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ScienceDirect
Biological Psychology 78 (2008) 20–28
BIOLOGICAL PSYCHOLOGY
www.elsevier.com/locate/biopsycho

Chronic stress, salivary cortisol, and α-amylase in children with asthma and healthy children

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Received 6 October 2007; accepted 12 December 2007
Available online 23 December 2007

Abstract

The present study examined whether chronic stress is related to daily life levels of salivary α-amylase (sAA), a marker for sympathetic activity, and cortisol in healthy children versus children with asthma.

Children's sAA and cortisol levels were measured repeatedly over 2 days. Chronic stress measures included interviews with children about chronic home life stress and interviews with parents about one marker of socioeconomic status, parental education.

Among children with asthma, higher chronic stress was associated with lower daily sAA output, while among healthy children, higher chronic stress was associated with flatter cortisol slopes.

In conclusion, chronically stressed children with asthma showed lower salivary α-amylase output, indicating lower sympathetic activity, and implying a possible mechanism for increased susceptibility to symptom exacerbations. In contrast, higher cortisol levels in healthy children with chronic stress may indicate, for example, an increased risk for infectious diseases. This dichotomy emphasizes the different biological effects of chronic stress depending on illness status.

Keywords: Salivary α-amylase; Cortisol; Childhood asthma; Chronic stress; Socioeconomic status

1. Introduction

Stress is known to activate two major biological systems, the hypothalamus-pituitary-adrenal (HPA) axis and the sympatho-adrenal medullary (SAM) axis. In humans, activation of the hypothalamus-pituitary-adrenal axis results in an enhanced secretion of the hormone cortisol. Cortisol has a typical circadian pattern with higher levels in the morning and lower evening levels (Van Cauter, 1995). Activation of the SAM axis, on the other hand, results in the release of epinephrine and norepinephrine from the adrenal medulla as well as norepinephrine from nerve terminals of the sympathetic nervous system (Goldstein, 2000; Kvetnansky and McCarty, 2000). While both cortisol and catecholamines can be measured in plasma, the field of psychoneuroendocrinology (PNE) has sought to develop non-invasive markers of both axes. In the case of cortisol, salivary cortisol has become a widely used and important tool (Kirschbaum and Hellhammer, 1994). Salivary cortisol levels correlate highly with serum levels (Kirschbaum and Hellhammer, 2007) and reflect the free/unbound fraction of total cortisol, which is thought to be the biological active fraction (Mendel, 1992; Pearson-Murphy, 2000).

The search for a similar non-invasive and easily obtainable marker of the SAM axis has raised salivary α-amylase (sAA) as a promising candidate. Salivary α-amylase is an enzyme important for carbohydrate digestion and its secretion is under strong neurohormonal control (i.e., released upon sympathetic stimulation; Baum, 1993; Smith, 1996). Strong evidence for the assumption of salivary α-amylase reflecting sympathetic activity has come from pharmacological studies. van Stegeren et al. (2006) were able to reduce stress-induced salivary α-amylase increases by application of a β-adrenergic receptor blocker and Ehlert et al. (2006) recently reported that stimulation of the sympathetic nervous system using the α2-adrenergic receptor antagonist yohimbine increased salivary α-amylase levels. Hence, salivary cortisol levels reflect the activity of the HPA axis, whereas salivary α-amylase activity can be considered a marker of sympathetic activity.

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doi:10.1016/j.biopsycho.2007.12.004

1.1. Relevance of salivary α-amylase and cortisol for health

Changes in salivary α-amylase and cortisol levels are thought to have implications for health. For example, two studies by Granger et al. (2006, 2007a) suggest a link between salivary α-amylase and disease. One study found that greater salivary α-amylase increases in response to laboratory challenges were observed in children with more parent-reported illnesses. The second study revealed that higher levels of salivary α-amylase after acute laboratory stressors were associated with increased health problems, such as respiratory problems, in children. With respect to cortisol, there is a large body of evidence linking it to disease. Changes in basal as well as stimulated cortisol levels are reported to be associated with different disease processes or susceptibilities towards different diseases (Chrousos, 1998), such that, for example, a decreased HPA axis activity is found in individuals with fibromyalgia (Chikanza et al., 1992) and atopic diseases (Buske-Kirschbaum et al., 1998).

In the present study, we compared salivary α-amylase and cortisol in children with asthma versus healthy children. Asthma was chosen because it is the most common chronic illness in childhood (Mannino et al., 1998), and has been linked to alterations in cortisol responses to acute laboratory stressors (Buske-Kirschbaum et al., 2003) as well as in autonomic nervous system activity (Kallenbach et al., 1985). Hence our first goal was to determine whether a chronic illness such as asthma would be associated with different patterns of daily life salivary α-amylase and cortisol in children.

1.2. Psychological factors linked to salivary α-amylase and cortisol

As mentioned above, psychological stress activates both the HPA axis as well as the SAM axis, which manifests as changes in cortisol and salivary α-amylase output. For example, salivary α-amylase has been found to respond to psychological stress (Bosch et al., 1996, 2003; Nater et al., 2006, 2005; Rohleder et al., 2006; Skosnik et al., 2000), a finding that is also true in children (summarized in Granger et al., 2006, 2007a,b). Interestingly, while a relatively large number of studies have investigated laboratory stress reactivity and salivary α-amylase, studies of salivary α-amylase variations during individuals' daily lives (basal levels) are relatively rare. Rohleder et al. (2004) reported salivary α-amylase in university students to show a diurnal pattern opposite that of salivary cortisol, with lowest values shortly after awakening followed by increases during the day. The same pattern was reported by others (Jenzano et al., 1987; Nater et al., 2007; Rantonen and Meurman, 2000). To our knowledge, however, there have been no studies investigating basal salivary α-amylase activity over the day in children and adolescents. Furthermore, not only are studies investigating basal salivary α-amylase levels rare, but even fewer studies have attempted to link it to psychological factors such as chronic stress. Only one study that we are aware of measured chronic stress and found a positive association with salivary α-amylase levels over the day in university students (Nater et al., 2007). One study that investigated differences in socioeconomic status (SES) in young children found salivary α-amylase reactivity to an acute stressor to be negatively associated with SES (Granger et al., 2006).

Many more studies have investigated the effects of acute stressors on cortisol secretion. Acute stressors are known to elicit a delayed increase in cortisol secretion with a slow decrease after the offset of the stressor, reaching baseline levels approximately 1 h later (for a review see Dickerson and Kemeny, 2004). However, the literature on the effects of chronic stress on basal cortisol levels is less consistent. Both elevated levels of diurnal cortisol output (Arnetz et al., 1987; Baum et al., 1983; Kosten et al., 1984; Schaeffer and Baum, 1984) as well as a reduced cortisol output (Heim et al., 2000; Miller et al., 2002; Vedhara et al., 2002; Yehuda, 2000) over the day were found to be associated with chronic stress. Furthermore, a recent meta-analysis highlighted the importance of taking stressor and person features into account (Miller et al., 2007). For example, stressors that elicited a flat diurnal cortisol profile with high afternoon and evening levels were characterized as ones that threatened physical integrity, involved trauma, and were uncontrollable.

The second aim of the present study was thus to compare the effects of chronic stress on basal salivary α-amylase and cortisol levels in children with asthma and healthy children, thus testing whether psychosocial factors could be linked to daily life profiles of these two biological markers.

1.3. Study aims

In summary, the goal of the present study was twofold. First, we aimed to compare basal salivary α-amylase activity and basal salivary cortisol levels in healthy children versus children with asthma. Second, we aimed to test the relationships between chronic stress and salivary α-amylase as well as cortisol. Chronic stress was defined in two ways in this study, the experiences of chronic stress in home life, as well as low family SES, based on parental education.

2. Methods

2.1. Subjects

A total of 92 children and adolescents were recruited from the Vancouver, BC community through advertisements in newspapers, magazines, and physicians' offices. 47 children and adolescents were physician-diagnosed with asthma according to NHLBI guidelines (NHLBI, 1997, 2002) and 45 children and adolescents were medically healthy. Children with any other chronic medical (besides asthma) or psychiatric illness were excluded. To assess this, during the telephone-screening interview, parents were asked about any chronic medical or psychiatric illnesses their child had as well as about any acute illnesses in the past month and any medications the child was taking. During their visit at the laboratory, parents were queried again about any major health problems, physical, emotional, or other types the child had. Furthermore, a differential blood count from the child was used to ensure that children were not having any acute health problems. Children with upper-respiratory illness during the past 4 weeks were rescheduled.

Table 1
Demographics of study participants

	Asthma	Healthy
Sex	31 male, 16 female	24 male, 21 female
Age	8–18	9–17
	(13.1 ± 2.6)	(13.1 ± 2.2)
Body mass index (kg/m^2)	14.1–32.5	15.9–29.4
	(21.7 ± 4.3)	(21.1 ± 3.8)
LSI–home life stress	1.5–4.0	1.0–4.5
	(2.4 ± 0.5)	(2.4 ± 0.8)
Years of parental education	11–27	11–27
	(16.8 ± 3.1)	(17.9 ± 3.7)
Asthma severity[a]	Mild intermittent: 9 Mild persistent: 16 Moderate: 14 Severe: 8	–
Asthma medication[b]	No medication: 6 β-Agonists: 39 Inhaled corticosteroids: 32 Both: 30	–

Means and standard deviations are given in parentheses; LSI, life stress interview (Hammen and Rudolph, 1999).
[a] Based on asthma medication use and asthma symptoms (Bacharier et al., 2004).
[b] Names and dosages of medications recorded directly from the label.

Table 1 summarizes information on sex distribution, age, and body mass index (BMI: kg/m^2) of all study participants as well as details on asthma severity and medication for children and adolescents with asthma. Neither sex nor BMI differed significantly between the two groups (sex: $\chi^2 = 1.52$, $p = .22$; BMI: $t_{90} = 0.75$, $p = .46$). Furthermore, age did not differ significantly between boys and girls in each of the two groups (asthma: $t_{45} = -1.03$, $p = .31$; healthy: $t_{43} = -1.53$, $p = .14$). We also did not find differences between healthy children and children with asthma with regard to ethnic descent ($\chi^2 = 11.24$, $p = .08$; European: 30 asthma/32 healthy; Chinese: 7 asthma/6 healthy; other Asian: 3 asthma/0 healthy; other: 3 asthma/6 healthy).

2.2. Protocol

Upon arrival at our research center, the study procedure was explained in detail and written consent was obtained from the accompanying parent. The child was asked for assent and then taken into a separate room and interviewed in depth regarding chronic stress (see below). Simultaneously, the parent was asked questions about the educational attainment (see below).

Subsequently, the child was familiarized with the Salivette device (Sarstedt, Nuembrecht, Germany) and asked to collect saliva samples over the course of the following 2 days by chewing on the cotton roll and move it around in the mouth for 60 s. On each day, saliva had to be collected 1, 4, 9, and 11 h after awakening. These sampling times were chosen both based on the recommendations by the MacArthur Research Network of Socioeconomic Status and Health (2000) with regard to cortisol sampling and further, to best reflect the salivary α-amylase daily profile established by Nater et al. (2007). *MEMS 6 TrackCap Monitors* (Aardex Ltd., Switzerland) were used to test compliance, defined as collecting a saliva sample within 1 h of the intended time. We found compliance to be satisfactorily high with 88.2%. We further checked how compliant subjects were based on their self-reported times of collecting saliva, and found that 86.7% collected their saliva samples within 1 h of the intended time, suggesting that participants self-report of compliance with saliva collection procedures was similar to electronic monitoring reports. To avoid false high or low values, participants were additionally asked to refrain from brushing their teeth, smoking, eating, and drinking (except water) at least 30 min prior to collecting saliva. After chewing on the cotton roll, the participants placed the roll in the Salivette device and then in a refrigerator as soon as possible. Samples were mailed back to the laboratory once data collection was finished.

2.2.1. Life stress interview

The UCLA life stress interview for children was used to assess the child's exposure to stressful experiences over the past 6 months (Hammen and Rudolph, 1999). This semi-structured interview covers chronic stress in various domains such as family relationships, friendships, school, and home life. Levels of chronic stress are rated by the interviewer in each domain with ratings ranging from no difficulties (1) to severe and persistent difficulties (5). The present study focused on home life stress, which captures factors such as parents' work stress and persistent health problems among family members. Dimensions of home and family life have consistently been found to predict biological markers in previous research (Chen et al., 2006; Miller and Chen, 2006).

The interview has been used successfully in children as young as eight, and has been shown to have good reliability and validity (Hammen, 1991).

2.2.2. Parent education

Parent education was measured as one indicator of family SES. The parent was questioned about the number of years of education each parent in the household had received. In two parent families, the higher of the two was used.

2.3. Biochemical analysis

Saliva samples were centrifuged at 800 × *g* for 5 min, transferred to deep-well plates, and stored at −30 °C until assayed. Cortisol has been shown to be stable at room temperature for a period of 2 weeks (Kahn et al., 1988) and salivary α-amylase for at least 96 h (Granger et al., 2006). Both cortisol and salivary α-amylase were shown to be flow-rate independent (Vining et al., 1983; Rohleder et al., 2006; respectively).

2.3.1. Salivary α-amylase

Salivary α-amylase activity was measured as described by Rohleder et al. (2006). In short, 20 μl of standards and diluted saliva (1:625) were incubated with 80 μl of substrate reagent (Roche Diagnostics, Mannheim, Germany) in a waterbath at 37 °C for 90 s. Optical density was measured at 405 nm. After second incubation (5 min) and measurement, increase in absorbance were calculated and transformed to salivary α-amylase concentrations using a linear regression calculated for each microplate (inter- and intra-assay variation <10%).

2.3.2. Cortisol

Free cortisol levels in saliva were measured in duplicates using a commercially available chemiluminescence assay (IBL, Hamburg, Germany). Inter- and intra-assay variation was below 10%.

2.4. Control variables

Medical variables potentially influencing biological measures in asthma were included as control variables. This included asthma severity and asthma medication use.

Asthma severity was classified as mild intermittent asthma, mild persistent asthma, moderate asthma, and severe asthma, according to the procedure described by Bacharier et al. (2004), i.e., based on the NAEPP/EPR2 Guidelines and the higher of symptom frequency and medication use.

Asthma medication was assessed by asking participants the number of times during the past 2 weeks they had used asthma medications. Asthma medications were divided into inhaled corticosteroids and β-agonists, and both the number of days a participant used inhaled corticosteroids and the number of days a participant used β-agonists were included as covariates.

Additional variables we controlled for in the present analyses were age and sex. Both were included because it is well-known that asthma shows gender differences in prevalence rates which changes over adolescence and early adulthood (Sears, 1998). This is even true for asthma morbidity outcomes, such as hospital admission rates (Skobeloff et al., 1992).

2.5. Statistical analysis

Data were analyzed using the Statistical Package for the Social Science Version 11.0.4 (SPSS Institute, Chicago, IL) by following a three-step approach:

(1) Preliminary analyses: To increase stability, salivary α-amylase and cortisol values were averaged over the 2 days. Further, to address substantial skews, sAA and cortisol data were transformed by natural log transformation ($\ln(x-1)$) (Tabachnick and Fidell, 2001). The slope of the regression line as well as the area-under-the-curves (AUC; trapezoid formula (Pruessner et al., 2003)) were calculated for cortisol and sAA daily profiles according to Pruessner et al. (2003).
(2) Salivary α-amylase and cortisol trajectories and group differences: With regard to the averaged sAA and cortisol trajectories, repeated measures ANOVAs were computed to reveal possible time, group, and time-by-group effects. Results were corrected by the Greenhouse–Geisser procedure where appropriate. To test for group differences in sAA and cortisol AUCs and slopes, univariate analyses were computed. Both types of analyses controlled for asthma severity, asthma medication, age, and sex. Asthma severity and asthma medication were thereby coded 0 for healthy children. Partial correlations including the above control variables were calculated to test for associations between cortisol and sAA AUCs and slopes.
(3) Psychological factors linked to salivary α-amylase and cortisol: To test for associations between physiological parameters and stress measures, two sets of hierarchical regressions were calculated. For healthy children, age and sex was controlled for (step 1) before adding the variable of interest (i.e., chronic home life stress, years of parental education; step 2). For children with asthma, all regressions additionally controlled for asthma severity and asthma medications (step 1), before entering one of the two stress measures (step 2).

For all analyses, p-values of $p < .05$ were considered significant.

3. Results

3.1. Preliminary analyses

The stability of cortisol levels over 2 days was rather low with significant correlations for the first two time points (+1 h: $r = .273$, $p = .013$; +4 h: $r = .325$, $p = .003$) and non-significant correlations for the last two samples (+9 h: $r = .066$, $p = .552$; +11 h: $r = .181$, $p = .101$). Hence, cortisol concentrations of the corresponding samples were averaged for the 2 days to increase stability. Interestingly, sAA levels showed a much higher stability with correlations between $r = .477$ ($p < .001$, +4 h) and $r = .653$ ($p < .001$, +11 h) (+1 h: $r = .560$, $p < .001$; +9 h: $r = .502$, $p < .001$). Nevertheless, these data were averaged as well in order to keep the following analyses comparable. Fig. 1 depicts the resulting trajectories of sAA and cortisol levels. Data were then tested for skewness. To address the considerable skew we found (sAA: skew = 1.72–2.69, S.D. = 0.25; cortisol: skew = 1.25–3.47, S.D. = 0.25), data were subjected to a natural log transformation (sAA: transformed skew = −0.62 to 0.03, S.D. = 0.25; cortisol: transformed skew = 0.07–0.99, S.D. = 0.25). These log transformed data were then used to calculate AUCs and slopes for both sAA and cortisol trajectories (sAA AUC: mean = 13.7, range = 5.2–21.7; sAA slope: mean = 0.02, range = −0.05–0.10; cortisol AUC: mean = 4.1, range = 0.4–8.9; cortisol slope: mean = −0.04, range = −0.08 to 0.03).

3.2. Salivary α-amylase and cortisol profiles and group differences

Controlling for age, sex, asthma severity, and asthma medication, repeated measures ANOVA using the averaged and log-transformed individual time point values revealed significant group differences in sAA concentration over time (group: $F_{1,85} = 3.96$, $p = .05$), with healthy children showing higher sAA values throughout the day. No significant changes over time or group-by-time interaction (time: $F_{3,255} = 0.37$, $p = .77$; interaction: $F_{3,255} = 0.182$, $p = .91$) were found. Cortisol trajectories, on the contrary, did show significant changes over time ($F_{3,255} = 2.82$, $p = .039$), but no group differences or group-by-time interaction (group: $F_{1,85} = 0.98$, $p = .33$; interaction: $F_{3,255} = 0.24$, $p = .87$). Similarly, when summary variables across the day were used, group differences were only found for sAA AUC (sAA AUC: $F_{1,85} = 4.15$, $p = .045$; sAA slope: $F_{1,85} = 0.21$, $p = .65$; cortisol AUC: $F_{1,85} = 1.35$, $p = .25$; cortisol slope: $F_{1,85} = 0.06$, $p = .81$).

Furthermore, there were no significant correlations between sAA and cortisol for either AUC or slope (AUC: $r = .13$ $p = .22$; slope: $r = -.07$, $p = .49$; partial correlations controlling for group, age, sex, asthma severity, asthma medication: AUC: $r = -.019$, $p = .86$; slope: $r = -.08$, $p = .45$).

3.3. Psychological factors linked to salivary α-amylase and cortisol

Next, separate regression analyses were computed for children with asthma and healthy children to test for associations between basal salivary measures (i.e., cortisol and sAA) and chronic stress measures (i.e., chronic home life stress and parental education). All regressions controlled for age and sex. Regressions computed for children with asthma additionally controlled for asthma severity and asthma medication.

3.3.1. Children with asthma

First, we tested whether chronic stress influenced sAA output variables in children with asthma. For this, sAA variables

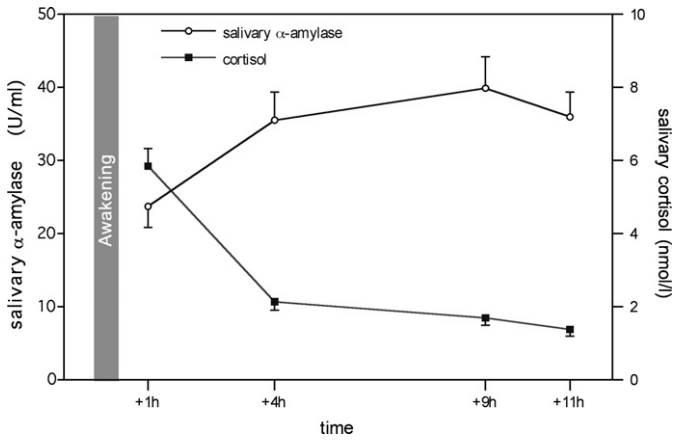

Fig. 1. Salivary α-amylase and cortisol trajectories in children ($n = 92$). Graph shows means and standard errors of values averaged over day 1 and day 2 (+1, +4, +9, and +11 h corresponds to 0928, 1248, 1729, and 1949 h, respectively).

Table 2
Regression analyses predicting salivary α-amylase and cortisol area-under-the-curves and slopes from chronic stress ratings

	Asthma ($n = 47$)			Healthy ($n = 45$)		
	β	t	p	β	t	p
Chronic home life stress						
sAA AUC	−.31	−2.30	.027*	−.09	−0.59	.56
sAA slope	.13	0.84	.41	−.22	−1.39	.17
Cort AUC	−.10	−0.65	.52	.09	0.61	.55
Cort slope	.07	0.45	.65	.43	2.98	.005**
Years of parental education						
sAA AUC	.29	2.04	.048*	.22	1.36	.18
sAA slope	.08	0.48	.63	.03	0.17	.87
Cort AUC	−.01	−0.07	.95	.19	1.24	.22
Cort slope	.11	0.66	.51	−.40	−2.69	.010*

Regression for healthy children controlled for age and sex; regression for children with asthma controlled for age, sex, asthma severity, and asthma medications. *Abbreviations*: sAA, salivary α-amylase; cort, cortisol; AUC, area-under-the-curve. *$p < .05$. **$p < .01$.

were regressed on scores of home life stress and years of parental education (see Table 2). We found a significant relationship between stress ratings and sAA AUCs, indicating that higher home life stress was associated with lower overall sAA output over the day in children with asthma ($\beta = -.31$, $p = .027$; see Fig. 2), explaining 9.3% of variance in sAA AUC values ($\Delta F_{(1,40)} = 5.28$). Furthermore, parental education was related to sAA AUCs as well, such that lower parental education predicted lower overall sAA output ($p = .048$, $\Delta R^2 = .075$, $\Delta F_{(1,40)} = 4.14$). No such associations were found for sAA slopes (home life stress: $p = .41$; parental education: $p = .63$).

In contrast, none of the cortisol output variables were associated with either home life stress (AUC: $p = .52$; slope: $p = .65$) or parental education (AUC: $p = .95$; slope: $p = .51$).

3.3.2. Healthy children

Next, we tested whether chronic stress influenced sAA output variables in healthy children (see Table 2). Contrary to the results in children with asthma, no associations between chronic home life stress and sAA output variables were found (AUC: $p = .56$, slope: $p = .17$). The same was true for parental education and sAA variables (AUC: $p = .18$, slope: $p = .87$).

However, both chronic home life stress and parental education did show strong relationships with cortisol slopes. Higher chronic home life stress was associated with flatter cortisol slopes in healthy children ($\beta = .43$, $p = .005$), explaining 16.6% of variance in cortisol slope values ($\Delta F_{(1,41)} = 5.89$). Furthermore, parental education was related to cortisol slopes, such that lower parental education predicted flatter cortisol slopes ($\beta = -.40$, $p = .010$, $\Delta R^2 = .14$, $\Delta F_{(1,41)} = 7.25$; see Fig. 3).

4. Discussion

This is the first study that we are aware of to examine simultaneously basal salivary α-amylase and cortisol trajectories throughout the day in healthy children as well as children with asthma. As expected, salivary α-amylase and cortisol were found to have opposite diurnal patterns. Furthermore, no significant correlations were found between daily salivary α-amylase levels and daily cortisol levels, emphasizing the distinctiveness of the two parameters being indicators of sympathetic activity versus HPA axis activity, respectively. Interestingly, only salivary α-amylase but not cortisol trajectories differed between children with asthma and healthy children. Additionally, there were differences in associations with chronic stress variables between children with asthma and healthy children. Among healthy children, having higher chronic home life stress and parents with less years of education predicted flatter cortisol slopes. In contrast, among children with asthma, higher chronic home life stress and having parents with less years of education were associated with a lower total amount of salivary α-amylase secreted over the day. Each of these findings will be discussed in more detail below.

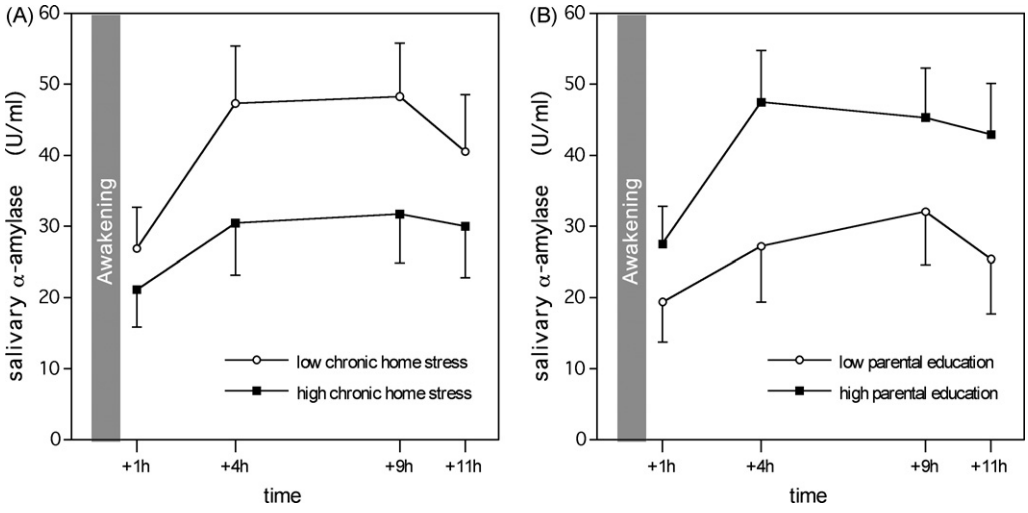

Fig. 2. (A) Chronic home life stress and (B) years of parental education predict salivary α-amylase trajectories (i.e., area-under-the-curve) in children with asthma ($n = 47$). Figures show means and standard errors. Ratings of chronic home life stress and years of parental education were categorized by median split.

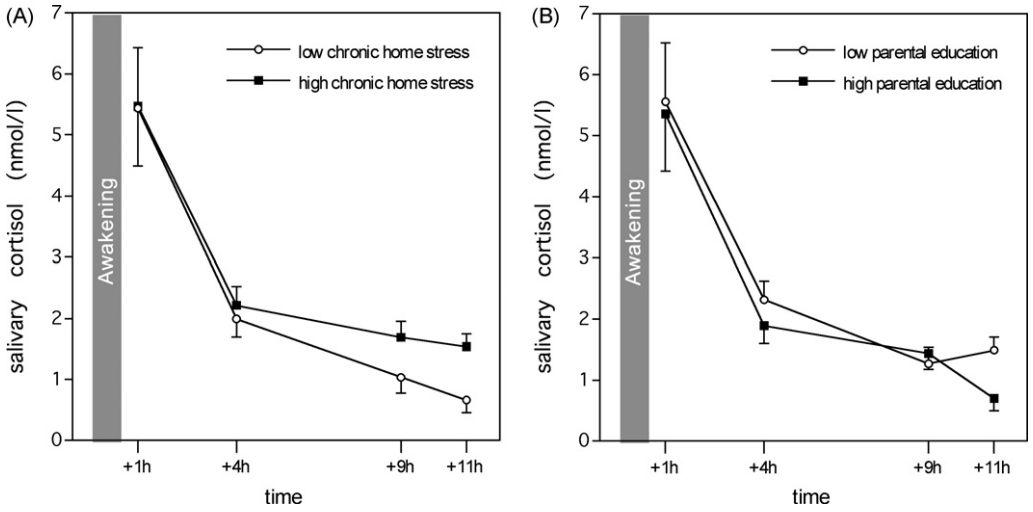

Fig. 3. (A) Chronic home life stress and (B) years of parental education predict cortisol trajectories (i.e., slopes) in healthy children ($n = 45$). Figures show means and standard errors. Ratings of chronic home life stress and years of parental education were categorized by median split.

4.1. Salivary α-amylase and cortisol profiles in children

The preliminary analyses showed that the stability of single salivary α-amylase measures was very high throughout the day. This finding argues for the usefulness of salivary α-amylase measures from a methodological point of view (e.g., practicality, cost effectiveness). In line with former stress-related findings (Chatterton et al., 1996; Granger et al., 2006; Nater et al., 2006, 2005), no correlation was found between basal salivary α-amylase and cortisol parameters. This endorses basal salivary α-amylase activity as a measure clearly distinct from cortisol with salivary α-amylase and cortisol outputs representing different systems, i.e., the SAM axis and the HPA axis, respectively. The daily pattern of salivary α-amylase and cortisol activity in children parallels previous findings as well (Rohleder et al., 2004), such that in adults, the trajectory of salivary α-amylase activity over the course of a day was opposite of that observed for cortisol, with lowest levels 1 h after awakening and increasing levels over the day.

Interestingly, compared to healthy children, children with asthma showed lower salivary α-amylase levels throughout the day. This finding suggests that salivary α-amylase levels may be an indicator that can distinguish chronically ill from healthy children. Furthermore, since lower salivary α-amylase levels indicate lower sympathetic activity, this suggests that increasing sympathetic activity may serve a protective function in asthma. This hypothesis is discussed in more detail below.

Children with asthma did not show any differences in cortisol trajectories compared to healthy children. Since altered cortisol levels have been found to have implications for health (see introduction), this lack of a difference is somewhat surprising. One hypothesis may be that compensatory processes (e.g., altered regulation of HPA activity by feedback inhibition; Dallman et al., 1994; De Kloet et al., 1998) are still successful because of the young age of this sample, such that overall daily output of cortisol is still similar across children with asthma and healthy children. We might than expect to see that over time as children age, compensatory processes may not be sufficient and differences in cortisol output may emerge, which would be expected to translate into differences in health outcomes. Alternatively, although cortisol levels do not differ, compensatory processes vary by illness status. For example, the sensitivity of target tissues, such as airway epithelial cells, to signals may be changed causing symptoms only in children with asthma (e.g., via changes in receptor expression level, hormone binding affinity, and/or repression by transcription factors; for cortisol see Bamberger et al., 1996; for catecholamines/sympathetic nervous system see Elenkov et al., 2000; Sanders et al., 1997).

4.2. Patterns of salivary α-amylase and cortisol with chronic stress

With respect to psychological variables, an interesting dichotomy was found whereby associations of chronic stress variables (i.e., chronic home life stress and years of parental education) with cortisol were found in healthy children but not in children with asthma, whereas associations of chronic stress variables with salivary α-amylase were found in children with asthma but not in healthy children. These findings could not be explained by nor were they moderated by sex or age (data not shown).

First, children and adolescents with asthma reporting high levels of chronic home life stress and having parents with less years of education showed lower salivary α-amylase activity throughout the day (AUC), suggesting lower sympathetic activity. In the clinical asthma literature, psychological stress is usually associated with negative clinical outcomes. Sandberg et al. (2000), for example, reported an increased risk for an asthma attack within 2 weeks of an acute negative life event in chronically stressed children with asthma. This raises the question of how elevated salivary α-amylase output might translate into changes in asthma outcomes. Asthma is a disease associated with increased parasympathetic activity (Kallenbach et al., 1985). In children and adolescents with persistent asthma, sympathetic activity may help counter-regulate asthma-associated increases in parasympathetic activity. If true, this would

argue for greater sympathetic activity being protective against asthma. Our findings of high chronic stress being associated with low sympathetic activity (low levels of salivary α-amylase) would then suggest a physiological vulnerability that could explain the clinical observations that high chronic stress is associated with greater asthma morbidity. This theory could also explain why associations with salivary α-amylase are only present in children with asthma but not healthy children, since it suggests that a chronic illness accompanied by changes in autonomic nervous system activity is necessary for detecting systematic variation in basal salivary α-amylase activity.

It should be noted that, in children with asthma, there were no associations between chronic stress variables and cortisol. One obvious explanation might be the medications that children and adolescents with asthma were on, especially inhaled corticosteroids used for long-term asthma control. However, patterns were the same regardless of whether we controlled for medication use or not.

Secondly, in healthy children, higher home life stress as well as parents having less years of education predicted flatter cortisol slopes. Thus far, reports linking blunted morning cortisol levels or flatter cortisol rhythms throughout the day with chronic stress exist mainly for adults. The types of chronic stressors that have been linked to flatter cortisol profiles in adults include burnout (Pruessner et al., 1999), work stress (Caplan et al., 1979), parenting a child with cancer (Miller et al., 2002), and SES (Cohen et al., 2006a,b). A study in adolescents, whose ages ranged from 15 to 19, reported a similar pattern of lower cortisol in association with low SES (Chen and Paterson, 2006). The meta-analysis by Miller et al. (2007) found that one of the characteristics associated with flat diurnal cortisol profiles was chronic stressors that were uncontrollable. From a child's point of view, chronic home life stress, which captures in part parents' work stress and persistent health problems among family members, may have a strong component of uncontrollability. Hence, consistent with the patterns found in adults, children showed a flatter cortisol profile under conditions of chronic stress as well. As mentioned above, changes in HPA activity are associated with increased susceptibility to various diseases (Chrousos and Gold, 1998). With regard to the immune system, for example, a lack of the modulatory activities of cortisol may put physically healthy but chronically stressed children at an increased risk for inflammatory diseases (Sapolsky et al., 2000).

Further emphasizing the dichotomy found in the present results, in healthy children, there were no associations between chronic stress and salivary α-amylase. Under acute stress conditions, in contrast, some evidence for SES-dependent differences in salivary α-amylase were found (Granger et al., 2006). However, as mentioned above, it might be that disease-related autonomic nervous system dysfunction is necessary to detect stable differences in salivary α-amylase.

4.3. Limitations

This study has several limitations: First, studies are needed which include other measures of autonomic nervous system (ANS) activity. Including measures of both sympathetic and parasympathetic activity would allow researchers to test the hypothesis of salivary α-amylase indicating sympathetic counter-regulation. Second, future studies including asthma outcome measures (e.g., clinical symptoms, pulmonary function) are needed to test the predictive value of salivary α-amylase. That is, are children and adolescents who have high salivary α-amylase more likely to have decreased asthma morbidity over time? If so, this would have important implications for the clinical assessment and management of asthma. Lastly, it has to be pointed out that in the present study, we operationalized chronic stress by measuring chronic home life stress and years of parental education. Since there are many other domains of chronic stress besides chronic home life stress as well as other ways to capture SES (e.g., resource-based indicators, such as family income or parental occupation, or prestige-based indicators, such as social status in the community), the present results are limited in terms of their generalizability.

4.4. Summary

In summary, healthy children and adolescents showed the expected pattern of a flatter cortisol rhythm being associated with higher levels of chronic stress. Over the long-term, such increased cortisol levels may have negative health effects, rendering healthy children, for example, at increased risk for infectious diseases. In contrast, in children and adolescents with asthma, high levels of chronic stress were associated with low levels of salivary α-amylase. This clearly distinct pattern of associations suggests that salivary α-amylase is more sensitive to social characteristics in chronic diseases involving altered ANS activity. Salivary α-amylase may thus serve both as a useful measure in PNE research on children and adolescents, as well as a relevant marker of sociobiological effects in asthma, since attenuated salivary α-amylase activity associated with chronic stress in children with asthma may indicate risk for future susceptibility to asthma attacks and symptom exacerbations. Ultimately, both findings emphasize that there are pronounced, but distinct, biological effects of chronic stress not only in children with asthma, but also in healthy children.

Acknowledgements

Funding for this study was provided by the Canadian Institutes of Health Research (CIHR) and the Michael Smith Foundation for Health Research (MSFHR).

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