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Understanding Cortisol Reactivity across the Day at Child Care: The Potential Buffering Role of Secure Attachments to Caregivers

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Abstract

Full-day center-based child care has been repeatedly associated with rising cortisol across the child care day. This study addressed the potential buffering role of attachment to mothers and lead teachers in 110 preschoolers while at child care. Using multi-level modeling and controlling for a number of child, family, and child care factors, children with more secure attachments to teachers were more likely to show falling cortisol across the child care day. Attachment to mothers interacted with child care quality, with buffering effects found for children with secure attachments attending higher quality child care. Implications for early childhood educators are discussed.

Previous work has shown that full-day, center-based child care is associated with increased reactivity of the hypothalamic-pituitary-adrenal (HPA) axis for many young children in the US (e.g., Tout, de Haan, Campbell, & Gunnar, 1998; Watamura, Donzella, Alwin, & Gunnar, 2003; Watamura, Sebanc, & Gunnar, 2002). Specifically, increasing cortisol from morning to afternoon at full-day child care, in contrast with decreasing cortisol across the day for these same children at home, has been repeatedly demonstrated for toddlers and preschoolers (Dettling, Gunnar, & Donzella, 1999; Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Watamura et al., 2003; Watamura, Kryzer, & Robertson, 2009). Although a focus on stress reactivity at child care is relatively new, efforts to understand this phenomenon have demonstrated that caregiving quality is important (Dettling et al., 2000; Sims, Guilfoyle, & Parry, 2006; Tout et al., 1998; Watamura et al., 2009). It is less clear whether particular child characteristics and relationship characteristics might be important for predicting which children are protected from responding to the challenge of child care with HPA reactivity. In particular, the theoretically important construct of attachment to both mothers and teachers as a potential buffering factor remains relatively understudied.

Human and non-human animals have an interconnected set of complex physiological systems for managing physical, cognitive, and socio-emotional challenges. One of these systems is the hypothalamic-pituitary-adrenal (HPA) axis. In humans, the primary product of this system is the steroid hormone cortisol (Hennessy & Levine, 1979). The HPA axis is

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particularly sensitive to situations that involve novelty, uncontrollability, or threats to the social self (Dickerson & Kemeny, 2004; Gruenewald, Kemeny, Aziz, & Fahey, 2004).

As part of the body's normal regulatory functions, cortisol follows a circadian rhythm, with adults and children demonstrating the highest values at waking, followed by a steady decline across the day, returning to its lowest levels at approximately midnight (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Watamura, Donzella, Kertes, & Gunnar, 2004). When assessed at home under baseline conditions, the mature adult rhythm is clearly evident by 36 months for children experiencing low-stress environments (Badanes & Watamura, 2008), suggesting that the increasing profile seen across the mid-section of the day in many children at child care may indicate context-dependent activation in the face of challenge.

Although it remains unclear what consequences (if any) this context-dependent activation may have, recent work has demonstrated that children who attend full-day child care *and* who show elevated cortisol levels may have lower antibody production. This work suggests the rising pattern may have consequences for physical health (Watamura, Coe, Laudenslager, & Robertson, 2010). Further, a recent analysis of the NICHD data set demonstrates that maternal insensitivity and longer child-care hours during the first three years of life (i.e., the factors that are putatively associated with concurrent elevated cortisol levels) are subsequently associated with lower awakening cortisol levels at age 15, suggesting possible long-term effects (Roisman et al., 2009).

With as many as 63% of American children under five experiencing some form of nonparental child care (U.S. Census Bureau, 2005), there has been an effort to understand the significance of child care experiences for children's stress-system development. In order to explain afternoon elevations in cortisol at child care, early work explored whether factors other than stress or challenge, such as changes associated with age, might elucidate the rising pattern at child care. Both Dettling et al. (1999) and Watamura et al. (2003) demonstrated a curvilinear developmental course for the rising pattern such that infants in child care and school-aged children older than five years of age were less likely to show the reversed pattern as compared to two-, three-, four-, and five-year-old child care attendees.

In addition to age, several studies have demonstrated that global classroom quality is an important index for predicting children's HPA reactivity at child care. For example, Gunnar and her colleagues (Gunnar, Kryzer, Van Ryzin, & Phillips, 2010) recently reported that child care quality as indexed by caregiver behavior (e.g. intrusive and overcontrolling care) was related to the rise in cortisol at child care. In an early study, Tout et al. (1998) found that 73% of the children studied showed a rising cortisol pattern across the day in centers scoring higher on the Early Childhood Environment Rating Scale (ECERS) (Harms & Clifford, 1980) as compared to 96% of children in the lower quality centers. In addition, Watamura et al. (2009) showed that better classroom interpersonal climates were related to reduced differences between home and child care cortisol levels. Similarly, in a home-based child care setting, Dettling et al. (2000) found that children's cortisol patterning across the day was related to the amount of focused attention and stimulation the child received from their teacher. These studies suggest that responsive, sensitive, and developmentally appropriate caregiving environments at child care may provide coping resources to the child facing stress and challenge. What is less clear at this point is whether HPA reactivity to child care is influenced by individual or dyadic factors such as teacher- child relationships, the child's home experiences, or the child's temperament. Work on these questions is much more limited and results have been inconsistent.

Attachment

Attachment theory proposes that differences in the organization of the child's early attachment relationships will emerge as a result of differences in the nature and quality of the patterns of interactions with a caregiver over the first few years of life (Ainsworth & Wittig, 1969; Bowlby, 1969/1982/1973/1980). These differences support the construction of distinct working models of attachment relationships and the self that carry forward to subsequent relationships (Cassidy, Kirsch, Scolton, & Parke, 1996; Sroufe, 1985).

Children's attachments to their caregivers have been broadly characterized along the dimension of security versus insecurity. More secure attachment is associated with higher levels of maternal sensitivity and responsiveness, and securely attached children are expected to develop working models of caregivers as trusting and supportive, and to therefore seek out caregivers for help and support in times of need and use them as a secure base from which to explore (Bretherton & Munholland, 1999). In contrast, insecure children may be unwilling or unable to use caregivers as a secure base from which to explore (Sroufe, Egeland, & Carlson, 1999).

Although parents typically serve as the primary attachment figure for the toddler or preschooler, several studies suggest that young children in families that use out-of-home child care also form attachment relationships with their teachers (Howes & Hamilton, 1992; Pianta, Nimetz, & Bennett, 1997). Through their daily interactions of supervision and instruction, teachers and young children develop close relationships characterized by proximity seeking, reassurance, and other secure-base behaviors (Barnas & Cummings, 1994; Howes & Ritchie, 1999). Goossens and van IJzendoorn (1990) rated teachers in one-on-one free play sessions and found them to be more sensitive than the mothers of the same one-year olds. However, this sensitivity appeared to decrease significantly in whole group child care settings where the caregivers' attention is divided (Goossens & Melhuish, 1996). Other variables associated with higher-quality teacher-child relationships include more hours spent at child care (Goossens & van IJzendoorn, 1990), teacher experience (Stuhlman & Pianta, 2002), female child gender (Birch & Ladd, 1997), non-minority children (Hamre & Pianta, 2001), and higher family resources (Ladd, Birch, & Buhs, 1999).

The availability of coping resources and effective coping behavior plays an important role in determining whether potentially threatening events stimulate elevations in cortisol (see Stansbury & Gunnar, 1995). Attachment security reflects one potential coping resource. Through their contingent and sensitive interactions with their caregivers, secure children may develop regulatory capacities that allow them to modulate stress reactions more effectively. Children who are securely attached may be better equipped to deal with the stressors associated with child care because they are better able to manage their emotional arousal within a social interaction (Parker & Gottman, 1989), have more positive interactions with their peers (Cohn, 1990), are more behaviorally and emotionally empathetic (Kestenbaum, Farber, & Sroufe, 1989), and are rated by teachers as having higher levels of social skills (Sroufe, 1983). However, because young children do not yet cope with impending threat very well on their own, of particular importance in coping with a potential threat is whether a responsive caregiver is present (Bowlby, 1973; Gunnar & Brodersen, 1992). For example, infants receiving their wellness inoculations were more likely to demonstrate cortisol elevations in response to the injection when they were insecurely attached to the parent bringing them to the appointment (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996).

Much of the previous work demonstrating associations between attachment security and HPA-axis activation has been conducted with infants using Ainsworth's Strange Situation paradigm with their primary caregiver (Ainsworth & Wittig, 1969; e.g., Ahnert, Gunnar,

Lamb, & Barthel, 2004; Hertsgaard, Gunnar, Erickson, & Nachmias, 1995). We know much less, however, about the associations between attachment security and cortisol responses to child care providers after prolonged or repeated separations from parents in preschoolers. Only one study published to date has assessed attachment security and cortisol levels after daily separations from a parent, and this was with infants and toddlers. In this work (Ahnert et al., 2004), salivary cortisol was assessed in the morning on days 1, 5, 9, and 5 months after child care entry (mean age = 14.9 months old) and dyads were coded for secure-base behaviors. Compared with insecure infants and toddlers, secure young children had lower cortisol levels during the adaptation phase to child care when the mother was present. However, young children from both attachment types demonstrated increased cortisol levels during the separation phase as compared to the adaptation phase.

Of course, infants and toddlers are not always in the presence of their primary caregiver during a stressful situation. In a laboratory study that allowed for the manipulation of supportive caregiving behaviors, infants who received individualized care from a babysitter that was less friendly, playful, and responsive during half-hour separations from their mothers demonstrated cortisol elevations, whereas the infants cared for by a more interactive and responsive babysitter showed no cortisol elevations (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992). Dettling and colleagues (2000) also demonstrated that children's cortisol patterns across the day at family child care were lower when they received more focused attention and stimulation from their caregivers, behaviors that parallel those of a secure attachment relationship. In addition, although attachment security was not assessed in their 2008 study, Lisonbee, Mize, Payne, and Granger found that teacher-reported overdependence, as measured by the Student Teacher Relationship Scale (Pianta, Steinberg, & Rollins, 1995), predicted cortisol increases from morning to afternoon at childcare. Further, relationship conflict as reported by teachers predicted cortisol increases during a teacher-child interaction. Higher dependence and more conflict may be indicative of a less secure attachment relationship.

The Current Study

The overarching purpose of the current study was to assess the role of attachment security to parents and teachers as a potential buffer against cortisol reactivity at child care. However, we know that a number of other child, family, and child care factors are related both to the attachment relationships that children form with adults and to their experience of non-maternal child care. To improve our ability to discuss the unique role of attachment security, we utilized a demographically diverse sample, assessed global classroom quality, asked mothers about their current depressive symptoms to capture the powerful effect of maternal depression on child outcomes (Cicchetti & Toth, 1998), and included teachers' ratings of children's temperament.

The current study examined three specific questions: 1) whether degree of attachment security to mother and, 2) whether degree of attachment security to lead teacher modifies cortisol changes over the child care day, and 3) whether the impact of secure attachment to mothers or teachers varied as a function of other important characteristics such as classroom quality. Our specific questions were addressed in models that included a number of theoretically or empirically important child, family, and teacher-level covariates so that we could estimate the unique importance (if any) of secure attachment. Therefore, to address the first question, we utilized linear regression to determine whether cortisol change scores across the day were predicted by security of attachment to mother, while controlling for starting cortisol values (morning cortisol), the length of time between morning and afternoon sampling, the child's age and sex, the family's demographic risk characteristics, the child's temperament, and maternal depressive symptoms. To address the second aim, it was necessary to account for the fact that children were nested within classrooms which would

mean that some of the variance in both the dependent (cortisol change) and independent (attachment to teacher; global classroom quality) variables could be at the classroom level. To handle this, we utilized hierarchical linear modeling. All variables used for aim one were retained, and we added attachment to lead teacher and global classroom quality as well. We hypothesized that secure attachment to the immediate caregiver (lead teacher) would be reflected in less cortisol reactivity to child care. We also hypothesized that secure attachment to mothers, given its importance in the early childhood period and its contributions to the security of other close relationships, would be related to less cortisol reactivity to child care, although we expected this effect to be more modest than attachment security to teachers who were present at the time of cortisol assessment. For our third aim, we tested for possible important interactions, although we were agnostic about their existence or direction.

Method

Participants

Participants were enrolled in one of 14 classrooms from one of six full-day child care centers. To ensure adequate representation of understudied populations, centers serving Head Start eligible and ethnically and racially diverse families were targeted. Because previous work has utilized university-affiliated child care centers, one such site was also included in the sample. To reduce variability due to global classroom quality, centers with the resources and philosophy to support high-quality early childhood education were deliberately chosen. All centers were average-to-above-average in quality as evaluated by the Early Childhood Environment Rating Scale-Revised (ECERS-R; Harms, Clifford, & Cryer, 1998) with a median score of 5.62 (out of 7). This step was deliberate and necessary as we did not want to simultaneously change two important sample characteristics – family demographics and center quality – thereby making any results that might differ from previous work uninterpretable. Because the effect of quality is well documented, we elected to hold quality high while examining stress and buffering among families experiencing more demographic risk factors.

Of the 222 children enrolled in the selected classrooms, parents of 166 children agreed to participate. Of these, 118 were eligible to participate in the study (i.e., did not have a developmental disability and attended child care for at least three days a week). Two children refused saliva sampling and six children were subsequently excluded for use of medications thought to interfere with cortisol assays ($n = 4$ inhaler for asthma, $n = 2$ allergy medication). Of the remaining full sample of 110 children, 53% were female, and children were 2.03 to 5.38 years of age (M age = 4.03, $SEM = .07$). Eight children in the study were siblings (all analyses were repeated without the siblings included in the sample and similar results were obtained).

Seventy-five percent of children were identified by parents as white and 25% as non-white (15% African American/Black, 8 % Asian American/Asian, and 2% American Indian/Alaskan). In addition, 45% of those identifying as white were also identified as non-Hispanic, with 55% identifying as Hispanic. Of these 55% Hispanic families, 24% reported that the child's mother was born in Mexico. For 14% of the Hispanic families (24 children), Spanish was identified as their primary language, and therefore all questionnaires and procedures were administered to them in Spanish. This study was approved by the Institutional Review Board at the University of Denver. Families were compensated \$55 for their participation, while teachers were compensated \$20 for each child participating in their classroom.

Measures

In this study, we were interested in whether a secure attachment to mother and/ or teacher buffers children against rising cortisol across the day at child care. To assess the unique influence of attachment to mothers and teachers, we also included measures of a number of demographic, child, family, and relationship factors which may influence children's stress sensitivity.

Attachment security—Security of attachment was assessed with the extensively used Attachment Q-Sort (AQS; Waters, 1995). The AQS contains 90 statements about a child's behavior in the context of interactions with a specific caregiver. The items were developed to provide a comprehensive characterization of the child's use of the caregiver as a secure base for exploration and as a safe haven when distressed (Ainsworth & Marvin, 1995). Because the AQS is based on observations of the child in their natural environments, it is considered by some to have better ecological validity than lab-based measures (Howes & Ritchie, 1999). It also allows for an economical examination of attachment beyond infancy and can be used with a broader age range. The AQS has been used with hundreds of parents and teachers (see van IJzendoorn, Vereijken, Bakermans-Kranenburg, & Riksen-Walraven, 2004 for a meta-analysis).

Primary caregivers (109 mothers, and 1 legal guardian grandmother; collectively referred to as mother) and teachers were asked to sort the 90-item cards into nine piles with roughly 10 cards in each pile. Items that are “more characteristic” or “like” the child are given high placements (i.e., categories 7 – 9), and items that are “less characteristic” or “unlike the child” are placed in the lower categories (i.e., 1-3). Items that are “neither characteristic nor uncharacteristic” are sorted in the center of the item distribution (i.e., categories 4-6). This fixed distribution of items has been shown to be easier to learn than a quasi-normal or uneven distribution (Waters & Deane, 1985). To generate attachment security scores for each participant and their mother and teacher, the Q-sort description of the child was correlated with the description of the hypothetical “very securely attached” child provided by Waters, Vaughn, Posada, and Kondo-Ikemura (1995) as suggested by the instrument authors (Waters & Deane, 1985). Total scores for attachment security to mother and lead teacher could thus range from -1 to 1, with a higher score reflecting higher attachment security. For this sample, the average attachment security scores with mother and teacher respectively were .37 (*SEM* = .02, range -.19 to .76) and .32 (*SEM* = .02, range -.33 to .71) indicating a moderate degree of congruence between these children and the hypothetically secure child on average and considerable variance across the sample. These scores are similar to those reported by a variety of researchers using the Q-sort with preschoolers (e.g., Teti, Nakagawa, Dass, & Wirth, 1991). Inter-rated reliability of this measure with mothers has been found to be approximately .88 (Cassibba, Van IJzendoorn, & D'Odorico, 2000; Moran, Pederson, Pettit, & Krupka, 1992; Nakagawa, Teti, & Lamb, 1992) while with caregivers it has been found to be lower at around .70 (Cassibba et al., 2000).

Cortisol—Saliva samples were collected from children at child care on three child care days at approximately 10am and 4pm for a total of six samples per child. For the first two classrooms (nine children), these unstimulated saliva samples were obtained using a 1.5 inch cotton dental roll that was then expressed into a vial. It has been established that cotton fibers retain cortisol, particularly for samples with low volume (de Weerth, Graat, Buitelaar, & Thijssen, 2003). To allow inclusion of the samples collected with cotton, the values were corrected by a factor of 1.4 nmol/L based on the retention of known cortisol concentrations using cotton rolls from the same lot used in this study. For the remaining 12 classrooms (101 children), saliva samples were obtained via synthetic salivette collection devices (Sarstedt, Nuembrecht, Germany). Saliva was extracted by centrifuging for 4 min at 2500 RPM. Vials

and salivettes were frozen at -20°C until data collection was complete. Samples were then defrosted and batched for assay in groups of 36 and were assigned to batches so that classroom and batch were not confounded, and so that all samples from the same child were analyzed in the same batch. Samples were sent to the Biochemical Laboratory, Psychobiology, University of Trier, Germany to be assayed. Cortisol levels were determined by employing a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFA; Hoferl, Krist, & Buchbauer, 2005). For samples retained in the analyses described below, the mean intraassay coefficients of variation (CV) for controls were 6.6% to 8.5%. For duplicates of the samples used in this study, the interassay CV was 5%.

For seven children, a total of 10 individual samples were excluded, five (two and three for two children) were excluded for reported dairy contamination (dairy products consumed within 30-minutes of sampling) and one sample each for five children was excluded because it was > 3 SD of the sample mean for that time point and highly dissimilar from that child's other values suggesting contamination. All other samples from these seven children were included.

Cortisol assays were performed in duplicate and averaged, resulting in two samples per day (mid-morning and mid-afternoon) on up to three days. Data were examined for skew; however, raw values were reasonably normal. Therefore, no log transformation was performed. Because we were interested in average child care cortisol patterns and not day-to-day variation, samples across days were averaged and then a difference score was computed subtracting the average afternoon value across the sampling days ($M = .13\ \mu\text{g/dL}$, $SEM = .01$, $\alpha = .74$), from the average morning value across the sample days ($M = .13\ \mu\text{g/dL}$, $SEM = .01$, $\alpha = .41$). Difference scores, ranged from $-.34$ to $.20\ \mu\text{g/dL}$ ($M = .01\ \mu\text{g/dL}$, $SEM = .01$). Due to the influence of starting values on change scores, the intercept (AM cortisol values) was controlled in all analyses.

Control Variables

Demographic variable—Parents were asked their child's birth date; their child's race using NIH categories (coded 1 = minority race, 0 = white/Caucasian); their child's ethnicity (Hispanic or non-Hispanic; coded 1 = Hispanic, 0 = non-Hispanic); and if Hispanic whether their mother was born in Mexico (for the purpose of the larger study; coded 1 = mother born in Mexico, 0 = mother not born in Mexico). Age was calculated by subtracting the first saliva sampling date from the child's birth date (for six children, birth date was unavailable and age as reported by teachers was substituted). These demographic variables were used as controls. Parents were also asked if their child used an inhaler for asthma or other reasons; whether their child had any allergies; if their child took any other medications on a regular basis (and what they were); if their child was currently ill; and whether their child had any other medical conditions that were not otherwise addressed. Data on medications and illnesses were used to exclude saliva samples from children taking allergy or asthma medications thought to interfere with cortisol assays (e.g. albuterol) and to exclude samples that were taken while the child was ill (taking antibiotics or with a fever) as noted above.

Child temperament—Lead teachers were asked to complete the Teacher-Children's Behavior Questionnaire (TCBQ; Gunnar, Tout, de Haan, Pierce, & Stanbury, 1997). This 10 scale, 50-item version was modified from the original CBQ (Rothbart, Ahadi, & Evans, 2000) with help from Mary Rothbart. Items are scored on a 7-point Likert scale with lower scores indicating items less representative of the child. The 10 scales produce three standard factors: negative affectivity (the average of standardized scores for anger, discomfort and sadness), $\alpha = .71$, effortful control (the average standardized scores for attention focusing,

inhibitory control and smiling/laughter), $\alpha = .61$, and surgency (the average standardized scores for activity level, high pleasure, impulsivity and shyness reverse scored), $\alpha = .83$.

Family variable—Maternal depression was assessed using the Center for Epidemiological Studies Depression scale (CES-D; Radloff, 1977). The CES-D is a 20-item self-report scale that measures symptoms of depression with a severity rating of zero to three. It is one of the most widely used and validated measures of depressive symptomatology for non-clinical samples (Orme, Reis, & Herz, 1986) with internal consistency of .85 in a non-clinical sample and .90 in a clinical sample (Roberts, 1980). The 20 items ($\alpha = .78$) were summed and the total symptom score was included as a continuous variable. Using the standard cutoff of 16, 46 mothers in our sample reported current depressive symptoms above the clinical cutoff.

A demographic risk variable was created based on the family's experience of financial strain (described below) and minority status (non-white race, Hispanic, and/or mother born in Mexico). Financial strain was assessed with the Family Finances questionnaire adapted from the NICHD Study of Early Child Care (2000) and consisted of one item addressing family size, two items assessing sources of income, one question asking about amount of income earned, and five items asking about insecurity of critical resources. For this study, we used these last five items as we felt it was the best reflection of the impact of poverty that was not dependent on an outsiders' assessment of what is livable. Items asked whether in the past 12 months, the following was true or false for the family: 1) Your family went without a telephone, 2) You didn't pay all or part of the rent/ mortgage because you did not have enough money, 3) Your family was evicted for failure to pay, 4) You didn't pay all or part of the gas/electric/oil bill, 5) You had your oil/gas/electricity turned off for failure to pay. These five items were summed and a dichotomous variable was created so that 1 = families with one or more financial risk factors and 0 = families reporting no critical resource unavailability. Participants received a score of 1 on the demographic risk variable if they had one or more financial risk factors and/or if they belonged to an ethnic minority group ($n = 62$), and a score of 0 if participants did not endorse any financial risk factors and did not belong to an ethnic minority group. For the purposes of parsimony, the minority status and the financial risk variables were combined into a single demographic risk variable. We have separately tested (results not reported here) the proposed models using the minority status and the financial risk variables. The main results regarding attachment security were virtually identical to those obtained with the combined demographic risk variable.

Classroom quality—Classroom quality was assessed using the Early Childhood Environment Rating Scale-Revised (Harms et al., 1998). The ECERS-R consists of 43 items that assess seven aspects of center-based care and education for children aged 2 ½ to 5. Detailed descriptions are provided for each item. Item scores are 1 (inadequate) through 7 (excellent). The ratings are designed to be based on a minimum of 2-hr observations. In the current study, observation times ranged from 6 to 30 hrs, and were completed by gold-standard trained observers. Average total scores were used in these analyses.

Additional measures—Arrival and departure times were obtained from the classroom on sampling days with samples excluded on days when this information was not available or when the child arrived late or left early. Nap awakening times were also recorded.

Procedure

Recruitment procedures and data collection occurred by center, one classroom at a time. During the first week in a classroom, researchers visited the classroom daily so that the children felt comfortable in their presence and so that researchers could become familiar

with the daily schedule of activities. At the end of the week, families were recruited and teachers signed consent forms. Researchers also demonstrated salivary collection methods to the children and teachers.

Salivettes were collected on a small group basis, generally for four to six days. Samples were collected mid-morning and mid-afternoon, as close to 10:00am and 4:00pm as possible while minimizing disruption to the classroom schedule. Both morning and afternoon samples were taken before snack or at least 30 minutes after snack or breakfast, and afternoon samples were taken at least 30 minutes after nap time. Given schedule differences in the various centers, both morning and afternoon sampling times were somewhat variable (mid-morning samples: $M = 9:50$, $SEM = .03$; range = 9:11 to 10:40; mid-afternoon samples: $M = 15:34$, $SEM = .05$; range = 14:25 to 16:37). If, as has been reported previously (Watamura et al., 2002), cortisol begins to rise across the morning period and then again between nap and late afternoon, restricting the interval between the two samples could artificially result in flatter cortisol patterns across the day. Morning sampling time was negatively related to cortisol values, $r(110) = -.41$, $p < .001$, indicating that later sampling time likely did not contribute to a flatter pattern in the sample. However, a positive trend was evident for afternoon sampling time, $r(109) = .18$, $p = .056$, indicating that the earlier sampling that occurred in two of the centers (six classrooms), may have contributed slightly to the flatter cortisol values seen for the sample average across the child care day. Therefore we controlled the average time between the am and pm samples in all models. Saliva sampling took approximately five minutes to complete and was structured around a game where participants shared imagined flavors for their salivettes. After each morning sampling, child temperature was obtained using a Genius™2 IR Tympanic Thermometer. Samples on days where the child's temperature was at or above 99.5°F were not used in analyses. If children were absent due to an illness, we waited at least two days after their return and until symptoms were cleared to collect samples.

Attachment—Lead teachers completed the AQS with support from the lead researcher for each child in the sample in their class. Each of the 90 items was read to the teacher by the researcher and then sorted into one of three roughly equal piles. During this division, teachers were encouraged to provide example behaviors for each sort and prompted to re-sort when piles were of unequal size. Following this initial division, the teacher further subdivided each of these three categories into three more categories as described earlier. Teachers were kept unaware of the constructs under investigation and were simply instructed to use the items to create best descriptors. Each AQS took approximately 30-40 minutes to complete.

During a home visit, the child's mother completed the AQS with support from the lead researcher or a trained research assistant. Procedures for sorting were exactly the same as described above with lead teachers.

Results

Analytic Plan

All analyses controlled for the effects of child and family demographic characteristics, teacher-reported temperament (surgency, negative affectivity, and effortful control), maternal depression, and time between morning and afternoon sampling. We further controlled for AM cortisol because we were predicting change scores and the amount of change may be dependent on AM cortisol values. We first explored whether attachment to mother predicted cortisol change score across the day at child care in a single regression model. To address our second aim of whether attachment security to teacher buffers children, multi-level models (using HLM version 6.0, Raudenbush, Bryk, & Cheong, 2004)

were used with the lead teacher as the level-2 nesting variable, all individual predictors as level-1 variables, and classroom quality as the level-2 variable. Although variance at the center level is possible, it was not significant in our models and was therefore excluded. We therefore estimated a two-level multilevel model for a continuous outcome variable (i.e., a model that estimates how security to teacher is associated with cortisol change scores). The estimated model had the following form (Raudenbush et al., 2004):

$$\text{Level 1: } Y_{ij} = \beta_0 + \beta_n X_n + r$$

$$\text{Level 2: } \beta_0 = \gamma_{00} + \gamma_{01} \text{ Classroom Quality}_j + u_0$$

$$\beta_n = \gamma_{n0} + \gamma_{n1} \text{ Classroom Quality}_j + u_n$$

where Y_{ij} is the outcome variable, cortisol change, for a focal child i in classroom j ; β_0 is the intercept; β_1 through β_n are the effects of the individual-level predictors X_n (i.e., control variables – age, sex, demographic risk, surgency, negative affectivity, effortful control, maternal depression, maternal security, and average AM cortisol, and the main study variable -- teacher security); and r is the residual variance. A set of models was tested to evaluate whether the β_0 through β_n coefficients are random or fixed (i.e., have zero variance). Coefficients with significant classroomlevel variance were set as random and were modeled as a function of classroom quality (γ_{n1}).

We first report descriptive statistics and simple bivariate correlations (see Table 1). All correlations were in the expected direction. Higher teacher security was associated with being female, having higher effortful control, lower negative affectivity, and with being securely attached to their mother.

Attachment to Mother and Cortisol Change Scores using Linear Regression

A linear regression was performed to predict cortisol change scores on the basis of maternal security and the nine control variables (age, sex, demographic risk, surgency, negative affectivity, effortful control, maternal depression, time between sampling, and morning cortisol). The model accounted for 50% of the variance in cortisol change scores. As expected, average morning cortisol predicted cortisol change scores across the day, $\beta = -.76$, $t(81) = -9.43$, $p < .001$. In addition, age was a significant predictor of cortisol change, $\beta = -.21$, $t(81) = -2.44$, $p < .05$. However, maternal security did not predict cortisol change scores across the day at child care in this linear regression model (see table 2 for the regression coefficients).

Maternal and Lead Teacher Attachment as a Predictor of Cortisol Change using HLM

The model-building procedure—The final model was derived in incremental steps (see Table 3). The first model (Model A) tested fixed effects for all study variables (control variables, as well as the key study variables). Age, AM cortisol, and teacher security emerged as the three significant correlates of the cortisol change. Next, a variance component (random effect) was added for each of the explanatory variables, one at a time; thus, testing whether the association between each variable and cortisol-change varies across classrooms. Based on this set of tests, the next model (Model B) simultaneously estimated variance components for all variables that had a significant variance component in an individual test. An alternative strategy would have been to test the variance components for all variables in a single model – a strategy that would have required testing a model with 14 extra parameters (one for each explanatory variable). Given the small sample size, we elected to first test each of the 14 variance components individually, and follow with a simultaneous test of variance components only for those variables that had demonstrated significant variance in individual testing. Based on individual tests (not reported in Table 3), AM cortisol, maternal depression, maternal security, and teacher security had significant

variability in their association with cortisol change. When these four variance components were added into a single model (Table 3, Model B), only the variability for maternal depression and maternal security remained significant. The third and final model (Model C) tested whether the between-classroom variability in the link between maternal depression and maternal security could be explained by classroom quality.

The final model—Results from the final model (Model C) indicated that participants who were younger, $b = -.03$, $p < .01$, had lower AM cortisol values, $b = -.70$, $p < .001$, and had lower teacher security, $b = -.07$, $p < .05$, were more likely to experience a pattern of rising cortisol across the child care day. Effects of maternal security and maternal depression varied across classrooms ($\chi^2(12) = 36.89$, $p < .001$ for maternal depression, and $\chi^2(12) = 33.87$, $p < .001$ for maternal security). However, only variability in the effects of maternal security was linked with classroom quality. As illustrated in Figure 1, children with lower maternal security exhibited a flat pattern of cortisol production across the day in classrooms that were both 1SD above and 1SD below the mean in quality, while children with high attachment security to their mothers exhibited falling cortisol in classrooms 1 SD above the mean in quality, but rising cortisol in classrooms 1 SD below the mean in quality.

Discussion

This study examined characteristics of the child, their environment at home and at child care, and their dyadic relationships with their caregivers as predictors of stress reactivity at child care. We first assessed whether a secure attachment to the mother was associated with decreased risk for stress reactivity across the day at child care without including relationship security to teachers or global classroom quality. Attachment security to mother in this model was not predictive of cortisol reactivity at child care. However, when we included relationship security to teachers and global classroom quality, attachment security to lead teacher predicted which children showed decreasing cortisol across the day even when controlling for a number of demographic, temperament, childcare, and family risk factors. Furthermore, in this final model, attachment to mother interacted with global classroom quality when predicting cortisol reactivity at child care.

Maternal Attachment and Cortisol Reactivity Across the Child Care Day

The null main effect for maternal security using linear regression was initially surprising given the previous laboratory (Gunnar et al., 1992; Nachmias, Gunnar, Mangelsdorf, & Parritz, 1996) and naturalistic (Ahnert et al., 2004; Gunnar & Brodersen, 1992) findings indicating that children who are more securely attached to their mothers are least at risk for experiencing stress reactivity as assessed by salivary cortisol. However, only one of these studies (Gunnar et al., 1992) assessed cortisol reactivity in the absence of the mother, and even in this study, the separation period was very brief (i.e., 30 minutes), as compared to the full-day, repeated separations experienced with child care. To our knowledge, Ahnert et al.'s (2004) transition study with infants is the only published study to date addressing attachment in relation to the longer, repeated separations typically experienced at child care. In the Ahnert study, both securely and insecurely attached infants showed higher morning cortisol in the first few months of out-of-home child care. However, differences between children with secure versus insecure attachment classifications were only related to HPA reactivity during the first 9-days of adaptation to child care when the mother was present. Their results suggest that infant and toddler HPA reactivity during the transition to child care in the mornings is buffered by secure attachment to the mother when she is present. Importantly, they also suggest that securely attached infants and toddlers do not necessarily regulate stress more effectively than insecurely attached young children in their mothers' absence. The preschoolers in our sample were only included in our sample if they had been in their

classroom for at least one month. Thus, it may be that once the transitional adjustment period is over, but when children are still relatively young and their working models of attachment may not be fully formed, attachment to mother does not have the same protective effect irrespective of the quality of the child care environment.

When classroom quality was added to the models and allowed to interact with the security of the child-mother dyadic relationship, an interesting interaction emerged. Children with the double protection of high security in their primary attachment relationship and high classroom quality showed the optimal decreasing cortisol pattern across the child care day. In contrast, children with low security in their primary attachment relationship showed a flat pattern across the child care day regardless of classroom quality, indicating that low maternal attachment security may prevent the optimal decreasing cortisol pattern even in high-quality child care environments. Finally, children with high security in their maternal attachment relationship but lower classroom quality showed increasing cortisol across the child care day, suggesting that children with better relationships with parents at this young age are more distressed by lower-quality child care. Perhaps the peer skills and self-confidence that a secure attachment is thought to confer provides these children with a set of coping resources that they are best able to utilize in a high-quality classroom that may share some features of their maternal relationship. In contrast, these skills and the working model that supports them may not yet be solidified enough to compensate for lower-quality care, and in fact because this care violates the expectations of caregivers these children have formed, children with secure maternal relationships react negatively to this discrepancy. This interpretation would fit with Boyce's model of context sensitivity (Boyce & Ellis, 2008), with some children benefitting more from more supportive environments but also suffering more from less supportive ones. However, it is important to keep in mind that all of the environments we studied were at least average in quality, and most were in the range considered good or excellent. Therefore, we do not know what role attachment security to mother would play when child care quality is low. However, the fact that we continue to see effects of quality even within this restricted range is important and suggests that future work should address the potential protective role of attachment security for children served in classrooms lower in global environmental quality. Based on early work (Tout et al., 1998), we might expect most children to exhibit cortisol increases in low-quality child care, regardless of attachment security to mother.

Attachment to Lead Teacher and Cortisol Reactivity across the Child Care Day

Our data suggest that children with higher security scores with teachers were more likely to exhibit falling cortisol across the child care day even when controlling for a number of demographic, temperament, child care, and family characteristics, including maternal relationships and global classroom quality. This finding replicates the previous work examining stress reactivity and attachment with substitute caregivers (Gunnar et al., 1992) and suggests that, at least for preschoolers, attachment security may be best able to serve a protective function against stress when that caregiver is present. Of course, it may also be the case that children rated by teachers as more securely attached are simply not as challenged by child care due to some other factor(s). For example, children with less reactive temperaments or better social skills may be both more likely to show the normative falling cortisol pattern across the child care day and more likely to form secure attachments (or be rated as such). In fact, temperament and attachment security as rated by teachers were modestly correlated (see Table 1). While we included temperament in the models, we did not include an assessment of peer or social skill. Nevertheless, this finding suggests that a good relationship with a lead teacher may buffer children at child care, and suggests a possible avenue for targeted teacher training.

Additional Findings

Of the nine controls used in this study, only age was a significant predictor of cortisol change score in our model. This finding is in line with previous work demonstrating that reactivity to child care is strongly influenced by the child's developmental level (Dettling et al., 1999; Watamura et al., 2003).

In addition to age, there was a positive trend for teacher-rated surgency to predict cortisol reactivity at child care. This finding mimics previous work examining temperament and stress reactivity in preschool that found similar associations between surgency and cortisol patterning across the day at child care (Davis, Donzella, Krueger, & Gunnar, 1999; Watamura et al., 2002; Watamura et al., 2009).

Limitations

There are several limitations of the current study that should be noted. First, although it has been shown that parents and teachers can be successfully trained to sort the Attachment Q-sort (De Wolff & van IJzendoorn, 1997), future research should include independent assessments of the child. In particular, ratings completed by individuals within an attachment relationship may be more accurately viewed as the adult's perspective on their relationship with the child than a pure measure of attachment irrespective of participant bias. Further, ratings may be influenced by other characteristics, most notably child temperament. In fact, this appears to be important in this study, and teacher ratings of child surgent or negative temperament were correlated with teacher ratings of their attachment security to the child. However, two lines of evidence suggest that child temperament alone does not account for the attachment-cortisol link. First, temperament alone has nearly always been assessed in studies of child care cortisol and as a main effect has yielded weak and inconsistent findings. Second, after controlling for teacher-rated temperament in the models, teacher-rated security was still important. Therefore, it is likely that child temperament is important for how they are viewed by teachers, may be important for the relationships they form, and likely interacts with other features of the environment when predicting cortisol reactivity. In addition, we did not assess attachment relationships with assistant teachers, who are likely to play an important role.

In addition, to more accurately assess the directionality of these findings, this research would ideally be longitudinal in nature, assessing attachment security in infancy using the Strange Situation and subsequently assessing stress reactivity to child care in the preschool period. It is also possible that due to our sample size of 110, we may not have had adequate power to detect the effects of some of our variables, and therefore, the null effects, particularly the lack of a main effect of maternal security irrespective of child care quality, should be interpreted with caution.

This study is one of the first to assess stress reactivity across the day at child care with an inclusive sample of both low socio-economic (SES) and Mexican-origin families. Children from these families are likely to have experienced more stressful life events than those children previously studied that come from a higher SES background and who have not experienced the unique challenges associated with immigration. Although this inclusion was deliberate and a number of demographic controls were utilized, future work with a sample that is inclusive and large enough to examine within-group processes is badly needed.

Conclusion

The findings from this study extend previous work examining cortisol reactivity in preschoolers attending full day child care. The data support previous findings that stress reactivity within this context is influenced by the age of the child. Further, they raise the

possibility of interactions between maternal characteristics and classroom quality. The findings also suggest that cortisol changes across the day are related to the child's attachment security to their lead teacher. These results are exciting as they demonstrate the important impact that teachers have on children in child care and provide a potential avenue to support buffering against stress reactivity within this normative developmental context.

Implications for Caregivers and Educators

This study and other similar work make explicit the importance of relationship quality for children's health and well-being. Further, they suggest that educating the whole child must involve socio-emotional support from caregivers. Children who must repeatedly utilize their body's stress management systems to manage the normative challenges of child care risk overexposure to potent hormones and possible negative consequences for physical and psychological health. These data clearly demonstrate that children who have secure relationships and who experience high-quality child care are less likely to recruit stress hormones to get through the day and may therefore be able to focus more of their resources toward the long-term goals of learning and growing. These data suggest that positive teacher-child relationships should be an explicit goal of early education programs and that supports should be provided to teachers so that they can overcome the difficulties of forming secure relationships with children who have negative or difficult temperaments. They also further reinforce the interconnections between family and child care systems and point to the need to provide integrated interventions across contexts.

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Research Highlights

- Full-day center-based child care has been repeatedly associated with rising cortisol across the child care day.
- This study addressed the potential buffering role of attachment to mothers and lead teachers in 110 preschoolers while at child care.
- Children with secure attachments to teachers were more likely to show falling cortisol across the child care day.
- Attachment to mothers interacted with child care quality, with buffering effects found for children with secure attachments attending higher quality child care.

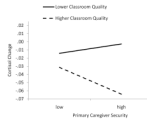


Figure 1. Estimated cortisol change by classroom quality and maternal attachment security, based on the values of classroom quality and maternal attachment security that are 1SD below and above their respective means.

Table 1
Intercorrelations Among Each Pair of Variables and Sample Size for Each Correlation

	1	2	3	4	5	6	7	8	9	10
1. Age	-									
2. Sex	-.15(108)	-								
3. Cortisol Change	-.01(108)	.08 (93)	-							
4. Surgency	.08(108)	-.32* (110)	-.07 (110)	-						
5. Negative Affectivity	.08 (108)	-.04 (110)	-.10 (110)	.01 (110)	-					
6. Effortful Control	.15 (108)	.23* (110)	.02 (110)	.21* (110)	-.44* (110)	-				
7. CES-D	.16 (99)	-.10 (99)	.01 (99)	.16 (99)	.20* (99)	-.14 (99)	-			
8. Maternal Security	-.16 (101)	.13 (101)	-.01 (101)	-.05 (101)	-.17 (101)	.14 (99)	-.16 (98)	-		
9. Teacher Security	-.08 (98)	.31* (110)	-.14 (110)	-.15(110)	-.35* (110)	.45* (110)	-.22* (99)	.29* (101)	-	
10. Demographic Risk	.12 (94)	.20 (94)	.21* (93)	-.11 (94)	.24* (94)	.08 (94)	.27* (94)	.07 (93)	-.01 (94)	-
11. AM Cortisol	-.30* (108)	-.01 (110)	-.71* (110)	-.05 (110)	.01 (110)	.00 (110)	-.09 (99)	.13 (101)	.16 (110)	-.14 (94)

* $p < .05$.

Table 2
Regression Coefficients for Maternal Security and Cortisol Change Score ($N = 92$)

	β	t
Constant		1.88
Age	-.21	-2.44*
Sex	-.07	-0.74
Demographic Risk	.13	1.42
Surgency	-.14	-1.53
Negative Affectivity	-.03	-0.29
Effortful Control	.08	0.82
Maternal Depressive Symptoms	-.01	-0.13
AM Cortisol	-.76	-9.43*
Time Between Samples	.05	0.58
Maternal Security	.01	0.11

* $p < .05$.

Table 3
Coefficients (Standard Errors) for Models of Cortisol Change on Teacher Security

Parameter	Model A fixed intercept and slopes		Model B random intercepts for maternal depression and maternal attachment		Model C maternal depression and maternal attachment intercepts as a function of classroom quality	
	B (SE)	t (80)	B (SE)	t (80)	B (SE)	t (78)
<u>Fixed Effects</u>						
Intercept, γ_{00}	.14 (.08)	1.63	.24* (.09)	2.65	.19* (.09)	2.06
Age	-.02* (.01)	-2.42	-.03* (.01)	-3.49	-.03* (.01)	-3.46
Sex	.00 (.02)	-.50	-.01 (.01)	-.65	-.01 (.01)	-.88
Demographic risk	.03 (.02)	1.64	.01 (.01)	.82	.01 (.01)	.53
Surgency	-.02 (.01)	-1.87	-.02 (.01)	-1.84	-.02 (.01)	-1.67
Negative affectivity	-.01 (.01)	-.59	-.01 (.01)	-.60	-.01 (.01)	-.91
Effortful control	.02 (.01)	1.55	.01 (.01)	1.21	.02 (.01)	1.57
Maternal depressive symptoms	.00 (.00)	-.19	.00 (.00)	.66	-.02 (.01)	-1.70
Class Qual., γ_{81}					.003 (.002)	1.75
AM cortisol	-.72* (.08)	-9.45	-.70* (.07)	-10.03	-.70* (.07)	-9.78
Time between cortisol collections	.01 (.01)	1.17	.00 (.01)	.09	.01 (.01)	.74
Maternal security	.02 (.03)	.65	.00 (.03)	.12	.71* (.30)	2.35

Parameter	Model A fixed intercept and slopes		Model B random intercepts for maternal depression and maternal attachment		Model C maternal depression and maternal attachment intercepts as a function of classroom quality	
	<i>B</i> (<i>SE</i>)	<i>t</i> (80)	<i>B</i> (<i>SE</i>)	<i>t</i> (80)	<i>B</i> (<i>SE</i>)	<i>t</i> (78)
Class Qual., $\gamma_{12.1}$						
Teacher security						
Intercept, $\gamma_{13.0}$	-0.07*	-2.20	-0.05	-1.66	-0.07*	-2.03
	(.03)		(.03)		(.03)	
<u>Variance Components</u>						
Level 1			σ^2_e	.002	.003	
Level 2						
In maternal dep.			σ^2_8	<.001*	.000*	21.01
In prim. careg. sec.			σ^2_{12}	.007*	.002*	22.47
						$\chi^2(11)$

* $p < .05$.