1.94	0	9	2.57	2.03	0	9
Peer problems scale	1.36	1.49	0	7	1.27	1.49	0	7
Prosocial behaviour = strength	6.46	1.45	1	8	6.89	1.27	1	8

Table 2: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and difficulties corrected for relevant confounders in boys (n=183)

Fixed effect	Coefficient	SE	t-value	df	p	Interpretation
Cortisol intercept	2.2680	0.3232	7.02	178	<0.001	
Age	0.0152	0.0094	1.62	178	0.107	Not significant
BMI z-score	0.0274	0.0234	1.17	178	0.243	Not significant
Parental education	-0.0307	0.0355	-0.87	178	0.388	Not significant
Happy	*-0.0277*	*0.0128*	*-2.16*	*178*	*0.032*	*-2.8%/scale point*
Time since wake up (= slope)	-0.1469	0.0027	-53.33	177	<0.001	-15.8%/hour
Age	0.0019	0.0023	0.84	177	0.400	Not significant
BMI z-score	0.0028	0.0006	4.35	177	<0.001	+0.03%/scale point
Parental education	-0.0017	0.0032	-0.543	177	0.588	Not significant
Negative events last 3 months	*-0.0001*	*0.0001*	*-2.01*	*177*	*0.045*	*-0.01%/scale point*
SDQ Emotional symptoms[(a)]	*-0.0037*	*0.0013*	*-2.74*	*177*	*0.007*	*-0.4%/scale point*
Cortisol awakening response	0.1935	0.0190	10.16	179	<0.001	+21.3% on T_{30}
Age	0.0297	0.0158	1.87	179	0.063	Not significant
BMI z-score	-0.0186	0.0051	-3.68	179	<0.001	-1.8%/scale point
Parental education	0.0012	0.0396	0.03	179	0.977	Not significant
Wake up time	-0.1065	0.0511	-2.08	182	0.039	-11.2%/hour
Day	0.0488	0.0281	1.74	182	0.084	Not significant

Note. Natural logaritmic transformed cortisol values were used as dependent variable. Time since wake up (centered as six hours post-awakening), cortisol awakening response dummy (value 1 for sample 30 mintues after wake up), day of testing dummy and wake up time were entered as level 1 variable. SDQ (strengths and difficulties questionnaire) and its subscales, child reported emotions (happy, angry, sad, anxious), total and negative life event scores (score for 0-3, 0-6, 0-9 and 0-12 months ago) and day-independent confounders (BMI z-score, age, parental education) were entered as level 2 predictor on each level 1 predictor. Variables in the final model represent the independent effect of each variable as all significant variables were entered simultaneously. Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: $B_{\%change}$=[exp(B)]-1.

Interpretation issue. Since the diurnal slope is negative, decreases in slope result in a steeper decline (=higher slope).

(a) Also the total SDQ score was significant (β=-0.0014; p=0.043). This data was not included in the same final model for reasons of multicollinearity.

Table 3: Hierarchical linear model showing association between children's cortisol and questionnaire data on life events, emotions and diffictulties corrected for relevant confounders in girls (n=202)

Fixed effect	Coefficient	SE	t-value	Df	p	Interpretation
Cortisol intercept	2.4114	0.4056	5.95	197	<0.001	
Age	0.0765	0.0282	2.712	197	0.007	+7.4%/year
BMI z-score	-0.0113	0.0213	-0.533	197	0.595	Not significant
Parental education	-0.0489	0.0322	-1.52	197	0.130	Not significant
SDQ Peer problems	*-0.0389*	*0.0178*	*-2.19*	*197*	*0.030*	*-3.8%/scale point*
Time since wake up (= slope)	-0.1457	0.0031	-46.70	197	<0.001	-15.6%/hour
Age	0.0034	0.0033	1.041	197	0.299	Not significant
BMI z-score	-0.0001	0.0019	-0.034	197	0.973	Not significant
Parental education	-0.0023	0.0026	-0.91	197	0.366	Not significant
Negative events last 3 months	*-0.0001*	*0.0001*	*-1.98*	*197*	*0.048*	*-0.01%/scale point*
Cortisol awakening response	0.2320	0.0247	9.38	198	<0.001	+26.1% on T_{30}
Age	-0.0371	0.0223	-1.66	198	0.098	Not significant
BMI z-score	0.0336	0.0146	2.29	198	0.023	+3.4%/scale point
Parental education	-0.0143	0.0272	-0.52	198	0.601	Not significant
Wake up time	-0.1966	0.0557	-3.53	201	0.002	-17.8%/hour
Day	0.0301	0.0367	0.82	201	0.414	Not significant

Note. Natural logaritmic transformed cortisol values were used as dependent variable. Time since wake up (centered as six hours post-awakening), cortisol awakening response dummy (value 1 for sample 30 mintues after wake up), day of testing dummy and wake up time were entered as level 1 variable. SDQ (strengths and difficulties questionnaire) and its subscales, child reported emotions (happy, angry, sad, anxious), total and negative life event scores (score for 0-3, 0-6, 0-9 and 0-12 months ago) and day-independent confounders (BMI z-score, age, parental education) were entered as level 2 predictor on each level 1 predictor. Variables in the final model represent the independent effect of each variable as all significant variables were entered simultaneously. Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: $B_{\%change}$=[exp(B)]-1.

Interpretation issue. Since the diurnal slope is negative, decreases in slope result in a steeper decline (=higher slope).

Table 4: Post-hoc analyses for the significant level 2 predictors stratified by sex: effect on morning (wake up) and evening cortisol

	Morning[(a)]			Evening[(b)]		
	Coefficient	p	Interpretation	Coefficient	p	Interpretation
Boys						
Happy	-0.0209	0.030	-2.1%/scale point	-0.0331	0.024	- 3.4%/scale point
Negative events last 3 months	0.0001	0.302	Not significant	0.0002	0.731	Not significant
SDQ Emotional symptoms	0.0017	0.038	+0.2%/scale point	-0.0092	0.503	Not significant
Girls						
SDQ Peer problems	-0.0428	0.035	-4.2/scale point	-0.0130	0.452	Not significant
Negative events last 3 months	-0.0001	0.063	Not significant	-0.0001	0.620	Not significant

Note Since cortisol values were log-transformed, the following transformation has been applied to the B coefficient for interpretation: $B_{\%change}$=[exp(B)]-1. The level 2 predictors were entered on their own.
SDQ= strengths and difficulties questionnaire
(a) Time not centered= wake up time
(b) Time centered at 12 hours post-awakening