Relationships between diurnal adrenocortical activity, children's attachment security and mothers' attachment representations

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This study investigated the relationship between diurnal adrenocortical activity and attachment quality (children's secure base behaviours and mothers' attachment representations). Forty-two children ranging in age from 18 to 26 months of age and their mothers participated in this study. Children and mothers' saliva samples were collected on two consecutive days (Saturday and Sunday), three times per day: awakening; 20m after awakening; bedtime. No significant relationships were found involving mothers' attachment representations. However, significant negative correlations between children and mothers' bedtime cortisol levels and children's attachment security were found.

Key words: Diurnal cortisol, Attachment, Secure base, Mothers' representations.

The hypothalamic-pituitary-adrenocortical axis (HPA) is responsible for the release of cortisol and its activity is regulated by the hypothalamus, which coordinates sensorial and cognitive inputs associated with stress regulation (Nelson, 2005; Stansbury & Gunnar, 1994). Daily activities that occur in a non-stressed state require a baseline of cortisol that shows a diurnal rhythm. This cortisol circadian rhythm is regulated by a circadian oscillator in the suprachiasmatic nuclei in conjunction with inputs from the limbic system, that together modulate adrenocortical activity, according to daily activities such as awakening, meals, napping or sleep (de Kloet, 1991; Stansbury & Gunnar, 1994). In humans, cortisol levels in the bloodstream have a strong diurnal variation, with 70% of the variability occurring during the day (Edwards, Clown, Evans, & Hucklebridge, 2001; Pruessner et al., 1997). The circadian rhythm of cortisol in adults on a normal day/night cycle is characterized by high early morning levels, that provide energy needed for action and stimulate the appetite for carbohydrates. These high levels are a result of cortisol peak levels, produced during the last hours of nightime sleep. After awakening, morning peaks decline strongly during the first hours and gradually thereafter. During the day, there is a temporary rise in cortisol around lunch time, which gradually falls down until nightime, where it reaches low levels, needed for rest (Stansbury & Gunnar, 1994). Thus, the awakening response is considered a safe biomarker in the study of the HPA dynamic functioning, as it shows a moderate to high, individual stability (Edwards et al., 2001; Pruessner et al., 1997). The diurnal rhythm of the HPA-axis and basal concentrations of cortisol change across development. The unbound fraction of salivary cortisol tends to be as high

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in newborns as in adults (Gunnar, 1992). However, newborns do not show the adult rhythm, expressing, instead, two peeks 12 hours apart (Gunnar & Donzella, 2002). Nevertheless, a daily rhythm in cortisol with high levels early in the morning and lowest concentrations in the evening is present from the earliest months (Larson, White, Cochran, Donzella, & Gunnar, 1998; White, Gunnar, Larson, Donzella, & Barr, 2000) emerging at about 3 months, when the single early morning peak in cortisol is reliably established (Gunnar & Donzella, 2002; Larson et al., 1998). During the first year, the infant's daily pattern shows differences when compared to the adult pattern, because it lacks a consistent decrease of concentrations over the middle portion of the day (Stansbury & Gunnar, 1994). This pattern is thought to be a result of a coordination of basal cortisol activity with napping and feeding schedules (Larson, Gunnar, & Hertsgaard, 1991). The expected small decrease in cortisol levels from mid-morning to mid-afternoon present in adults is observed only around four years of age (48 months) (Gunnar & Donzella, 2002), not in younger children (Larson et al., 1991), reflecting the development of mature sleep/wake patterns at this period of development (Gunnar & Donzella, 2002). No differences are found in children's HPA-axis diurnal rhythm and cortisol concentrations, associated with gender and body composition (Knutsson et al., 1997). However, relational aspects, such as the caregiver's quality of care and attachment relationship have been associated with children's adrenocortical regulation. Children with a secure attachment do not exhibit increases in cortisol levels when the attachment figure is present, unlike the insecure ones, more likely to show increases in the presence of the attachment figure, in relation to different types of stressors (medical examinations and maternal separations) (Gunnar, Brodersen, Krueger, Buss, & Rigatuso, 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998). The impact and effects of early experiences on HPA-axis responses seem to extend into adulthood. Attachment anxiety is negatively correlated with cortisol response to awakening and positively correlated with cortisol response to stressors in a group of adult women (Quirin, Pruessner, & Kuhl, 2008). Individuals who experienced early loss experiences (parents' divorce or death; detached relationship with surviving parent), particularly before the age of 14, show reduced cortisol responses upon awakening time (Meinlschmidt & Heim, 2005) and higher cortisol concentrations, during the exposure to stressful situations, compared to individuals without the same life history (Luecken, 2000). However, to best of our knowledge mothers' diurnal HPA-axis activity in response to their children's attachment security has not yet been fully investigated. Similarly, we are not aware of any studies on children's diurnal adrenocortical activity in response to their mothers' attachment representations. Some studies suggest that there might be a significant relationship between these two aspects, since parental experiences (maternal depression, financial overload, parental psychiatric illness) expressed during the first year of children's life have been linked to stress responses in the offspring (Essex, Klein, Cho, & Kalin, 2002; Warren et al., 2003). Moreover, maternal attachment representations have been associated with their children's (18-26 months) cortisol response to stressors (episodes of fear, positive affect, anger), in a marginal significant way (Roque, Veríssimo, Oliveira, & Oliveira, 2012). It is reasonable to expect that there might be significant relationships between mothers' attachment representations and their children's diurnal cortisol activity, as well as between mothers' adrenocortical activity and their children's attachment security, due to shared genetic background, environment (Spangler, 1991) and heritability of daytime cortisol levels and cortisol reactivity (Steptoe, van Jaarsveld, Semmler, Plomin, & Wardle, 2009).

The main goal of this study was to investigate the relationships between diurnal adrenocortical activity and attachment quality (children's secure base behaviours and maternal attachment representations) in a group of young children and their mothers. We expected to find:

1) Significant relationships between children's diurnal cortisol levels and their attachment security to the mother. In particular, we expected to find positive correlations between

children's cortisol responses to awakening and recovery to awakening and their attachment security to the mother (Meinlschmidt & Heim, 2005; Quirin, Pruessner, & Kuhl, 2008). On the other hand, we expected to find a negative correlation between children's bedtime cortisol response and attachment security, since the last may work as a buffer against daily-stress and contribute to the decline of cortisol levels at bedtime, needed for rest (Stansbury & Gunnar, 1994);

2) Significant relationships between children's diurnal cortisol levels and mothers' attachment representations, since there is evidence that there are significant relationships between parental experiences and attachment representations and children's cortisol levels (Essex, Klein, Cho, & Kalin, 2002; Roque, Veríssimo, Oliveira, & Oliveira, 2012; Warren et al., 2003). We expected to find positive correlations between children's cortisol responses to awakening and recovery to awakening and their mothers' attachment security. On the other hand, we expected to find a negative correlation between children's bedtime cortisol response and their mothers' attachment security;
3) Significant relationships between mothers' diurnal cortisol levels and their attachment representations. In particular, we expected to find positive correlations between mothers' cortisol responses to awakening and recovery to awakening and their attachment security (Meinlschmidt & Heim, 2005; Quirin, Pruessner, & Kuhl, 2008). On the other hand, we expected to find a negative correlation between mothers' bedtime cortisol response and attachment security (Stansbury & Gunnar, 1994);
4) Significant relationships between mothers' diurnal cortisol levels and their children's attachment security, due to shared genetic background and environment (Spangler, 1991). We expected to find positive correlations between mothers' cortisol responses to awakening and recovery to awakening and their children's attachment security (Meinlschmidt & Heim, 2005; Quirin, Pruessner, & Kuhl, 2008); and a negative correlation between mothers' bedtime cortisol levels and their children's attachment security (Stansbury & Gunnar, 1994).

Methods

Participants

Forty-two child-mother dyads (24 boys and 18 girls), all Caucasian, from bi-parental families participated in the study. Children's age ranged from 18 to 26 months of age (M=21.5; SD=2.01). Nineteen children had siblings and 21 were first-born. Thirty-six children attended day-care, spending there from seven to 11 hours (M=6.71; SD=2.92) each weekday. Children's age when first entered day-care ranged from four to 24 months of age (M=8.72; SD=4.68). Mothers' age ranged from 25 to 43 years (M=33.64; SD=4.22) and fathers' age from 26 to 55 years old (M=36.24; SD=6.18). Mothers' level of education ranged from nine to 19 years (M=15.02; SD=3.13) and fathers' from four to 19 years (M=13.62; SD=3.77). All parents worked outside the home, except for two mothers. Participants represented a range of socioeconomic status backgrounds, as reflected by parental education and were recruited from public and private daycare centers. All participants were healthy at the time of assessment and there were no premature children.

Materials and procedures

All procedures were carried out with the adequate understanding and written consent of the participants (mothers).

Attachment Behaviour Q-set (AQS; version 3.0). The Attachment Behaviour Q-set (AQS; Waters, 1995) evaluates the quality of the child's secure base behaviour towards the mother or other figures in an ecologically valid context, namely, the children's home, during a period of 2h. The 90 items of this instrument are distributed on a scale of 9 points, ranging from "extremely characteristic" to "extremely uncharacteristic". Mothers became aware of this work through an informed consent, left at their children's daycare. The AQS home visits were scheduled with the mother in a time of day when any other members of the family or friends were present at home. The visits were conducted by two observers that were trained not to disturb interactions in progress or interfere in domestic routines. The observers' agreement was analyzed through Spearman Brown correlations (M=.80). Individual Q-sorts, resulted from a mean between the descriptions of the two observers. Children final attachment score was obtained through a Pearson correlation between the child's individual Q-sort and the security criterion value of the "ideal child" (Waters & Deane, 1985). This correlation represents the place occupied by children on a security continuum. This value ranges between -1.0 and 1.0. Children who are able to use the mother or other figure as a secure base receive a higher value, while the least able to do it, receive lower values. In most normative samples, security scores average about .35 (Bost, 2006). The coders of infant attachment behaviours were in blind to maternal attachment status. This study uses the AQS for child attachment, instead of the Strange Situation (Ainsworth, Blehar, Waters, & Wall, 1978) procedure. Both measures are used in the field and both have proved to be valid measures to access quality of attachment. The validity of the AQS using observers, but not self-reported, has been clearly confirmed in a meta-analysis (van IJzendoorn, Vereijken, Bakermans-Kranenburg, & Riksen-Walraven, 2004) and it was included in the same category, in terms of quality, as that of the Strange Situation and the Adult Attachment Interview. Previous studies with Portuguese samples supported the utility and validity of the AQS in the Portuguese culture (Veríssimo, Monteiro, & Santos, 2006; Veríssimo, Monteiro, Vaughn, Santos, & Waters, 2005). Also, and very important, the Strange Situation is not recommended for the age level of our participants (Ainsworth et al., 1978).

Adult attachment representation narratives. The "Adult attachment representation narratives" (Waters & Rodrigues-Doolabh, 2004) is an instrument developed to gain access and analyze adult attachment representations and secure base scripts in possible daily and anxious scenarios related with the attachment relationship. The secure base script is described by a series of events which define the attachment relationship in terms of a balance between proximity towards the attachment figure and exploration behaviours, shown by the child or the adult. These events are: (1) the secure base (parent or partner) supports one's exploration; (2) the secure base remains available and responsive in case of need; (3) a threatening conflict and obstacle appears, which leaves the individual feeling anxious and fearful; (4) the individual searches and looks for comfort in the secure base and/or the secure base comforts the individual; (5) the conflict and threat are resolved; (6) proximity and contact with the secure base comforts the individual in an effective way and helps him/her to deal with the resulting anxiety; (7) the individual returns to his/her initial activity or changes it in a tranquil way (Posada et al., 1995). There are six narratives (four stories with attachment content and two used for control purposes). In each one of them are presented four groups of suggestive words, developed to guide the production of the narratives. The narratives are scored on a 7-point scale. The highest values are assigned when the script is elaborated, reveals knowledge and sensitivity concerning the emotional state of others, and reinterprets the meaning of the obstacle/conflict (suggested by the group of words) in a positive way. The narratives were assessed in a counterbalanced way, in a separate day from the AQS home visits and emotional situations and were scored by two trained observers, blinded to the results of the Q-sort home observations. Rater agreement was calculated as the intraclass correlations across rater-pairs. The intraclass correlations ranged from .64-.85 with over 85% of scores being within one scale point. Spearman-Brown reliability estimates story groups ranged from .83-.95. The final score for each story was the average across raters. An overall score was calculated by averaging scores over all the stories. Cronbach's alpha for the overall scores was .84. In this study we used the Narratives instead of the Adult Attachment Interview (AAI; George, Kaplan, & Main, 1984). Both the AAI and the narratives are valid measures in the study of attachment. Even if the secure base script is a recent measure, the validity has been clearly demonstrated. Correlation between AAI results and the Narratives' results are high. Several papers already showed that the measure is stable and correlated (as the AAI) with the quality of attachment between mother and child (Coppola, Vaughn, Cassibba, & Costantini, 2006; Vaughn et al., 2006, 2007).

Children and mothers' salivary cortisol levels. The cortisol responses were assessed from saliva, using Sarstedt's salivette kits in mothers and Salimetrics' sorbettes in children. All saliva samples were frozen within two hours after the collection. The samples were centrifuged (3000 rpm) at 10ºC, during 20 min. The assessment of cortisol was done by using luminoimmunoassay (LIA) kits (IBL, Hamburg, Germany). The mean intra and inter-assay coefficients of variation were 5,5% and 6,8% respectively. Children and mothers' saliva samples were collected on two consecutive days (Saturday and Sunday). Cortisol samples were collected three times during each day: (1) Immediately after awakening and before the first meal; (2) Twenty minutes after the first collection; (3) Immediately prior to bedtime (Adam, 2004; Stenius, Theorell, Lilja, Scheynius, Alm, & Lindblad, 2008). Children and mothers' awakening samples were collected between 08.00h and 09.30h in the morning. Children's bedtime samples were collected between 20.30h and 22.00h, and mothers' were collected between 22.30h and 24.00h in the evening. Children and their mothers had no food or drinks, except for water, before the morning collections and at least two hours before the last sample (bedtime). In children, saliva samples collected on Saturday and Sunday were significantly correlated in awakening [$r(42)=.59$, $p<.05$]; 20m after awakening [$r(42)=.68$, $p<.05$] and bedtime [$r(42)=.36$, $p<.05$] samplings. In mothers, saliva samples collected in Saturday and Sunday were also significantly correlated in awakening [$r(42)=.88$, $p<.05$]; 20m after awakening [$r(42)=.67$, $p<.05$] and bedtime [$r(42)=.30$, $p<.05$] occasions. Thus, final cortisol levels used in subsequent analysis resulted from a mean between Saturday and Sunday samplings, both in children and mothers. In order to quantify children and mothers' "recovery to awakening" parameter (Adam, 2004), difference scores (20m after awakening response – awakening response) were computed for each participant. The samples were analyzed at the Integrative Behavioural Biology Lab at ISPA – Instituto Universitário, Lisbon, Portugal.

Statistical analysis

In order to test possible significant differences in children and mothers'cortisol levels across the day, two sets of repeated measures ANOVAs were conducted. Cortisol sampling moments (awakening; 20m after awakening; bedtime) were used as within-subjects variables.

When results were significant, relevant differences were tested with pairwise comparisons analyses. To examine significant differences between children and their mothers' cortisol responses across the day, a set of multiple paired samples *t*-tests were conducted. Finally, to assess biobehavioural relationships, bivariate correlation analyses were conducted between children and mothers' attachment measures and cortisol parameters: awakening; recovery to awakening (20m after awakening – awakening responses) and bedtime responses.

Results

Preliminary results

In order to verify if demographic variables (children's age; number of hours spent at daycare each weekday; children's age when first entered daycare; parents' age and education) were related to attachment or cortisol parameters, several bivariate correlation analyses were conducted. When it comes to attachment security, there were no significant associations of any of these demographic variables with children or mothers' AQS scores and representations, respectively. On the other hand, significant correlations were found between adrenocortical reactivity, age and parental education. Children's age was significantly correlated with mothers' cortisol recovery to awakening response [$r(42)=.38$, $p=.01$]; and mothers' age was significantly correlated with their bedtime cortisol response [$r(42)=-.35$, $p<.05$]. Mothers and fathers' level of education were significantly correlated with mothers' cortisol recovery to awakening response [$r(42)=.34$, $p<.05$; $r(42)=.37$, $p=.01$, respectively]. With respect to children's gender, independent *t*-tests revealed no gender differences concerning attachment security [*M* boys=.40, *SD*=.26; *M* girls=.50, *SD*=.26; $t(40)=1.24$, $p>.05$]; cortisol awakening levels [*M* boys=4.68, *SD*=2.81; *M* girls=4.07, *SD*=1.70; $t(40)=.81$, $p>.05$]; 20m after awakening [*M* boys=5.26, *SD*=2.33; *M* girls=5.79, *SD*=2.81; $t(40)=.67$, $p>.05$]; recovery to awakening [*M* boys=.61, *SD*=2.70; *M* girls=1.72, *SD*=2.11; $t(40)=1.44$, $p>.05$] and bedtime [*M* boys=.82, *SD*=.67; *M* girls=.80, *SD*=.92; $t(40)=.07$, $p>.05$] levels.

A repeated measures ANOVA analysis revealed that children showed significant differences in their cortisol responses across the day [$F(2,82)=89.33$, $p<.001$]. Pairwise comparisons analysis showed a significant increase in children's cortisol levels from awakening to 20m after awakening responses ($p<.05$); and significant decreases from awakening and 20m after awakening responses to bedtime responses ($p<.001$; $p<.001$, respectively). In the mothers' case, a repeated measures ANOVA revealed significant differences in their cortisol responses across the day [$F(1{,}54; 62{,}58)=89.33$, $p<.001$]. We used the Greenhouse-Geisser test of within-subjects effects, since the repeated measures ANOVA's assumption of sphericity was not assumed [$W=.70$, $\chi^2(2)=14.28$, $p>.05$]. Pairwise comparisons analysis showed no significant differences between mothers' awakening and 20m after awakening responses ($p>.05$). However, significant decreases in cortisol from awakening and 20m after awakening responses to bedtime responses were found ($p<.001$; $p<.001$, respectively). Finally children's AQS results and mothers' attachment representations were significantly correlated [$r(41)=.69$, $p<.001$].

Relationships between children and mothers' cortisol responses across the day. A set of multiple paired samples *t*-tests, revealed no significant differences between children and their mothers in cortisol awakening levels [*M* children=4.42, *SD*=2.39; *M* mothers=4.52, *SD*=3.05; $t(41)=.19$, $p>.05$]; recovery to awakening [*M* children=1.09, *SD*=2.50; *M* mothers=0.67, *SD*=1.88; $t(41)=.80$, $p>.05$] and bedtime [*M* children=.81, *SD*=.78; *M* mothers=1.09, *SD*=1.99; $t(41)=1.17$, $p>.05$] levels (Table 1). Bivariate correlations between children and mothers' cortisol parameters (awakening; recovery to awakening; bedtime) were conducted in order to examine any possible relationships. Awakening cortisol in mothers and children showed no significant relationship. However, significant correlations between both members of the dyad were found for bedtime cortisol responses [$r(42)=.73$, $p<.01$]. Moreover, children's cortisol recovery to awakening response was significantly correlated with mothers' bedtime cortisol levels [$r(42)=.47$, $p<.01$]; and children's bedtime cortisol response was significantly correlated with mothers's cortisol recovery to awakening response [$r(42)=-.41$, $p=.01$].

Table 1

Means and standard deviations for children and mothers' cortisol levels at awakening; 20 m after awakening; recovery to awakening and bedtime moments

	Children (ng/ml)		Mothers (ng/ml)	
Cortisol levels	*M*	*SD*	*M*	*SD*
Awakening	4.42	2.39	4.52	3.05
20m after awakening	5.49	2.53	5.19	3.01
Recovery to awakening	1.09	2.50	.67	1.88
Bedtime	.81	.78	1.09	1.99

Relationships between children's cortisol parameters and attachment security to the mother (AQS). In order to test if children's attachment security was significantly related to their cortisol parameters, several bivariate correlations were conducted between AQS results and cortisol parameter. A significant correlation between children's attachment security to the mother and their cortisol levels at bedtime was found [$r(42)=-.31$; $p<.05$]. No significant correlations between children's attachment security and their cortisol awakening and recovery to awakening levels were found.

Relationships between mothers' cortisol parameters and children's attachment security (AQS). A significant correlation between mothers' cortisol levels at bedtime and their children's attachment security was found [$r(42)=-.37$; $p<.05$]. No significant correlations were found involving mothers' cortisol awakening and recovery to awakening levels.

No significant correlations were found between children's cortisol parameters and mothers' attachment representations, or between mothers' cortisol parameters and mothers' attachment representations.

Discussion

The objective of this study was to investigate the relationships between diurnal adrenocortical activity and attachment quality (children's secure base behaviours and maternal attachment representations) in a group of young children and their mothers. In this study, salivary cortisol was measured during two consecutive days (Saturday and Sunday) in children and their mothers. Three cortisol parameters were analyzed: awakening, recovery to awakening and bedtime cortisol responses. Bedtime cortisol levels were negatively correlated with children's attachment in both members of the dyad. These results suggest that the quality of the attachment relationship between the mother and the child may work as a buffer against diurnal stress, particularly, during bedtime periods, prior to sleep, needed for rest and children's development at this young age (18-26 months).

Our results are similar to other studies that have shown the existence of a significant relationship between HPA-axis activity and children's attachment quality. In particular, insecure attachment has been correlated with higher salivary cortisol levels after stressful situations (medical examinations, maternal separations) (Gunnar et al., 1996; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998). However, to the best of our knowledge, no significant results between children's attachment security and mothers' diurnal cortisol levels have been published before. The new results presented here suggest that children's attachment quality has an impact both on children and mother physiology. Previous research has shown that the emotional state of the mother is mirrored both on their own cortisol levels, as well on their children's, probably due to genetic similarities and similar environmental conditions (Spangler, 1991; Stenius et al., 2008; Steptoe et al., 2009).

Significant correlations between children's attachment and dyad's cortisol levels were found only at bedtime and not at awakening or recovery to awakening periods. Some studies have suggested that evening cortisol levels are more dependent on environmental factors, whereas morning levels seem to be more dependent on genetic variables, both in children and in adults. In fact, heritability was highest (60%) for cortisol levels during samples taken about 45 minutes after awakening in a group of twin pairs children. No significant genetic contributions were found for evening samples (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003; Kupper et al., 2005). The HPA-axis evening response may be under the influence and regulation of attachment security, both in children and in adults, which may explain the absence of significant genetic contributions during this period. Our study did not assess sleep quality during bedtime, however, significant relationships (i.e., negative correlations) found between children's attachment security and evening cortisol levels in children and their mothers raise an interesting question about how attachment quality may eventually influence sleep quality through its impact on the HPA-axis activity during evening periods, in both members of the dyad – a question worth being analyzed in future research.

Higher levels of cortisol in the bloodstream during the evening period may be associated with higher levels of stress, anxiety and difficulties in sleep. In fact, there is an indication of increased cortisol secretion associated with poor sleep quality (Hatzinger et al., 2008), increased subjective sleep problems and shorter sleep duration (El-Sheikh, Buckhalt, Keller, & Granger, 2008) in children and significant associations between cortisol levels and chronic aspects of sleep in adults (Eek, Karlson, Garde, Hansen, & Ørbæk, 2012). Attachment security may work as a buffer against stress and high levels of anxiety built over the day, promoting lower levels of cortisol, needed for rest and sleep in both members of the dyad. In fact, attachment anxiety is associated with higher levels of self-reported sleep difficulties for men and women (Carmichael & Reis, 2005).

In preschool children, attachment security is related to sleep quality indices (e.g., Sleep Activity, Wake Minutes after Sleep Onset, Sleep Efficiency, Vaughn et al., 2011). These findings are particularly important, since sleep quality has been associated with cognitive development in young infants (more motor activity in sleep and more fragmented sleep pattern is associated with lower mental developmental) (Scher, 2005) and with caregiving quality (Brummett et al., 2006). The relationship between children's attachment quality and cortisol levels in the evening, may not only benefit the dyad's sleep quality but also increase the mothers' emotional availability during this important period, by reducing their levels of stress. This is particularly important, given that maternal emotional availability at bedtime predicts infant sleep quality (Teti, Kim, Mayer, & Countermine, 2010).

No significant correlations were found between mothers' attachment representations and cortisol levels, neither in mothers nor in children. Past research has found a significant relationship between adult attachment and cortisol response to awakening (Quirin, Pruessner, & Kuhl, 2008). The lack of correlations may be an expression of a statistical power problem, due to the low number of participants in this study or to the use of different attachment assessment methodologies. In the future it would be interesting to replicate this study using different attachment methods and compare it with the results of this work. Moreover, this study only presented three cortisol parameters (awakening; recovery to awakening; bedtime), which may have diminished the possibilities of finding other significant correlations between attachment and cortisol, throughout the day.

In the future, work on this topic should increase not only the number of participants, but also the number of cortisol parameters during the day. Finally, this study was developed during weekends and not weekdays. Most work on this topic has been done on weekdays or has not specified this aspect. This is particularly important since during weekdays multiple external stressors (schedules; work overload; social interactions quality), increase the dyad's levels of emotional stress and may influence HPA-axis diurnal rhythm in both members of the dyad. In fact, relationship functioning and work demands predict individual differences in diurnal cortisol patterns in women and greater hours of maternal employment is associated with lower morning cortisol in mothers of two-years-old children (Adam & Gunnar, 2001). For both men and women, evening cortisol is lower than usual on higher-workload days (Saxbe, Repetti, & Nishina, 2008). The presence of these external stress variables may mask the real influence of attachment security on diurnal adrenocortical activity and should be further investigated in the future.

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Este estudo investigou a relação entre a actividade adrenocortical diurna e a qualidade da relação de vinculação (comportamentos de base segura das crianças e representações de vinculação maternas). Quarenta e duas crianças entre os 18 e os 26 meses e respectivas mães, participaram neste estudo. As amostras de saliva das crianças e das mães foram recolhidas durante dois dias consecutivos (Sábado e Domingo), três vezes por dia: ao acordar; 20m após o acordar; deitar. Não foram encontradas relações significativas envolvendo as representações de vinculação maternas. No entanto, foram registadas correlações negativas significativas entre os níveis de cortisol das crianças e das mães ao deitar, e a segurança da vinculação das crianças.

Palavras-chave: Cortisol diurno, Vinculação, Base Segura, Representações maternas.

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