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Cortisol patterns at home and child care: Afternoon differences and evening recovery in children attending very high quality full-day center-based child care

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ABSTRACT

Previous work has found that many young children show different patterns of production of the hormone cortisol, which is sensitive to stress and challenge, on days when they are at child care compared with days when they are at home. At home, preschool age children typically show a decreasing pattern of cortisol production across the day which is the expected diurnal rhythm for this hormone. At child care many children show a rising pattern of cortisol production across the day. Lower child care quality has been associated with larger child care cortisol increases from morning to afternoon. The current study examined salivary cortisol at mid-morning, mid-afternoon, and evening on child care and weekend days in children attending the highest quality child care centers. Child-level classroom quality assessments were obtained using the Modified-Observational Rating of the Caregiving Environment. The rising pattern across the child care day was replicated, although in a smaller proportion of the children than previously reported. Variation in the quality of the child's niche or microclimate predicted these cortisol increases. When children returned home from child care, cortisol levels returned to levels observed on non-child care days even for children who showed the rising cortisol pattern during child care.

1. Introduction

During the early childhood period, children experience challenges across all developmental domains, often simultaneously, and are developing physical and psychological strategies for managing these challenges. Thus, at the same time that they are developing cognitive skills such as literacy and numeracy and learning facts about the world, they are learning self-help skills, learning to navigate social relationships, learning fine and gross motor skills, and learning to recognize and communicate their feelings and needs. In addition, stress-sensitive physiological systems are also maturing over the early childhood years. Studies in non-human animals have shown that challenges imposed on these stress systems early in life may have important consequences for later behavior and long-term physical and psychological health.

Animals have complex physiologic systems for managing cognitive, physical, and psychological challenges. The two primary systems are the brainstem norepinephrine/sympathetic-adrenomedullary (NE–SAM) system and the hypothalamic–pituitary–adrenal (HPA) system (see Gunnar & Davis, 2003 for review). These systems balance growth, restoration, and preparation for the future with the allocation of resources for facing current needs. At least for humans, these systems have a protracted developmental period. The HPA-axis exerts many of its effects via a potent steroid hormone, cortisol. Exposure to both exogenous and endogenous forms of cortisol during development can have important effects on the body, brain, and behavior, as well as on

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the HPA-axis itself (Edwards & Burnham, 2001). Work with non-human animals clearly indicates that caregiving experiences during early development can be particularly important (Liu et al., 1997; Sanchez, Ladd, & Plotsky, 2001).

Advances in non-invasive techniques for measuring hormones have resulted in a burgeoning literature using physiologic assessments in adults and children. Studies using measurements of the hormone cortisol, including studies with children, are increasingly common. As the primary hormonal product of the HPA axis, many investigators have been interested in cortisol because it is sensitive to stress and challenge and is implicated in the pathway from stress to disease (Charmandari, Kino, & Chrousos, 2004; McEwen, 2000). Cortisol also has a number of other features that make it attractive in psychological research. Compared with adrenaline, cortisol reactivity is indicative of more sustained challenge, as one of its roles involves preparing the body for further adrenaline-mediated responses (McEwen & Lasley, 2002). Cortisol is also influenced by cognitive processes such as perceived control and support (Kirschbaum, Klauer, Filipp, & Hellhammer, 1995). A recent meta-analysis of 208 studies with adults suggests that, at least in laboratory settings, the largest cortisol changes and longest recovery times are due to stressors that are both uncontrollable and that contain social-evaluative components (Dickerson & Kemeny, 2004). Cortisol can also be easily and reliably assessed in both adults and children from saliva (Kirschbaum & Hellhammer, 1994).

In the absence of acute stress, cortisol follows a circadian rhythm. For adults, highest values are typically seen at waking, followed by a sharp decline and then a more gradual decline across the day, returning to nadir at approximately midnight (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Infants and toddlers show a morning peak and evening nadir, with a flatter pattern of cortisol production from mid-morning to mid-afternoon (Larson, White, Cochran, Donzella, & Gunnar, 1998; Price, Close, & Fielding, 1983; Watamura, Donzella, Kertes, & Gunnar, 2004). By the preschool and early school period most children show the expected decrease across the middle of the day at home (Bruce, Davis, & Gunnar, 2002; Davis, Donzella, Krueger, & Gunnar, 1999; Dettling, Gunnar, & Donzella, 1999).

A pattern of rising cortisol production across the day at child care has been repeatedly found for the majority of children studied, with a decreasing pattern across the day for these same children at home (Dettling et al., 1999; Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Watamura, Donzella, Alwin, & Gunnar, 2003; Watamura, Sebanc, & Gunnar, 2002). In the one study that assessed infants and toddlers, infants did not show a consistent difference in cortisol production across the day between home and child care, whereas toddlers showed the rising pattern at child care which has been found repeatedly for preschool age children (Watamura et al., 2003). Similarly, in studies assessing older, school-aged children, no rise across the day has been found when children are in school or at a summer school-based child care program (Bruce et al., 2002; Davis et al., 1999; Dettling et al., 1999). Thus, the rising cortisol pattern across the child care day is seen for toddlers and preschoolers and suggests a context-dependent activation of the HPA-axis limited to the early childhood period that may have important consequences for the developing child.

The quality of the caregiving the child receives and the quality of the relationship the child has with his or her caregivers are likely to be important sources of support for the child. Child care quality has been associated with many positive outcomes for children (Love et al., 2003). However, data from the NICHD Study of Early Child Care suggest that the quality of care children receive in the United States is often poor or merely adequate, with positive caregiving estimated to be very or somewhat uncharacteristic of the care received by 61% of young children (NICHD Early Child Care Research Network, 2000).

The effects of early experience in non-human animals depend on the caregiving context, such that optimal caregiving may buffer the effects of early stressors (Levine & Wiener, 1988; Sanchez et al., 2001). For humans, there is a much less complete understanding of the potential sensitive periods, buffers, and long-term effects of stressors during childhood. Interestingly, in a laboratory study that manipulated caregiving, infants cared for by babysitters who were less interactive and responsive during half-hour separations from mothers showed cortisol elevations, whereas infants cared for by more interactive and responsive babysitters showed no cortisol elevations (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992). In several studies published to date assessing child care quality and cortisol, higher quality child care was associated with less of a rise in cortisol at child care. Tout et al. (1998) found that a smaller proportion of children showed the rising profile at a higher quality center as compared with a lower quality center (although both were of at least good quality). Dettling et al. (2000) found that more focused attention and stimulation by family child care providers was associated with less of a rise across the day at child care. Sims et al. (2006a,b) have demonstrated that toddlers and preschoolers at high quality child care centers in Australia show decreasing cortisol across the day, whereas those in lower quality centers show the rising pattern found in the U.S. samples. Thus, the limited evidence available for children, which is supported by the non-human animal literature, suggests that the responsiveness and sensitivity of caregivers during early childhood may serve as a buffer to environmental stressors or result in fewer or less intense stressors.

In all U.S. child care centers studied to date, over 60% of children showed rising cortisol from mid-morning to mid-afternoon at child care, and in some centers nearly every child showed this pattern (Dettling et al., 1999; Dettling et al., 2000; Tout et al., 1998; Watamura et al., 2002). This relation seemed to be partially explained by variations in quality, although all centers were in the good to excellent range. In a paper examining the subcomponents of the Australian quality rating system, Sims et al. (2005) provide evidence that characteristics of warmth, individualized programming, family relationships, equity, and continuity of care may be particularly important factors influencing cortisol reactivity. The present study examined cortisol patterning in the highest quality child care environments available (see Method for characteristics) to identify whether the rising cortisol pattern would be replicated in children in these settings.

The majority of assessment instruments for child care quality provide a global classroom quality assessment, based largely on structural characteristics. However, it may be that individual children within a classroom have different experiences with caregivers and other children that are important for their development and their perception of challenge. In fact, it may be the case that differences at several levels of the child's environment are important. At least three levels seem worth considering: 1) the classroom-level, including structural variables, which has received considerable attention in the child care literature, 2) the child's niche or microclimate, including the child's interaction with their play partners and the caregiving this smaller group receives, and 3) the child-level, specifically the nature of the one-on-one caregiving the child receives.

The intermediate level, the niche or microclimate, has been most overlooked in the literature. However, from the child's perspective, experiences at child care may be largely a function of with whom the child plays and where in the room the child spends the majority of his or her free play time. The same teacher's caregiving style (e.g., degree of community building or controlling behavior) is likely influenced by what area of the room that teacher is in. For example, the dramatic play corner may elicit more efforts to help children learn to resolve their own conflicts (and conflicts themselves may be more frequent) than the project area. In turn, the project area might elicit more controlling behavior, as there are more rules involved with projects like cooking than in dramatic play. Similarly, play partners influence both each other and the caregiving the peer group receives. A child that frequently plays with children who are more aggressive, for example, may experience more negativity, both from the aggressive child, and from the efforts of others to manage the aggressive child.

The present study used a quality assessment instrument, the Modified Observational Ratings of the Caregiving Environment (M-ORCE; Gunnar, Kryzer, Phillips, & Vandell, 2001), which provides a quality rating for each child, including both measures of caregiving directed to the target child and that directed to the group.

Previous work with children in child care has not examined cortisol levels in the hours before or after child care. However, whether children who show rising cortisol from morning to afternoon at child care continue to show elevations into the evening hours is an important question. If the afternoon elevations reflect a sustained alteration of the HPA-axis, we might expect to find continued elevations in the evening hours. If, however, afternoon elevations reflect more temporary and situation-specific elevations, then we would expect cortisol to return to baseline evening levels when children return home. Because the concern about daily afternoon elevations stems from concern about the cumulative exposure to higher than normal circulating cortisol in the daytime hours, continued elevations into the evening hours would raise further concerns about the potential long-term effects of home versus child care patterning differences. To address this question, evening cortisol on child care and weekend days was also assessed in the present study.

2. Method

2.1. Participants

2.1.1. Settings

The population of interest was three- to six-year-old children attending high-quality full-day center-based child care. In a previous study (Watamura, 2004), child care centers in a small city in upstate New York were studied longitudinally through interviews, observation of professional development activities, and with the Early Childhood Environment Rating Scale— Revised (ECERS—R; Harms, Clifford, & Cryer, 1998). Based on this earlier study, three centers were identified as the highest quality centers serving children in a full-day center-based environment. Two of the settings were at ceiling (7) on the ECERS—R, and the third center was in the good range (5). In addition, centers were chosen to maximize quality characteristics beyond those assessed with the ECERS—R. Six of seven classrooms in the sample had at least one teacher with a master's level education in early education or child development. Teachers and directors at all three centers participated in intensive professional development programs that included one-on-one mentoring, monthly topical group meetings, regular visits by a staff development specialist, and mini-conferences on early education and developmental topics. Half the sample was drawn from a full-day University laboratory school child care, where teachers are both caregivers for children and teachers of undergraduates studying early childhood education. Lastly, the teachers and centers were located in resource-rich environments, with many special materials, field trips, and a high degree of parent involvement. Each classroom served no more than 16 preschoolers per day, with one or two lead teachers and one to three regular assistant teachers.

2.1.2. Children

There were a total of 120 eligible three- to six-year-old children in the classrooms at the time of the study. Criteria for exclusion were (a) in the current classroom for less than 1 month ($n = 2$), (b) diagnosed Pervasive Developmental Disability such as Autism ($n = 2$), (c) non-English speaking in an English-only classroom ($n = 1$) or (d) attending child care for less than 30 h/week ($n = 13$). This last exclusion criterion reflected our interest in children in full-time child care. Of the 120 potential participants, parents or guardians of 79 children (36 female) enrolled in the study. Of the enrolled children, four were siblings. Six children (2 female) refused saliva sampling. There were no differences between refusers and non-refusers on sex, age, or caregiving summary ratings (all $ps > .20$). Data from children who refused saliva sampling were excluded from all analyses. Seven children (2 female) were excluded due to illness ($n = 1$; flu), medications ($n = 4$; medication for asthma), or excessively high cortisol values likely due to medications, illness, or sample contamination ($n = 2$; values greater than 3.5 μg/dL). There were no sex, age, or caregiving rating differences between children with medications or illness and those with no known medications or illness, $ps > .25$.

Of the 65 children with cortisol and observational data, 45 families completed the demographic survey included in the weekend data collection kit. Families reported their child's race as American Indian or Alaska Native (3; 7%), Asian or Asian-American (7; 16%), White or Caucasian-American (29; 64%), both Black or African-American and White or Caucasian-American (4; 9%), and both Asian or Asian-American and White or Caucasian-American (2; 4%). In addition, families reported their child's ethnicity as Hispanic or Latino (7; 17%), or non-Hispanic or Latino (34; 83%). Families of 10 children reported that their child's first language was not English and an additional eight families reported that their child spoke a language in addition to English. Forty-two families reported their annual income as above $25,000 and 3 as under $25,000. In addition, 8 families reported receiving and 36 reported not receiving a child care subsidy.

Five families reported that their child was adopted, four internationally and one from within the United States. Children were adopted between 8.5 and 18 months of age. Because this seemed to be a high percentage of adopted children, cortisol was compared for the adopted versus the non-adopted children using a Mann–Whitney test. Cortisol levels averaged across all time points were lower for the adopted children (.07 vs. .13), $U = 26$, $p = .001$. Salivary cortisol was lower at each time point for the adopted children, although this difference was significant only for Child Care Afternoon, $U = 47$, $p = .009$. Four of the five adopted children showed a flat pattern across the day at child care (all decreasing, but the change was less than .05ug/dL), and the fifth child showed a rising pattern. The main analyses described below involving relations with cortisol were unchanged if the 5 adopted children were excluded. Because we have not assessed adoption status in previous samples, and because none of the 5 children were being treated for adoption-related health or behavior problems, we included these children in the analyses described below.

2.2. Measures

2.2.1. Cortisol

Saliva samples were collected from children at child care and at home, with the goal of obtaining samples on two child care and two home days at 10:30 am, 3:30 pm, and 8 pm, for a total of 12 samples per child. Median sampling times at child care were 10:48 am (range: 9:16 am to 11:23 am) and 3:48 pm (range: 3:09 pm to 4:40 pm). The median reported evening sampling time on child care days was 8:01 pm (range: 7:00 pm to 10:35 pm). Median reported sampling times on weekend days were 10:07 am (range: 8:50 am to 11:55 am), 3:44 pm (range: 1:51 pm to 6:00 pm), and 8:06 pm (range: 7:05 pm to 10:00 pm). At each time point, samples taken more than 1 h from the intended sampling time did not differ from the remaining samples, *ps* > .55, therefore all samples were included. The saliva was expressed into vials and stored at − 80 °C until data collection was completed. For assay, samples were assigned to batches so that all samples from the same child were in the same batch, and classroom and batch were not confounded. The samples were assayed at the Biochemical Laboratory, Department of Psychobiology, University of Trier, GE. Cortisol levels in saliva were determined using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA). Cortisol assays were performed in duplicate on .15 to .75 ml of whole saliva. For samples retained in the analyses described below, the mean intraassay coefficient of variation (CV) was 4.93% and the interassay CV was 5.65% to 8.16%.

2.2.2. Caregiving and experiences in child care

To assess caregiving and experiences at child care, a Modified version of the Observational Ratings of the Caregiving Environment (M-ORCE) was used because it assesses the quality of the caregiving environment for each child, with a particular focus on the child's experience of challenge/stress. To assess the caregiving environment in a large national study of early child care, the NICHD Early Child Care Research Network developed an observational instrument appropriate for use in homes and centers, the Observational Ratings of the Caregiving Environment (ORCE; NICHD ECCRN, 1996). For the purposes of a similar study on family child care, the ORCE was modified (M-ORCE, Gunnar et al., 2001). The modifications were designed to add variables expected to reflect the child's experience of challenge/stress in the child care setting. Added variables included measures of the degree of cohesion in the caregiving environment (positive and negative community) and the target child's negative affect (angry, anxious/vigilant, sad/withdrawn). In the present study, the M-ORCE was used to evaluate the child care environment for each child during two 44-minute observations, one in the morning and one in the afternoon. The qualitative ratings completed at the end of the observation period included several items addressing caregiving to the target child and several items addressing caregiving and climate of the group.

2.2.3. Additional measures

To assess possible confounding variables and to collect information on exclusion criteria, several additional pieces of information were collected. These data were collected in the classroom by research assistants or via questionnaires completed by parents.

2.2.3.1. Attendance. On sampling days children's arrival and departure times were recorded from the parent sign-in sheet to determine hours of attendance. Median day length for children with data included in the cortisol analyses was 8 h 17 min (range: 5 h 30 min to 9 h 40 min). As there was only one instance of a day length less than 6 h (1 day for one child), and all children attended child care 5 days/week, no children were excluded due to observed attendance less than 30 h/week.

2.2.3.2. Current health. Cortisol levels are elevated in children during some types of illness, particularly illness accompanied by a fever (Nickels & Moore, 1989; Zelnik, Kahana, Rafael, Besner, & Iancu, 1991). To assess concurrent illness children's temperatures were taken with an ear thermometer on saliva sampling days. Children with ear temperatures above 99.5 were not sampled, or samples were not used if sampling occurred prior to taking the child's temperature. Children were also asked about symptoms, and obvious symptoms such as a runny nose or cough were recorded. If children were absent due to illness, we waited several days after their return and until symptoms were cleared to collect samples.

2.2.3.3. Classroom schedule. Sampling times were selected so that they were at least 30 min after snacks to avoid post-meal surges in cortisol (Gibson et al., 1999; Ward et al., 1995) and at least 1 h before lunch to avoid anticipatory or midday surges (Follenius, Brandenberger, & Hietter, 1982). Similarly, children were sampled before gym or outside time to avoid aerobic levels of activity (Kirschbaum & Hellhammer, 1994), and at least 30 min after nap to avoid nap-time cortisol decreases (Watamura et al., 2002).

2.2.3.4. Health history questionnaire. The Health Questionnaire asked parents about the child's age, health history, family stressors, and adoption status. Data on illness and medications were used to exclude samples from children who take medications thought to interfere with cortisol assays or samples taken while children were ill with a fever or flu as reported above. In addition, families were asked to respond to the following question by circling none, 1, 2, or 3 or more: “Has your family experienced any major stressors in the last 6 months, for example, a move, birth of sibling, death in the family, divorce, separation, remarriage, death of a pet.” Data on the number of stressors in the past 6 months were available for 41 children for whom cortisol data were used. Of these, 22 families reported no stressors, 15 reported one stressor, and 3 reported two stressors. No children included in the cortisol analyses experienced 3 or more stressors in the past 6 months.

2.3. Procedure

2.3.1. Recruitment procedures

Center recruitment was conducted via e-mail, phone, and personal visits. Families were recruited through letters placed in their parent mailboxes at the centers. For 3 to 5 days after letters were distributed, research assistants were available during pick-up time to answer questions and recruit families.

2.3.2. Classroom data collection procedures

During the first week in a new classroom, the researchers spent time playing with children, becoming familiar with teachers, and learning the classroom schedule and rules. In the following week, the researchers demonstrated the saliva sampling procedure and the earphones and recorders that would be used during the M-ORCE observations to children and teachers. After demonstrating the procedures, saliva sampling and M-ORCE observations began.

2.3.2.1. Saliva sampling. Group saliva sampling in a classroom generally occurred over 3–5 days. If individual children had low volumes or had been absent or ill, they were sampled individually at a later time. Saliva samples were taken mid-morning and mid-afternoon, as close to 10:30 am and 3:30 pm as possible without disrupting classroom routines and avoiding daily snack, lunch, nap, gym, and outside time. Saliva samples were collected by research assistants using simple, well-established procedures (Gunnar & White, 2001; Watamura et al., 2003; Watamura et al., 2002). However, in the present study no flavor or other substance, such as Kool-Aid™, was used to stimulate saliva flow, and samples were collected during timed 2-minute intervals. Saliva was expressed into plastic vials using a mechanical compression device and a needleless syringe. Child care samples were frozen until assayed.

2.3.2.2. Classroom observations. The M-ORCE was completed during one morning and one afternoon observation period for each child within 1 month of classroom saliva sampling. EMK (lead M-ORCE coder in M. Gunnar's laboratory at the University of Minnesota) trained SEW, who trained all research assistants using both taped and live sessions. Whenever possible, two observers simultaneously coded the same child and consensus codes were used. This occurred for 53% of observations. Observers who coded independently had reached reliability with SEW. The average kappa for all behavior codes and raters was .78, and the range for items by coder was .41 to .97. Percent agreement for the qualitative items across all coders was 69% for exact agreement and 99.5% for agreement within 1 scale point.

2.3.3. Home data collection procedures

2.3.3.1. Sample collection. Parents were asked to collect saliva samples on two weekday evenings at 8 pm, and on two weekend days at mid-morning, mid-afternoon, and 8 pm. On the first full day of saliva sampling at child care, parents were given a kit containing sampling materials, instructions for taking one saliva sample, and a daily checklist. They were asked to take the sample that evening if possible, and if not possible, on another child care evening (Monday through Thursday). The child was given a child's watch with the alarm set for 8 pm. Parents were asked to sample their child at 8 pm, when the watch alarm sounded, for a 2-minute interval, using the watch to time the sample. When the first sample was returned, the second evening kit was put in the parent's mailbox. At the end of the sampling week, parents were given a weekend kit to collect 2 days of three samples per day at approximately 10 am, 3:30 pm, and 8 pm on two separate (consecutive or non-consecutive) weekend days.

Parents were asked to refrigerate the samples immediately and promptly return them to a small refrigerator at the child care center. Saliva samples were picked up daily from the child care centers; the saliva was expressed and frozen on the same day. All home kits included a cell phone number and e-mail address for both daytime and weekend questions, and parents were encouraged to contact us if they had any questions or concerns.

2.3.3.2. Compliance. As cortisol follows a circadian rhythm, accurate recording of the timing of the saliva samples is important. Previous research has indicated that compliance with requested sampling times varies across participants (Kudielka, Broderick, &

Kirschbaum, 2003). To encourage compliance, the importance of the time of sampling was emphasized in the directions included with the sampling materials. For the evening sample on days children attended child care, the digital child's watch was used as a reminder and encouragement of compliance. For the weekend samples, 94% of families were given sampling materials with compliance caps or compliance boxes. Compliance caps are a commercially available product (APREX Corp., Union City, CA), which resemble a prescription bottle. A digital device in the cap of the bottle records the time and date when the cap is opened. The cotton dental rolls used for sampling were placed inside the bottle. Compliance boxes were custom designed for this study (Cayuga Design, Ithaca NY). A large outer box contained all sampling supplies and a smaller recording box. The smaller box contained a circuit which recorded the time and date when the box was opened or closed. The compliance boxes were a considerable improvement over the compliance caps, as they were cheaper, did not resemble prescription bottles and thus were less intimidating and more likely to be returned, had a replaceable battery, and allowed the data to be read by any personal computer. In all cases, families were asked to record the time and date of each sample, and were told that some kits contained compliance tracking devices. Of the 45 families who returned weekend data and are used in the analyses described below, 36 also had compliance data available. The times recorded by parents and compliance devices differed by a median of 3 min (range: 0 to 153 min). This study was approved by the Institutional Review Board of Cornell University.

2.4. Missing data and data reduction

2.4.1. Cortisol

For five children, a total of 11 individual samples (< 2%) were excluded due to ear temperatures over 99.5 °F (2 children, 7 samples), dairy contamination (1 sample), unclear labeling (1 sample) or distress following a physical accident (1 child, 2 samples). Usable cortisol and observational data were available for 65 children (31 female) enrolled in 7 classrooms. These children ranged in age from 2 years 8 months to 5 years 4 months ($M = 4$ years 3.5 months).

Of the children who participated in saliva sampling at child care, after the above exclusions, 5 children had only morning and afternoon samples at child care, 15 children had morning, afternoon, and evening samples from child care days only, and 45 children had morning, afternoon, and evening samples on both child care days and on at least one weekend day. Using the Freeman–Halton extension of the Fischer Exact test, the distribution of males and females across these groups was not different, $FI = 3.69$, $p = .16$. Similarly, the groups did not differ in age, $F(2,62) = .29$, $p = .75$. There was a trend for Afternoon Climate to be different among the groups, $F(2,61) = 2.66$, $p = .08$. Tests of simple effects indicated that children with only morning, afternoon and evening samples from child care days received a lower climate rating than did children with morning, afternoon, and evening samples both at child care and at home ($M = .64$ vs. $M = 1.00$). Children were compared on the two cortisol levels available for all three groups, Child Care Morning and Child Care Afternoon. Child Care Afternoon cortisol differed among the three groups, $F(2,61) = 5.03$, $p = .01$. Tests of simple effects indicated that children with only morning, afternoon and evening samples on child care days had higher afternoon cortisol at child care than did children with morning, afternoon and evening samples on both child care and weekend days ($M = .24$ µg/dL vs. $M = .15$ µg/dL).

All cortisol assays were performed in duplicate and the results averaged. These values were then averaged within context by time point, creating six cortisol levels reflecting average mid-morning, mid-afternoon, and evening cortisol on child care days, and mid-morning, mid-afternoon, and evening cortisol on weekend days (see Table 1). Cortisol values at each time point were examined for positive skew. No skew was evident, so non-transformed values were used in all analyses. In addition, four change variables were calculated (see Table 1 for means and SEM). Child Care Change was the average mid-afternoon child care level minus the average mid-morning child care level. Similarly, Weekend Change was the average mid-afternoon weekend level minus the average mid-morning weekend level. In both cases, a positive change reflects an increase in cortisol across the day. Difference in Change was Child Care Change minus Weekend Change. Child Care Rise represented whether children showed a rising, flat, or falling pattern of cortisol production across the day at child care. Changes equal to or greater than .05 were used as a conservative estimate of change following the cut-off used in Watamura et al., 2002. That cut-off was selected to exceed the intra-assay CVs and thus reflect a reliable rise. Lastly, Overall Mean Cortisol was the average of the six time point cortisol levels.

Table 1
Mean salivary cortisol concentrations (µg/dL) and SEM.

Cortisol sample	Mean (SEM)	Sample size
Child care morning	.15 (.01)	$n = 64$
Child care afternoon	.16 (.01)	$n = 64$
Child care evening	.06 (.01)	$n = 59$
Weekend morning	.14 (.01)	$n = 49$
Weekend afternoon	.11 (.01)	$n = 50$
Weekend evening	.05 (< .005)	$n = 48$
Overall mean cortisol	.12 (.01)	$n = 65$
Child care change (afternoon–morning)	.01 (.01)	$n = 64$
Weekend change (afternoon–morning)	− .03 (.01)	$n = 48$
Difference in change (child care change–weekend change)	.04 (.02)	$n = 47$

Note: SEM stands for Standard Error Mean.

2.4.2. M-ORCE ratings

Two summary measures were created from each of the morning and afternoon M-ORCE qualitative ratings, one to reflect child-level caregiving, and one to reflect group-level climate (see Table 2). Sensitivity to the target child was the mean of the standardized ratings of sensitivity, positive regard toward the child, negative regard toward the child (reverse scored), detachment (reverse scored), and intrusiveness (reverse scored). Climate was the mean of the standardized ratings of positive emotional climate, positive community building, expressed community, chaos, negative emotional climate (reverse scored), negative community building (reverse scored), and over-control (reverse scored).

Interestingly, within this high quality sample, chaos was only coded as either 1 or 2. The manual for the M-ORCE indicates that a code of 1 for Chaos should be assigned when "…the setting is not at all chaotic. There is clear structure and organization to the environment and activities." A code of 2 should be assigned when "…the setting has brief moments of chaos. There is structure and organization the majority of the time with just some instances of chaos." It is not clear that a rating of 1 would always be preferable to a rating of 2. To maintain a firm structure in a preschool classroom at all times would require some rigidity on the part of teachers and would require significant attempts to curb children's normal range of behavior. Thus, "some lack of structure" might reflect a balance between guiding and protecting children while letting them explore, navigate relationships, and test their limits. Thus, we made the a priori decision not to reverse score chaos. Item analyses were computed for the Sensitivity and Climate variables. For the Sensitivity variables, the morning and afternoon alpha coefficients were .76 and .65, and for the Climate variables they were .57 and .54, respectively.

3. Results

3.1. Preliminary analyses

Bivariate correlations between age and the six variables representing cortisol values averaged within time for each context, the four derived cortisol change variables, and Overall Mean Cortisol were not significant, *ps* $> .25$. There were also no sex differences on any of the cortisol variables, *ps* $> .20$. Similarly, none of the bivariate correlations between age and the four M-ORCE summary ratings were significant, *ps* $> .35$. Care providers were more sensitive to girls than boys based on the summary sensitivity score obtained in the morning, $t(62) = 2.33$, $p < .05$, but not for the one obtained in the afternoon, $t(62) = .57$, *ns*. There were no other sex differences, *ps* $> .25$. Therefore, age was not included in subsequent analyses and sex was included only in analyses involving Morning Sensitivity.

3.2. Cortisol patterning at child care and home

3.2.1. Daytime

To test whether the previously reported morning to afternoon difference was replicated, a Context (Child Care Day, Weekend Day) × Time (Morning, Afternoon) ANOVA with repeated measures on both factors was computed for the cortisol data. There were no main effects of Time or Context. However, there was a Time by Context interaction, $F(1,46) = 5.36$, $p < .05$, $\eta_p^2 = .10$. Tests of simple effects indicated that afternoon levels were higher at child care than at home on the weekend, mean difference .04 μg/dL, *SEM* diff .01, $t(46) = 2.92$, $p < .01$, whereas morning levels were not different, mean difference .00 μg/dL, *SEM* diff = .01, $t(46) = .062$, *ns* (see Fig. 1). There were no differences between classrooms, $F(6,40) = 1.52$, *ns*, or centers $F(2,44) = .06$, *ns*.

Children were classified as exhibiting a rising, falling or flat pattern across the day using changes of .05 μg/dL or greater between morning and afternoon cortisol levels. At child care, 20% of children showed a falling pattern from morning to afternoon, 45% showed a flat pattern, and 34% showed a rising pattern. At home, 42% of children showed a falling pattern from morning to afternoon, 42% showed a flat pattern, and 17% showed a rising pattern. Based on a Wilcoxon Signed Ranks Test, cortisol rise scores were more positive (rising > flat > falling) at child care, $z = 2.70$, $p < .01$.

3.2.2. Evening

Child care evening and weekend evening cortisol levels were not different, .05 μg/dL vs. .04 μg/dL, $t(46) = .53$, *ns*, (see Fig. 1). In both contexts evening levels were much lower than either morning or afternoon levels, as would be expected given the diurnal rhythm in this hormone. Mean difference between morning and evening was .09 μg/dL, *SEM* .02, $t(58) = 6.02$, $p < .001$ on child care days and .10 μg/dL, *SEM* .01, $t(45) = 10.31$, $p < .001$, on home days. Mean difference between afternoon and evening was .11 μg/dL, *SEM* .02, $t(58) = 6.68$, $p < .001$, on child care days and .07 μg/dL, *SEM* .01, $t(45) = 7.56$, $p < .001$, on home weekend days.

Table 2
Mean and range for M-ORCE caregiving ratings ($n = 64$).

M-ORCE caregiving rating	Mean (SEM)	Range
Morning sensitivity	− .03 (.10)	− 5.03 to .77
Afternoon sensitivity	.02 (.08)	− 2.44 to .91
Morning climate	.96 (.06)	− 2.41 to 1.60
Afternoon climate	.90 (.07)	− 1.30 to 1.52

Note: M-ORCE and SEM stand for Modified Observational Ratings of the Caregiving Environment, and Standard Error Mean.
Values are averages of standardized qualitative item ratings.

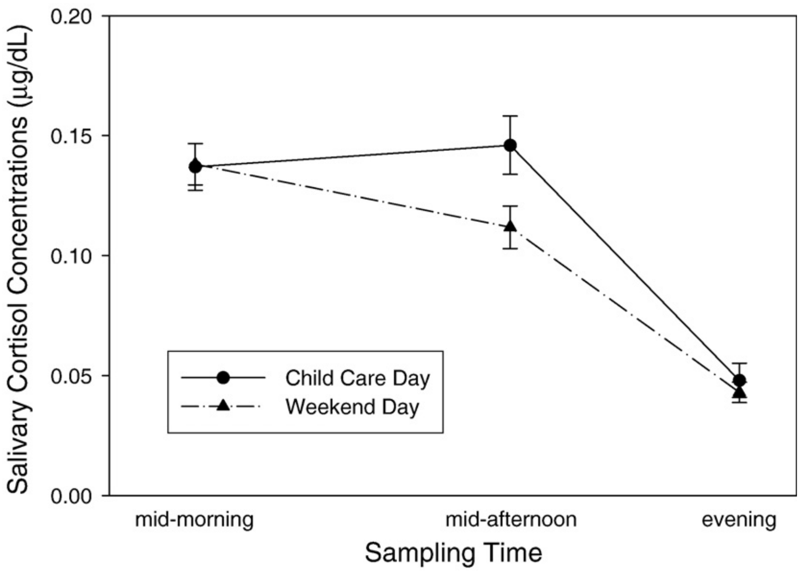

Fig. 1. Morning, afternoon, and evening salivary cortisol (mean ± SEM) at child care and at home ($n = 45$).

Forty-five percent of children showed a difference of .05 µg/dL or greater in the afternoon between home and child care, whereas only 13% showed a difference of .05 µg/dL or more in the evening, McNemar exact test, $p < .01$. For children showing a rising pattern from morning to afternoon at child care, there was also no difference in evening cortisol on child care versus weekend days, .05 µg/dL vs. .04 µg/dL, $t(16) = .11$, *ns*.

3.3. Relations between cortisol patterning and child care observations

Correlations examining relations between caregiving ratings, cortisol at child care, and the difference in cortisol change between home and child care were computed (see Table 3). There was a positive relation between ratings of caregiver's Afternoon Sensitivity and Child Care Morning cortisol, $r = .26$, $p < .05$, so that higher cortisol in the morning was related to greater caregiver sensitivity in the afternoon. There was also a trend for both Morning Climate and Afternoon Climate to be positively related to morning cortisol at child care, $r = .23$, and $r = .22$, $ps < .10$, respectively.

Using the variable assessing home/child care cortisol patterning differences, there was a negative relation between M-ORCE ratings of Morning Climate and the Difference in Change scores, $r = -.32$, $p < .05$, such that children with higher climate ratings at child care in the morning showed less of a difference between cortisol change at child care compared to home (see Fig. 2). Using the ordinal Child Care Rise variable, there was a negative relation between Child Care Rise and Morning Climate, $r = -.27$, $p < .05$, so that children with higher Morning Climate ratings had less positive cortisol rise scores (falling < flat < rising) at child care.

For the Climate variables, some items (i.e. Negative Community Building) did not occur with sufficient frequency to contribute to the composite rating, and no one individual item was responsible for the correlations with Difference in Change. Instead, most items had low correlations (.1 to .3) in the expected direction.

To assess whether the Morning Climate variable helped to explain some of the variance in the overall cortisol pattern, a Context (Child Care Day, Weekend Day) × Time (Morning, Afternoon) Analysis of Covariance (ANCOVA) with repeated measures on both

Table 3
Correlations between M-ORCE caregiving ratings and cortisol variables.

	Child care Morning cortisol	Child care Afternoon cortisol	Child care Rise	Difference in cortisol change (Child care–weekend home)
Morning Sensitivity[a]	.09 $df = 60$	.11 $df = 60$	−.06 $df = 60$	.22 $df = 44$
Afternoon Sensitivity	.26* $n = 63$	.07 $n = 63$	−.21 $n = 63$	−.10 $n = 46$
Morning Climate	.23** $n = 63$	.04 $n = 63$	−.27* $n = 63$	−.32* $n = 47$
Afternoon Climate	.22** $n = 63$	.07 $n = 63$	−.18 $n = 63$	−.10 $n = 46$

Note: M-ORCE stands for Modified Observational Ratings of the Caregiving Environment.
[a] Partial correlations are reported for Morning Sensitivity controlling sex.
* $p < .05$; ** $p < .10$.

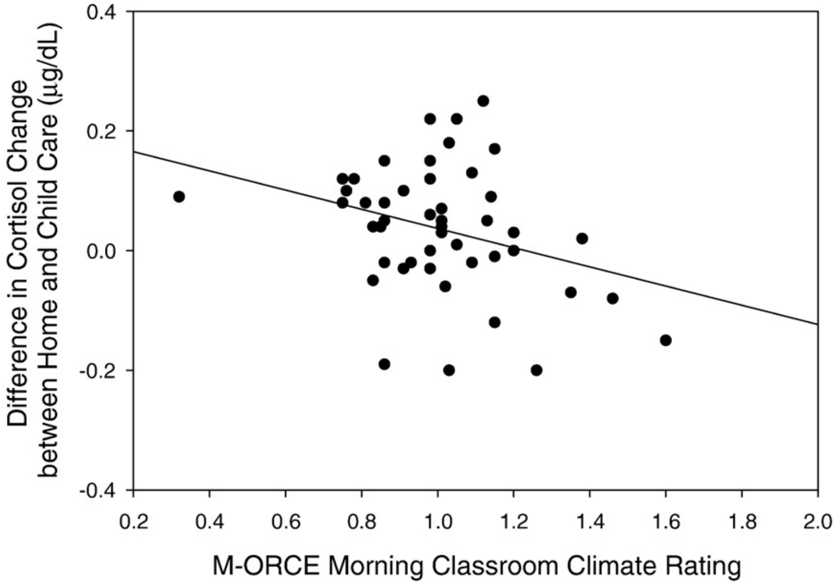

Fig. 2. Scatterplot of morning climate and difference in change (afternoon–morning) between child care and home days ($n = 46$).

factors and Morning Climate as a covariate was computed. A Time × Context interaction, $F(1,45) = 7.40$, $p < .01$, $\eta_p^2 = .14$, and a Time × Context × Morning Climate interaction, $F(1,45) = 5.18$, $p < .05$, $\eta_p^2 = .10$, indicated that the cortisol pattern across the day on child care and weekend days differed, and that this difference varied according to classroom climate. Children who received higher Morning Climate scores tended to have decreasing cortisol in both contexts, whereas children who received lower climate scores tended to have increasing cortisol at child care, but decreasing cortisol at home.

4. Discussion

This study had three principal aims. The first was to assess whether the previously reported rise in cortisol across the day at child care compared to at home was replicated in a sample of children attending the highest quality center care. Using the full sample, the mean change in cortisol from morning to afternoon at child care in the present study was smaller than reported by Dettling et al. (1999) and Watamura et al. (2002), .01 μg/dL versus .08 μg/dL and .15 μg/dL, respectively. However, a smaller proportion of children in the present study showed the rising pattern (.05 μg/dL or greater) across the day at child care: 34% compared with 83% of 3–6 year olds in the Watamura et al. (2002) study. For those children who showed a rising pattern (increase greater than .05 μg/dL), the magnitude of the rise was .13 μg/dL in the present study and .18 μg/dL in the Watamura et al. (2002) study. As demonstrated by the work of Sims et al. (2005, 2006a,b) in Australia, the aspects of quality that we used to select the current study classrooms such as warmth, individualized programming, family relationships, equity, and continuity of care may be particularly important for cortisol reactivity. Although direct comparison across studies is obviously not appropriate, taken together, the results from the U.S. studies done to date suggest that the proportion of children exhibiting a rise during the day at child care may vary according to center quality, but that for children who exhibit a rise it may be of similar magnitude, regardless of center quality.

The second aim was to determine whether assessments of classroom climate and caregiving styles were related to differences in cortisol changes across the day at child care versus at home. Interestingly, even within these very high quality child care centers we found differences in children's child care cortisol responses that were related to classroom climate. It was not simply that some classrooms had better climates and thus children in those classrooms had less of a difference in cortisol at child care and at home. Rather, within classrooms a better climate rating for a child was related to less of a difference between home and child care.

While it makes sense that niche or microclimate would be important, it was surprising that the variables reflecting caregiving specifically directed to the target child were not related to cortisol patterning. This may have been an artifact of our presence. In general it is possible for teachers to guess which child is being observed. Subtle changes of caregiving in response to observers is likely, and teachers frequently made comments about trying to “stay out of the way” of the observation, which may have resulted in less direct attention to the target child. The climate ratings would have been relatively protected from this kind of influence, although global changes in teacher's behaviors simply because observers were present could not be avoided.

The third aim was to assess cortisol levels in the evening hours on child care and weekend days to determine whether the afternoon elevations were followed by a return to low evening levels, or whether the elevations continued into the evening hours. Looking either at the overall mean levels or the individual child data, there was no evidence for a difference between child care days and home days in the evening. The means were not different, and in both contexts, nearly every child had values of .10 μg/dL or below. The return to low levels in the evening, combined with the normal decreasing pattern on weekend days suggests that the elevated levels are restricted to weekday, daytime hours, and therefore that the underlying rhythm is not disturbed. While we still do not know whether the small observed elevations in the afternoon on child care days have any negative effects, it is clear that, at least for children in high quality environments, the elevations are confined to the time at child care. This would minimize any potential negative effects by limiting the body's cumulative exposure to higher circulating cortisol levels.

Finally, the small sample of adopted children was notably different from the larger sample of non-adopted children. Their mean cortisol levels were half that of the other children, and they were lower at every time point measured, although this was a significant difference only for afternoon levels at child care. Generally low cortisol levels and the absence of an elevation in the afternoon could reflect a suppressed HPA-axis, which is indicated by low baseline cortisol and an attenuated response to challenge. However, studies specifically addressing early institutional care have not consistently found differences in cortisol for these children (Gunnar & Kertes, 2005). Although obviously a very small sample, the current data suggest further investigations may be warranted and that adoption history should be assessed as a possibly important variable in studies involving non-referred children.

With regard to the full sample of children in this study, it is important to note that the small elevations seen in the afternoon, followed by a return to low levels in the evening and a decreasing pattern on weekend days suggest that the HPA-axis is functioning normally for these children. For most children, the elevations are small, and limited to the time of day which is most challenging. To the best of our understanding, our stress systems have evolved to do just this, exhibit rises of the appropriate magnitude to meet a challenge, and then return to baseline as quickly as possible. In fact, as with other areas of research on stress systems, what we should expect is a U-shaped function relating cortisol levels and negative outcomes. Both showing no elevation when it is needed and showing large elevations because of excessive environmental demands or personal characteristics should be related to negative outcomes. In contrast, showing moderate elevations in response to appropriate challenges should be associated with positive outcomes. The question for which we still do not have an answer is whether the challenge or challenges that stimulate this small rise at child care are appropriate and therefore related to positive outcomes. It is not inherently surprising or worrisome that children meet more challenges on an average child care day than they do on an average weekend day. And it is not surprising that higher quality child care, particularly care that is individualized, would result in more appropriate challenges than would lower quality child care. It may be that environments that result in elevations every day for most children may on average have costs, whereas environments that result in elevations on some days for some children may on average have beneficial outcomes.

Having established that patterning differences exist in U.S. samples for at least some children even in centers of the highest quality, three next steps seem necessary. First, it is still unclear whether the small elevations observed are associated with any negative outcomes. Both longitudinal assessments and additional measures (e.g. assessment of immune function) will be necessary to determine if there are any negative consequences, and whether these consequences are modified by characteristics of the settings or children studied. Second, a better understanding of what causes these elevations, and whether they occur at a particular developmental stage regardless of previous child care experience or whether the onset and magnitude are related to age of entry into non-maternal child care, is needed. It is possible that children in particular developmental periods are more sensitive to the challenges involved in full-day center care. This hypothesis is supported by evidence that infants have not reliably shown patterning differences (Watamura et al., 2003), and that school-age children do not show a rising pattern across the day at school (Bruce et al., 2002). Lastly, work examining cortisol patterning in children experiencing increased stress and challenge outside of child care and children experiencing low quality child care is badly needed to further understand early variations in HPA-axis activity and their consequences for health and development.

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