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Running head: PARENTS' STRESS AND CHILDREN'S CORTISOL

Parents' psychological stress over time may affect children's cortisol at age 8

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Parents' psychological stress over time may affect children's cortisol at age 8

**Objective** To examine possible relations between parents' psychological stress and children's saliva cortisol levels in connection with a mild stressor (drawing a blood sample).

**Method** Parenting stress and serious life events at birth, age 1, age 2, age 5 and age 8 were assessed. 82 paired saliva samples collected from their 8 year old children just before and 30 minutes after children's blood was drawn were analyzed.

**Results** Repeated measures general linear models indicated a significant decrease of cortisol levels in relation to blood being drawn and a significant relation between higher parenting stress at child age 1 and at age 8, and elevated cortisol levels. A *t* test showed that cortisol levels after a blood draw were significantly higher in children whose parents reported a serious life event at age 8.

**Conclusion** Parenting stress could be a relevant factor for children's adjustment of the HPA axis with long-term effects and leave children more vulnerable to experiences of stress.

**Key words** parenting stress; serious life events; saliva cortisol; blood sample

Parents' psychological stress over time may affect children's cortisol at age 8

Cortisol has been established as a biological marker for the experience of stress in adults (e.g., Dickerson & Kemeny, 2004; Van Eck, Berkhof, Nicolson, & Sulon, 1996) and children (e.g., Gunnar & Donzella, 2002; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), as it is the main hormone released as a result of hypothalamic-pituitary-adrenal (HPA) axis activation. The HPA axis is activated in stressful situations, and through the release of cortisol and other hormones, it activates and concentrates the body's resources. Whereas short term activation of the HPA axis can help the person deal with demands, long term activation can harm the body (Charmandari, Tsigos, & Chrousos, 2005; McEwen, 2008).

Cortisol secretion increases as a biological response to stressful circumstances and returns to normal levels afterwards in all age groups (Kudielka et al., 2004). Cortisol secretion also shows variations throughout the day, with a higher cortisol level in the morning (Van Cauter, Leproult, & Kupfer, 1996), peaking at about 30 minutes after awakening, and thereafter declining to a lower level in the evening. This natural diurnal fluctuation becomes established within the individual some time before the second year of life (Gunnar & Donzella, 2002). Cortisol levels in the morning are highly individual (Rosmalen et al., 2005) and may depend in part on heritability (Bartels, De Geus, Kirschbaum, Sluyter, & Boomsma, 2003).

Several authors argue that the HPA axis is adjusted early in life through parent – child interaction in response to stressors (Essex, Klein, Cho, & Kalin, 2002; Flinn, 2006; Gunnar & Donzella, 2002; Repetti, Taylor, & Seeman, 2002; Taylor, Lerner, Sage, Lehman, & Seeman, 2004). Biological adjustment of the HPA axis to social and emotional stress early in life may be an explanation why growing up in stressful environments is a risk factor for mental and physical ill-health (Taylor et al., 2004). Flinn (2006) argued that a difficult social environment or trauma early in life increases the responsiveness of the HPA axis to socially stressful situations, which results in a stronger cortisol response for socially stressful situations later in

life. For infants, low maternal responsiveness is associated with elevated cortisol levels (Blair et al., 2008; Mörelius, Nelson, & Gustafsson, 2007) and children who are exposed to severe abuse or violence in the family have lasting altered responses of the HPA axis (Flinn, 2006; Pendry & Adam, 2007; Taylor et al., 2004). Is it true that even children in stressed families from the general population have an altered HPA axis that leads to extended stress reactions?

Parents have a prominent role in how infants' and young children's stress responses develop (Essex et al., 2002; Repetti et al., 2002; Taylor et al., 2004). One focus of the current study is, whether parents' experiences of psychological stress during the child's first years of life are related to HPA axis activity and responsiveness later in childhood. Our conceptual reasoning is that parents who experience stress may create a more stressful environment for their child (Essex et al., 2002; Mörelius et al., 2007; Östberg, 1998) and are less likely to teach their child how to handle stress (Gunnar & Donzella, 2002; Repetti et al., 2002). Hence, children of stressed parents may develop an increased baseline HPA axis activity and a stronger response of the HPA axis in stressful situations compared to children whose parents are not stressed.

Some aspects of parents' psychological stress can be measured by assessing parenting stress (Östberg, Hagekull, & Wettergren, 1997) and parents' experience of serious life events (e.g., Holmes & Rahe, 1967; Williamson et al., 2003). Parenting stress is the experience of stress in the domain of parenting and it is a stable construct (Östberg, Hagekull, & Hagelin, 2007) over several years. In general, serious life events give rise to some general life stress over a longer period of time (Williamson et al., 2003). Hence, both parenting stress and experience of serious life events represent long-term, perhaps even chronic stress of the parents rather than some short-term, transient stress. Children have to adjust to the constant stress of their parents and accordingly, HPA axis activity adjustments may be long-lasting. Experience of stress in the child's first year of life may have lasting effects throughout

childhood and even beyond as this is a sensitive time for the development of HPA axis functioning (Gunnar & Donzella, 2002). Experiences of stress later in childhood may still be important for adjustments of HPA axis activity but may not have the lasting effect as the HPA axis is not as sensitive as in the first year of life.

In the current study, parents' psychological stress (assessed as parenting stress and parents' experience of serious life events) during the child's first 8 years of life was examined in relation to children's morning cortisol levels before and thirty minutes after a mild stressor (a blood sample was drawn). It was hypothesized that baseline levels of children's saliva cortisol (assessed with a sample before a mild stressor was administered) increase in relation to higher parenting stress and parents' experience of serious life events. Furthermore, it was hypothesized that an increase in cortisol levels (assessed as the difference in cortisol level before and after a mild stressor was administered) would be observed as a response to the stressor in children whose parents reported more psychological stress. The relation between parents' psychological stress and children's cortisol was expected to be observed both longitudinally and cross-sectionally.

## Method

### *Participants*

The current study was part of the All Babies in Southeast Sweden-project (ABIS), which aims to study causes of type 1 diabetes by following a general population cohort of 17 000 children born between October 1997 and October 1999 from birth to adolescence. Parents completed questionnaires at the child's birth, when the child was 1 year old, 2-3 years, 5-6 years, and 8 years old (referred to as birth, age 1, age 2, age 5, and age 8). Children who were born between October 1997 and October 1998, who attended one of thirteen schools in a medium sized Swedish city (with about 140,000 inhabitants), and participated in ABIS at age 8 were eligible for the current study. Overall, 165 children were eligible and 126 children

decided to participate. There were 53 (46 %) boys in the sample and the mean age of the children was 7 years 10 month ( $SD = 4.7$  month). Mothers were between the ages of 21 and 43 years ( $M = 30.5$ ,  $SD = 4.7$ ) and fathers between the ages of 20 and 56 years ( $M = 32.5$ ,  $SD = 5.5$ ) when their child was born. Nine mothers and 10 fathers were born abroad (missing data  $n = 4$ ). Children lived either with both parents ( $n = 101$ ), with the mother only ( $n = 1$ ), with the mother and a stepfather ( $n = 2$ ), or alternating between the mother's and the father's home ( $n = 8$ ; missing data  $n = 14$ ). Forty-seven mothers and 45 fathers had a university education of at least 3.5 years (missing data  $n = 10$ ) when their child was 8 years old.

Some longitudinal data are missing. At age 1, 89 families; at age 2, 83 families; at age 5, 81 families returned questionnaires. Generally, questionnaires were completed by the mothers, but some were answered by fathers, age 1:  $n = 3$ , age 2:  $n = 8$ , age 5:  $n = 10$  and age 8:  $n = 15$ . In one family the father answered all questionnaires.

Participants not differ significantly concerning children's, mothers', or fathers' age, mothers' or fathers' education, custody status, proportion of parents born abroad, or parenting stress when compared to the children who were eligible for the current study but did not participate (data not shown). However, a higher proportion of experience of serious life events was reported at age 2,  $\chi^2(1) = 7.10$ ,  $p < .01$ , but not at other time points (data not shown) by parents whose children were eligible but did not participate. Furthermore, no significant differences concerning children's, mothers', or fathers' age, custody status, proportion of parents born abroad, parenting stress, or experience of serious life events were found comparing the current sample to all other participants of the ABIS 8-year follow-up (data not shown). However, a significantly higher proportion of parents in the current sample had attended university for 3.5 years or longer (mothers:  $\chi^2[1] = 22.41$ ,  $p < .001$ , fathers:  $\chi^2[1] = 40.82$ ,  $p < .001$ ) compared to all other participants in the ABIS 8-year follow-up.

### *Procedure*

Parents were asked to participate in ABIS before the birth of their child. When parents consented to participate they were asked to fill out questionnaires that included measurements for psychological stress, environmental factors, and health for both parents and children at the first health care check-up at their local well-child clinic about one week after their child's birth. Up to age 5 questionnaires were given to the accompanying parent at the regular health care check-ups at the well-child clinics. These clinics are used by > 99 % of Swedish parents. The parents filled out the questionnaires either during the visit at the clinic (but without any assistance) or later at home. At age 8, questionnaires were sent home to the families and returned by mail.

School nurses at the 13 schools asked children to participate in the current study and obtained informed consent from parents and children. Children were then asked to meet the research staff together with the school nurse in her office at each school. Instructions to children stated that they should not eat or brush their teeth the morning of the testing. Parents had received an anesthetic cream beforehand and had been asked to apply the cream on the child's arm before the child went to school. Saliva was collected before (cortisol sample 1) and thirty minutes after (cortisol sample 2) a venous or, if the child preferred, capillary blood sample was drawn. All venous blood samples were collected under local anesthesia by a trained research nurse. The authors of the current article explained the procedure on the day of testing to the children, recorded data and collected the saliva samples.

### *Ethical Considerations*

Parents received written and oral information and were invited to watch a video film about ABIS before they gave their consent to participate in ABIS. ABIS was approved by the research ethics committees of the Faculty of Health Sciences at Linköping University, Sweden and of the Medical Faculty, at Lund University, Sweden. Parents of children

participating in the current study were informed and asked permission for their child to participate in the current study prior to the day of testing at their school. On the day of testing, children were reminded that they could withdraw from the study at any time as well as decline from participating in certain parts (e.g. not provide a blood sample). When children appeared nervous, extra care was taken to comfort them and to ask if they wanted to proceed.

### *Measures*

#### *Parenting stress.*

Parenting stress was assessed with the Swedish Parenting Stress Questionnaire (SPSQ; Östberg et al., 1997) at age 1, age 2, age 5, and age 8. Good internal consistency (Cronbach's  $\alpha > .86$ ) has been reported for this instrument in three different samples (Östberg et al., 1997). This instrument is comprised of five subscales tapping the dimensions incompetence, spouse relationship problems, role restriction, social isolation, and health problems. At age 1 and age 2 the mean for parenting stress was based on all 34 items. At age 5 and age 8, due to space restrictions in the ABIS questionnaire, the subscales for social isolation and health problems were not assessed and the mean for parenting stress was based on the remaining 23 items. On each item a 6-point Likert-type response scale was used ranging from 1 (*strongly disagree*) to 6 (*strongly agree*). Analyses were based on the mean of all answered items and means were treated as missing if six or more items were unanswered. Higher means reflected more parenting stress after reversing some items according to the manual. For descriptive and psychometric details see Table 1.

#### *Parents' experience of serious life events.*

Parental experience of serious life events at birth was assessed with the following yes / no question: "Have you been exposed to something which you perceive as a serious life event during pregnancy (e.g. "death of a relative", "divorce")?". At age 2 and age 5, parents were first asked "Have you been exposed to something which you perceive as a serious life event

since your child was born?" Following this item parents were provided with a checklist (based on Holmes & Rahe, 1967) where they could mark which of the following serious life events they had experienced: "death of a relative", "divorce", "serious illness in the family", "serious accident in the family", "exposed to violence", and "becoming unemployed". If they had experienced any other type of serious life event they could state the event in an extra space provided. At age 8, the checklist for serious life events was further extended. Again, parents were first asked "Have you been exposed to something which you perceive as a serious life event in the last two years?". Following this item parents were provided with a checklist asking for above mentioned events and additionally: "death of spouse", "death of your parent", "self fallen seriously ill", "serious illness of a significant other", "experience of a custody trial for one's child/-ren". For analyses at all ages, two independent groups were compared: parents that reported a serious life event were compared to parents that did not report a serious life event, based on the initial yes/no question at each time point.

#### *Children's emotions.*

Children's emotional responses to the blood sampling were assessed with the Color Analogue Scale from 1 to 10 (CAS; Hicks, Von Baeyer, Spafford, Van Korlaar, & Goodenough, 2001). Children were asked how afraid they were before the blood sample was drawn, and afterwards how painful and unpleasant the drawing of the blood sample had been. Higher scores indicated more adverse responses.

#### *Children's cortisol levels.*

Cortisol levels were assessed from the children's saliva. Children chewed on an absorbent cotton dental roll for about two minutes and the dental roll was then stored in the plastic container provided. After all children had been tested at a school, saliva samples were transported back to the lab and centrifuged at 1500 G for 4 minutes in room temperature, in order to separate saliva from the cotton dental roll and then stored at - 20° Celsius until the

analyses were done. Staff at the laboratory of the Medical Center at Linköping University, Sweden analyzed the saliva samples with the Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, State College, PA, USA) according to manufacturers' instructions (sensitivity: 0.33 nmol/l – 82.77 nmol/l).

#### *Confounding variables.*

Possible confounding variables were: if children had had something to eat or drink, had brushed their teeth, or had been running or cycling within the last 30 minutes (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). These data were collected by asking the children. Another important variable was the time of day when saliva samples were collected (Van Cauter et al., 1996) and the time was recorded when saliva samples were taken.

#### *Statistical Analyses*

Samples with cortisol levels outside the approved sensitivity range as indicated by the manufacturer were removed from analyses ( $n = 3$ ) as they were not reliable. Descriptive statistics were reported on the actual cortisol values, but these were positively skewed (cortisol sample 1: skewness = 2.46,  $SE = 0.22$ ; cortisol sample 2: skewness = 2.95,  $SE = 0.23$ ). Hence, cortisol values were transformed with the natural logarithm (LN) for statistical analyses (transformed cortisol sample 1: skewness = 0.48,  $SE = 0.22$ ; transformed cortisol sample 2: skewness = 0.47,  $SE = 0.23$ ), as is common with saliva cortisol values (e.g., Kelly, Young, Sweeting, Fischer, & West, 2008; Van Eck et al., 1996) and which yielded a better normal distribution than a square root transformation.

In order to use time of day for cortisol sample collection as a variable in statistical analyses, minutes were transformed to a scale of 100 (by  $\text{minute} * 100/60$ ). Reported means for time of day have been transformed back to a scale of 60 for readability (Table 2). General linear models (GLM) were used to estimate the relations between cortisol sample 1 for time of day (entered as covariate), and gender, having eaten, brushed teeth, or exercised (entered as

fixed factors), as these could confound the relation between psychological variables and cortisol values.

Relations between children's emotions (fear, pain, unpleasantness, all entered as covariates) and cortisol values were studied with repeated measures GLM. Independent sample *t* tests were used to compare cortisol values in relation to parents' experience of serious life events. Furthermore, relations between psychological variables (parenting stress as covariate; serious life events as a factor) and cortisol values were tested with univariate and repeated measures GLM. Univariate models tested a direct relation between psychological variables and cortisol values with either cortisol sample 1 or cortisol sample 2 as dependent variable, whereas repeated measures models adjusted for the within subject variance of cortisol values and interactions between cortisol values and independent variables were employed. Significance of interaction terms indicates a relation between independent variables and change in cortisol values from sample 1 to sample 2.

SPSS software version 15.0 for Windows, Release 15.0.0 (6 September 2006) was used for statistical analyses. Due to internal attrition sample sizes vary among the analyses, in particular in the longitudinal analyses.

## Results

### *Psychological Variables*

Parenting stress was stable between follow-ups for the current sample (age 1 to age 2:  $r(60) = .70$ , age 2 to age 5:  $r(64) = .58$ , age 5 to age 8:  $r(76) = .63$ , all  $p < .01$ ). Descriptive statistics are shown in Table 1. Experience of serious life events before birth were reported by nine parents (7.4 % of valid cases, missing data  $n = 4$ ), at age 2 by 14 parents (17.1 % of valid cases, missing data  $n = 44$ ), at age 5 by 30 parents (37.5 % of valid cases, missing data  $n = 46$ ), and at age 8 by 29 parents (25.9 % of valid cases, missing data  $n = 14$ ).

Children provided a venous blood sample ( $n = 94$ ), a capillary blood sample ( $n = 19$ ), a venous and capillary blood sample ( $n = 4$ ), or no blood sample ( $n = 9$ ). Most children did not find having their blood drawn scary ( $M = 2.8$ ,  $SD = 2.5$ ), painful ( $M = 1.8$ ,  $SD = 2.6$ ), or unpleasant ( $M = 1.6$ ,  $SD = 2.2$ ). The capillary blood drawn ( $M = 2.9$ ,  $SD = 2.7$ ) was experienced as more painful than the venous blood drawn ( $M = 1.4$ ,  $SD = 2.5$ ),  $t(110) = 2.46$ ,  $p = .02$  (two-tailed),  $d = 1.55$ . No significant differences were observed for how scared children were or how unpleasant the procedure was comparing venous and capillary blood drawn (data not shown). No significant relations between emotional responses, that is being scared, feeling pain, finding blood drawn unpleasant, and cortisol levels were observed, all  $p > .05$ , all  $r < .17$ .

#### *Cortisol Levels*

Time of day was related to cortisol sample 1,  $\beta = -0.35$ ,  $t = 4.4$ ,  $p < .001$ , and after adjusting for time of day, children who had brushed their teeth had higher cortisol levels for sample 1,  $F(1,114) = 6.19$ ,  $p = .01$ , whereas no difference was found for having eaten,  $F(1,114) = 1.77$ ,  $p = .19$ , or having exercised,  $F(1,114) = 1.80$ ,  $p = .18$ . Children who did not provide a blood sample had lower cortisol levels,  $F(1,114) = 6.74$ ,  $p = .01$ . Therefore, children who had brushed their teeth,  $n = 29$ , or who did not provide a blood sample,  $n = 8$ , were removed from further analyses, leaving for the cortisol sample 1,  $n = 84$ , for cortisol sample 2,  $n = 83$ , and for paired samples,  $n = 82$  cases for analyses. Descriptive statistics for cortisol levels are shown in Table 2. Cases removed from the cortisol analyses did not significantly differ on parents' psychological stress, age, education, and origin, compared to the cases included in the final analyses (data not shown).

Repeated measures GLM analyses with cortisol as a within-subject variable and time of day as a covariate showed that cortisol levels decreased from cortisol sample 1 to sample 2,  $F(1,80) = 8.52$ ,  $p < .01$ ,  $\eta_p^2 = .096$ , and that there was a significant interaction between time of

day and decrease in cortisol levels  $F(1,80) = 8.25, p < .01, \eta_p^2 = .093$ , as visualized in Figure 1.

Parent's psychological stress was either reported by the mother or the father but no significant differences were found between mothers' or fathers' reports of parenting stress or reports of serious life events (data not shown). There were no significant differences between children's cortisol levels and which one of the two parents reported on psychological stress (data not shown). Furthermore, adjusting models for which parent reported on stress did not change the relations observed between parent's psychological stress and children's cortisol levels (data not shown). Due to the differences in parents' education between the current sample and all other participants in ABIS 8-year follow-up, cortisol levels and parenting stress were examined in relation to parents' education but no significant relations were found (data not shown).

#### *Parents' Psychological Stress and Children's Cortisol*

Crude and adjusted GLM analyzing the relation between parenting stress and cortisol levels are shown in Table 3. Repeated measures GLM analyses controlling for the within-subject variance in cortisol levels and between subject variance for time of day and children's gender also showed a significant relation between parenting stress at age 1 and cortisol levels,  $F(1, 53) = 4.88, p = .03, \eta_p^2 = .084$ , with a significant increase in cortisol sample 1,  $\beta = 0.37, t = 2.7, p = .01, \eta_p^2 = .118$ , but not in cortisol sample 2,  $\beta = 0.21, t = 1.3, p = .19, \eta_p^2 = .032$ . No relation was found between parenting stress at age 2,  $p = .19, \eta_p^2 = .034$ , or at age 5,  $p = .11, \eta_p^2 = .053$ , and cortisol levels. However, parenting stress at age 8 was related to cortisol levels,  $F(1, 74) = 8.38, p < .01, \eta_p^2 = .102$ , both in sample 1,  $\beta = 0.34, t = 3.1, p < .01, \eta_p^2 = .113$ , and sample 2,  $\beta = 0.25, t = 2.0, p = .046, \eta_p^2 = .053$ . Experience of serious life events, as tested by an independent sample  $t$  test did not show a significant difference in cortisol levels, except for serious life events reported at age 8 that related to higher cortisol levels in cortisol

sample 2 (details are shown in Table 4). After adjusting for time of day the relation between experience of serious life events and cortisol sample 2 was still significant,  $F(1, 69) = 5.40$ ,  $p = .02$ ,  $\eta_p^2 = .073$ . Repeated measures GLM analyses controlling for the within-subject variance in cortisol levels and time of day did not indicate any relations between serious life events and cortisol levels at any age, all  $p > .05$ , all  $\eta_p^2 < .04$ . Testing parenting stress and serious life events at age 8 in the same repeated measures GLM showed a significant relation between an increase in parenting stress and higher cortisol levels,  $F(1,67) = 5.71$ ,  $p = .02$ ,  $\eta_p^2 = .078$ , and a significant interaction between cortisol level and serious life event,  $F(1,67) = 4.80$ ,  $p = .03$ ,  $\eta_p^2 = .067$ . The interaction indicates that an experience of a serious life event related to an increase in cortisol levels, whereas no experience of a serious life event related to a decrease in cortisol levels, after adjusting for parenting stress.

### Discussion

In the current study we examined whether parents' psychological stress was related to their children's cortisol level before and after children experienced a mild stressor (blood drawn from their arm). Parents' psychological stress was assessed as parenting stress and the experience of serious life events. The main findings were that parenting stress related significantly to increased cortisol levels in children longitudinally and cross-sectionally. A significant relation was found between parents' experience of serious life events and children's cortisol levels cross-sectionally. Cortisol levels did not increase systematically as a reaction to the drawing of blood, and the children did not find the drawing of blood scary, painful, or unpleasant, suggesting that the intended stressor (having blood drawn) was actually not perceived as stressful.

The longitudinal relation found between parenting stress at age 1 and cortisol levels at age 8 supports the idea that children's HPA axis activation is adjusted early in life with regard to early experiences of stress (Essex et al., 2002; Flinn, 2006; Gunnar & Donzella, 2002). Early

experiences of stress may have a lasting effect on the HPA axis, resulting in an increased activity with constantly elevated levels of cortisol. In humans, evidence for this longitudinal effect has mainly been shown for children who experienced harsh family environments, including abuse or trauma (Taylor et al., 2004). Our results, on the other hand, indicate that children of stressed parents in the general population show a longitudinal adjustment. This is in line with evidence presented by Essex et al. for a longitudinal relation between parenting stress experienced by mothers during their children's first year of life and children's cortisol levels at age 4.5 years. However, Essex et al. concluded that maternal depression was the main explanation for the higher cortisol levels.

Conceptually, parenting stress may indicate social and emotional stress for the child, as parenting stress is linked to unresponsiveness (Östberg, 1998) and insecure child attachment (Robson, 1997), and creating a stressful environment for the child (Mörelus et al., 2007). Children may develop an increased activation of the HPA axis as their parents create a more stressful environment for them and/or fail to support them during experiences of stress. Parenting stress experienced in the first year of life seems to be related to lasting HPA axis adjustments and may leave children more vulnerable to experiences of stress later in life.

Lack of a significant relation between parenting stress at age 2 and cortisol levels at age 8 and a trend towards a significant relation between parenting stress at age 5 and cortisol levels at age 8 suggest that parenting stress in the first year of life would be more important for lasting adjustments of HPA axis activity than parenting stress during the preschool years. Hence, our results support a sensitive period for HPA axis adjustments in infancy (Gunnar & Donzella, 2002). Nevertheless, a cross-sectional relation at age 8 suggests that concurrent parenting stress is important for HPA axis activity, too, but may or may not have the lasting effect as parenting stress in the first year of life.

It is interesting to note that after adjusting for parenting stress, children whose parents experienced serious life events increased in cortisol levels, whereas children whose parents did not experience serious life events at age 8 decreased in cortisol levels. The differences in children's cortisol reactions in relation to parents' experience of serious life events support the notion that parents under stress do not help their children to adjust stress responses and HPA axis activity. If parents are stressed children may be more vulnerable to experience stress outside the family leaving children more exposed to stress for example during a blood draw at school. Our results points to the importance of considering parents when helping children in clinical settings.

Children volunteered to participate in the current study and did not find the drawing of blood scary, painful, or unpleasant. As an anesthetic cream was used it was expected that children did not feel any pain. Nevertheless, a clinically interesting finding was that drawing blood from children who volunteer a blood sample is not systematically related to increased cortisol levels and may not be perceived as stressful. This was unexpected but is interesting for clinical research both for methodological and ethical reasons.

In large scale epidemiological studies parents' psychological stress has been used as a proxy for children's psychological stress early in life (e.g., Sepa, Wahlberg, Vaarala, Frodi, & Ludvigsson, 2005). Our results suggest that parenting stress in childhood may not only be a proxy for psychological stress experienced by the child but may relate to the child's actual cortisol levels. As genetic and environmental factors interact, further epidemiological research should consider heritability of HPA axis functioning (Bartels et al., 2003) together with parental behavior and stress when trying to explain adjustments of HPA axis activity.

#### *Methodological Considerations*

Further studies might investigate the effects of parenting stress on the whole diurnal fluctuation. For this investigation measuring cortisol levels just after waking up would be a

better baseline measure than the first sample we collected, especially since our samples were collected in a social situation that was not common for the child. Also, time of day for saliva sample collection varied and the time of day was used to adjust models statistically for this variation. However, parenting stress was related to cortisol levels in both samples, suggesting that parenting stress can be related to a general increase in cortisol levels.

All parents received the questionnaires during the clinic visits up until age 5, some chose to fill out the questionnaire at the clinic while others chose to fill it out at home. Since the difference was not judged to be of any importance, no information was gathered on where the questionnaires were filled out. However, it may be important if the responding parent was the mother or the father. Analyses did not indicate any systematic influences of parental gender. Unfortunately, too few fathers participated so mothers and fathers cannot be directly compared. Hence, parents' psychological stress may be important for children's cortisol levels, but we cannot conclude whether there is a difference between mothers' and fathers' influence.

Attrition analyses were performed but did not indicate systematic attrition or selection bias except an attrition related to serious life events at age 2 and higher education of parents in the current sample. It is unfortunate that attrition was related to serious life events, as this was of particular interest and can be a reason for the lack of a longitudinal relation between serious life events and children's cortisol levels. Education, on the other hand, was expected to be higher as the study was conducted in a city with a large university. Education did not relate to parenting stress nor to cortisol levels and did not account for the relation found between parenting stress and cortisol levels.

### *Conclusions*

When parents experience parenting stress during the child's first year of life their children may develop a lasting increase in HPA axis activity. Due to this sensitive period it may be

important to help parents to lower their stress levels. Since concurrent parenting stress also was related to children's cortisol levels, it may be beneficial for children if their parents are also supported, when school children in stressful circumstances are given a clinical intervention. Furthermore, for methodological, ethical, and clinical reasons it is interesting to note that children who volunteer a blood sample under local anesthetics do not find this procedure stressful. Finally, parenting stress may be a relevant factor for adjustment of their children's HPA axis with long-term effects, leaving the children more vulnerable to experiences of stress.

## References

- Bartels, M., De Geus, E. J. C., Kirschbaum, C., Sluyter, F., & Boomsma, D. I. (2003). Heritability of daytime cortisol levels in children. *Behavior Genetics*, *33*(4), 421-433.
- Blair, C., Granger, D. A., Kivlighan, K. T., Mills-Koonce, R., Willoughby, M., Greenberg, M. T., et al. (2008). Maternal and Child Contributions to Cortisol Response to Emotional Arousal in Young Children From Low-Income, Rural Communities. *Developmental Psychology*, *44*(4), 1095-1109.
- Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response, *Annual Review of Physiology*, *67*, 259-284.
- Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355-391.
- Essex, M. J., Klein, M. H., Cho, E., & Kalin, N. H. (2002). Maternal stress beginning in infancy may sensitize children to later stress exposure: effects on cortisol and behavior. *Biological Psychiatry*, *52*(8), 776-784.
- Flinn, M. V. (2006). Evolution and ontogeny of stress response to social challenges in the human child. *Developmental Review*, *26*(2), 138-174.
- Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, *27*(1-2), 199-220.
- Hicks, C. L., Von Baeyer, C. L., Spafford, P. A., Van Korlaar, I., & Goodenough, B. (2001). The Faces Pain Scale - Revised: Toward a common metric in pediatric pain measurement. *Pain*, *93*(2), 173-183.
- Holmes, T. H., & Rahe, R. H. (1967). The social readjustment rating scale. *Journal of Psychosomatic Research*, *11*(2), 213-218.

- Kelly, S. J., Young, R., Sweeting, H., Fischer, J. E., & West, P. (2008). Levels and confounders of morning cortisol collected from adolescents in a naturalistic (school) setting. *Psychoneuroendocrinology*, *33*(9), 1257-1268.
- Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. *Psychoneuroendocrinology*, *29*(1), 83-98.
- McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology*, *583*(2-3), 174-185.
- Mörelius, E., Nelson, N., & Gustafsson, P. A. (2007). Salivary cortisol response in mother-infant dyads at high psychosocial risk. *Child: Care, Health and Development*, *33*(2), 128-136.
- Pendry, P., & Adam, E. K. (2007). Associations between parents' marital functioning, maternal parenting quality, maternal emotion and child cortisol levels. *International Journal of Behavioral Development*, *31*(3), 218-231.
- Repetti, R. L., Taylor, S. E., & Seeman, T. E. (2002). Risky families: family social environments and the mental and physical health of offspring. *Psychological Bulletin*, *128*(2), 330-366.
- Robson, A. L. (1997). Low birth weight and parenting stress during early childhood. *Journal of Pediatric Psychology*, *22*(3), 297-311.
- Rosmalen, J. G. M., Oldehinkel, A. J., Ormel, J., De Winter, A. F., Buitelaar, J. K., & Verhulst, F. C. (2005). Determinants of salivary cortisol levels in 10-12 year old children; A population-based study of individual differences. *Psychoneuroendocrinology*, *30*(5), 483-495.

- Schwartz, E. B., Granger, D. A., Susman, E. J., Gunnar, M. R., & Laird, B. (1998). Assessing Salivary Cortisol in Studies of Child Development. *Child Development*, 69(6), 1503-1513.
- Sepa, A., Wahlberg, J., Vaarala, O., Frodi, A., & Ludvigsson, J. (2005). Psychological stress may induce diabetes-related autoimmunity in infancy. *Diabetes Care*, 28(2), 290-295.
- Taylor, S. E., Lerner, J. S., Sage, R. M., Lehman, B. J., & Seeman, T. E. (2004). Early environment, emotions, responses to stress, and health. *Journal of Personality*, 72(6), 1365-1393.
- Van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology and Metabolism*, 81(7), 2468-2473.
- Van Eck, M., Berkhof, H., Nicolson, N., & Sulon, J. (1996). The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. *Psychosomatic Medicine*, 58(5), 447-458.
- Williamson, D. E., Birmaher, B., Ryan, N. D., Shiffrin, T. P., Lusk, J. A., Protopapa, J., et al. (2003). The Stressful Life Events Schedule for children and adolescents: Development and validation. *Psychiatry Research*, 119(3), 225-241.
- Östberg, M. (1998). Parental stress, psychosocial problems and responsiveness in help-seeking parents with small (2-45 months old) children. *Acta Paediatrica*, 87(1), 69-76.
- Östberg, M., Hagekull, B., & Hagelin, E. (2007). Stability and prediction of parenting stress. *Infant and Child Development*, 16(2), 207-223.
- Östberg, M., Hagekull, B., & Wettergren, S. (1997). A measure of parental stress in mothers with small children: dimensionality, stability and validity. *Scandinavian Journal of Psychology*, 38(3), 199-208.

Table 1

*Descriptive statistics for parenting stress for all follow-ups*

Measure and time points	Descriptive statistics			Psychometric properties	
	<i>n</i>	<i>M</i>	<i>SD</i>	Cronbach's alpha	<i>n</i> of items
Parenting stress					
Age 1	85	2,50	0,61	0,89	34
Age 2	81	2,58	0,60	0,89	34
Age 5	79	2,70	0,70	0,88	23
Age 8	115	2,47	0,60	0,86	23

Table 2

*Descriptive statistics concerning cortisol levels (in nmol/l), time of day, and time delay between sample collection (in minutes)*

Descriptive statistic	Cortisol levels			Time of day	Time delay between samples
	Sample 1 ( <i>n</i> = 84)	Sample 2 ( <i>n</i> = 83)	Difference Sample 2 - Sample 1 ( <i>n</i> = 82)		
<i>M</i>	3,46	3,43	-0,03	8:56 AM	0,34
<i>SD</i>	2,53	2,97	2,70	0,38	0,06
Minimum	0,70	0,70	-6,90	7:48 AM	0,22
Maximum	15,20	21,60	15,60	10:30 AM	0,49

Table 3

*Univariate and adjusted general linear models (GLM) examining the relation between parenting stress and cortisol levels (in nmol/l)*

Measure and time point	Cortisol Sample 1					Cortisol Sample 2				
	<i>n</i>	<i>r</i>	$\beta$	<i>t</i>	<i>p</i>	<i>n</i>	<i>r</i>	$\beta$	<i>t</i>	<i>p</i>
Univariate GLM										
Parenting stress										
Age 1	57	.36	0,39	2,91	< .01	58	.20	0,23	1,55	.13
Age 2	56	.21	0,23	1,58	.12	56	.06	0,08	0,47	.64
Age 5	54	.25	0,21	1,82	.07	53	.11	0,11	0,80	.43
Age 8	80	.30	0,28	2,73	< .01	79	.19	0,20	1,71	.09
Adjusted GLM										
Parenting stress										
Age 1	57	.36	0,37	2,66	.01	57	.17	0,31	2,35	.02
Age 2	56	.21	0,23	1,64	.11	55	.10	0,20	1,48	.14
Age 5	54	.25	0,22	1,86	.07	53	.11	0,23	1,90	.06
Age 8	80	.30	0,28	2,66	< .01	78	.22	0,30	2,98	< .01

*Note.* Adjusted GLM for cortisol sample 1 are adjusted for gender

and time of day and GLM for cortisol sample 2 are adjusted for gender, time of day, and differences in cortisol levels in sample 1 and sample 2.

Table 4

*Cortisol levels (in nmol/l) for children whose parents did or did not report a serious life event and independent sample t tests examining the differences in cortisol levels*

Measure and time point	Cortisol Sample 1					Cortisol Sample 2				
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>t</i>	<i>p</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>t</i>	<i>p</i>
Serious life event										
At birth				1,46	.15				0,40	.69
No	72	3,61	2,69			72	3,54	3,15		
Yes	7	2,24	0,56			7	2,77	1,21		
Age 2				-0,46	.65				-0,74	.46
No	46	3,81	3,21			46	3,73	3,71		
Yes	9	3,56	1,32			9	3,67	1,57		
Age 5				-1,31	.20				-0,52	.61
No	35	3,46	2,53			35	3,67	3,92		
Yes	18	4,53	3,57			18	3,83	2,62		
Age 8				-1,05	.30				-2,15	.04
No	55	3,42	2,52			55	3,12	2,37		
Yes	19	3,91	3,01			19	4,60	4,53		

Figure Caption

*Figure 1.* Means for saliva cortisol levels (in nmol/l) are shown in relation to time of day (a.m.) for saliva sample collection. Children were tested twice, with a 30 minute delay, and lines represent mean changes for saliva cortisol levels. *n* indicates the number of children who provided a saliva sample at that time of day.

