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The Rise in Cortisol in Family Daycare: Associations With Aspects of Care Quality, Child Behavior, and Child Sex

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Abstract

We examined the increase in salivary cortisol from mid-morning to mid-afternoon in 151 children (3.0-4.5 yrs) in full-time home-based daycare. Compared to cortisol levels at home, increases were noted in the majority of children (63%) at daycare, with 40% classified as a stress response. Observations at daycare revealed that intrusive, over-controlling care was associated with the cortisol rise. For girls, the cortisol rise was associated with anxious, vigilant behavior, while for boys the rise was associated with angry, aggressive behavior. Child behavior did not mediate or moderate relations between care quality and the cortisol rise, except for evidence that boys scoring low on angry-aggressive behavior were more sensitive to variations in warm-supportive care than boys scoring high on this behavior.

Years of study of the impact of child care on children's development has led to the general conclusion that whereas child care can confer benefits (e.g., Ahnert & Lamb, 2003; Love et al., 2003; Phillips, McCartney, & Sussman, 2006), long hours in care may have some negative impacts that may be more pronounced for experiences in child care settings of poorer quality (e.g., Belsky et al., 2007; Gunnar & Donzella, 2002; NICHD ECCRN, 1998, 2001, 2003). Specifically, higher quality care has been associated with more positive peer interactions and lower levels of externalizing behavior (Howes et al., 1996; NICHD ECCRN, 1998, 2001; Votruba-Drzal, Coley, & Chase-Lansdale, 2004) and long hours in care are associated with the emergence of behavior problems in childhood (Belsky et al., 2007; NICHD ECCRN, 2001, 2003). Although the association between quantity of care and externalizing behavior is generally maintained in models with controls for family background and child care quality, a recent re-analysis of data from the NICHD Study of Child Care and Youth Development has documented that this association is moderated by the quality of care children experienced prior to school entry (NICHD ECCRN, in press). Hours of child care over periods when the child was in care was a stronger predictor of externalizing behavior at 24 and 54 months in lower quality as compared to higher quality care.

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In attempts to understand the mechanisms through which child care may operate to increase risks of negative developmental outcomes, researchers have begun to explore relations between child care and activity of the hypothalamic-pituitary-adrenocortical (HPA) system. The HPA system produces cortisol, a steroid hormone that plays critical roles in adaptation to stressors (for discussion of the HPA axis and the neurophysiology of stress, see Gunnar & Quevedo, 2007). Chronic or frequent activations of this neuroendocrine system early in life have been shown in animal models to increase fearfulness and impair behavior and physiological regulatory competence (Gunnar & Quevedo, 2007).

Interest in child care as a stressor capable of activating the HPA axis can be traced to a serendipitous finding published a decade ago (Tout, de Haan, Campbell, & Gunnar, 1998). Tout and colleagues were studying associations between day-to-day variability in cortisol levels and children's socioemotional behavior. When they examined their morning and afternoon cortisol data, they found that most of the children showed a rise in cortisol from morning to afternoon and they did so on most of the days they were in child care. This was remarkable as the typical basal pattern of cortisol production between mid-morning and mid-afternoon tends to be level or declining for children of this age (Watamura, Donzella, Kertes & Gunnar, 2004).

Since publication of the Tout et al. (1998) study, there have been a number of studies examining cortisol activity at child care. Two recent meta-analyses have summarized those findings (Geoffroy, Côté, Parent, & Séguin, 2006; Vermeer & van IJzendoorn, 2006). Both papers concluded that the rise in cortisol from morning to afternoon at child care has been convincingly documented and that this rise is not seen in the same children when they are at home on non-child care days. Both meta-analyses also concluded that age is a relevant factor, with larger increases being observed among the younger children (2- and 3-year-olds) as compared to the older children (4- and 5-year-olds). However, the two meta-analyses differed with regard to two critical issues: (1) whether child care quality influences the magnitude of the cortisol stress response over the day at child care, and (2) whether elevations in cortisol at child care are associated with children's temperament or behavior in the care setting.

Regarding quality of care, the first meta-analysis (Geoffroy et al., 2006) concluded that it has been shown conclusively that cortisol levels are higher and rise more over the day in poorer quality child care, whereas the second meta-analysis (Vermeer & van IJzendoorn, 2006) noted that nearly all of the child care studies of cortisol activity have been conducted in relatively high-quality care settings and thus child care quality cannot be the primary factor determining increases in cortisol at child care. The Vermeer and van IJzendoorn (2006) analysis was restricted to studies of children in child care, whereas the Geoffroy et al. (2006) analysis included a broader range of social settings. Thus a conservative conclusion is that the relation between care quality and the cortisol increase over the child care day has not been fully explored.

Indeed, only a few studies that have directly examined increases in cortisol in relation to measures of child care quality. Legendre (2003) examined structural characteristics of eight child care centers and found that both the presence of large (> 15) numbers of children and more than four adults, in addition to a wide age spread, were associated with greater cortisol increases. Sims, Guilfoyle, and Parry (2006) examined child care settings and grouped them as high quality, satisfactory and unsatisfactory. Among 3- to 5-year-old children, significant *decreases* in cortisol over the day were noted for high quality centers, whereas in unsatisfactory centers, significant *increases* were noted. To our knowledge, there has been only one study examining whether care quality is associated with cortisol activity in family daycare settings. Dettling, Parker, Lane, Sebanc and Gunnar (2000) examined a small

number of family daycare settings targeting one child in each setting. They noted that poorer quality of care using items from the Observational Rating of Care Environments (NICHD ECCRN, 2000) was associated with larger increases in cortisol, but found no evidence that group size in these daycare homes was a significant factor. Thus the evidence that quality of child care affects the rise in cortisol at child care is not conclusive, especially with regard to family daycare. Furthermore, the association between the facets of quality, including structural characteristics (e.g., group size, age-range, ratio of caregivers to child) and child-provider interactions (i.e., process measures of quality) needs further exploration. The first two goals of the study were to 1) determine whether cortisol increases over the day in family daycare settings and 2) determining whether increases over the child care day in these settings were associated with structural or process measures of child care quality.

The third goal was to examine relations between child behavior and the rise in cortisol over the child care day. As noted, the two meta-analyses also differed in their conclusions about child behavior. The Geoffroy et al. (2006) analysis concluded that children with difficult temperaments exhibit higher cortisol levels at child care, whereas the Vermeer and van IJzendoorn (2006) analysis concluded that the association between children's emotional dispositions and the rise in cortisol has yet to be shown conclusively. Again the difference in conclusions lies partly in the fact that the two meta-analyses included different studies, with the Geoffroy et al. (2006) analysis including studies of children in settings other than child care. Because the question pertains to child behaviors associated with the rise in cortisol over the child care day, focusing specifically on full-day child care settings is essential to answering the question.

Based on the human and animal literature, there are two aspects of child behavior that might impact HPA axis stress responses in child care settings: anxious, vigilant behavior and angry, aggressive behavior. Regarding anxious vigilance, neuroscience evidence on the neural pathways activating stress hormone reactions documents the importance of input from the amygdala and distributed systems involved in fear and anxious vigilance (see review, Rosen & Schulkin, 1998). Thus, it has long been suspected that children who are more anxious and vigilant would be more vulnerable to producing activation of the HPA axis (e.g., Kagan, Reznick, & Snidman, 1987). The neuroscience evidence regarding angry and aggressive behavior is mixed. Whereas there is some evidence that children extreme in these behaviors (e.g. Oppositional Defiant Disorder) have lower than normal basal and response levels of cortisol (see for review, van Goozen, Fairchild, Snoek, & Harold, 2007), there is also evidence that among preschool-aged children aggressive and under controlled behavior in peer group settings is associated with higher cortisol levels (see meta-analysis, see Alink et al., 2008). This latter finding may partly reflect cortisol increases in response to peer rejection, which occurs more among children who are aggressive and undercontrolled (Gunnar, Sebanc, Tout, Donzella & van Dulmen, 2003). Thus, the third goal of the study was to examine how patterns of anxious, vigilance and angry, aggressive behavior at child care might relate to cortisol stress reactions over the day in family daycare settings.

In examining these aspects of child behavior, we were aware that boys and girls might exhibit these anxious, vigilant and angry, aggressive behaviors with different frequencies. In particular, boys have often been noted to be more physically aggressive with peers (Crick et al., 2006; Fabes, Martin, & Hanish, 2003), although girls may exhibit relational aggression somewhat more than boys (Card, Stucky, Sawalani, & Little, 2008; Crick et al., 2006). To the extent that these behaviors reflect challenges the children were having in these settings, then their different prevalence might also be related to gender differences in patterns of association with cortisol increases over the child care day. Indeed, studies of cortisol-behavior associations for children in group settings do report sex differences in patterns of findings, although the direction of effects is not consistent. Several of the studies report

associations for one sex but not the other, and sometimes for different measures for each sex within the same study (Dettling, Gunnar, & Donzella, 1999; Tout et al., 1998). While these sex differences might be spurious, they may also reflect differences in how emotional dispositions influence children's experiences in peer group settings. For example, we have noted that preschool girls who are popular with other girls tend to score higher on measures of negative emotionality and lower on measures of assertiveness, whereas boys who are popular with other boys tend to be more exuberant and higher in assertiveness (Sebanc, Pierce, Cheatham, & Gunnar, 2003). These findings may also reflect differences in how care providers respond to boys versus girls with different temperaments and behavioral styles (Phillips et al., under review). Thus, the fourth goal of the study was to examine whether gender moderated the relations between child behavior and the child care cortisol stress response.

Finally, we were also interested in how these patterns of child behavior might operate in relation to child care quality measures in statistical models predicting the rise in cortisol over the child care day. Both mediating and moderating relations were explored. Regarding mediating relations, the theoretical model for these relations assumed that in order for care quality to impact activity of the HPA system it must have an emotional-behavioral impact on the child. Furthermore, the aspects of behavior impacted must reflect or connect with neural systems capable of activating the HPA stress response. This model is fairly straight-forward for anxious vigilance: children displaying more anxious, vigilant behavior were expected to be experiencing more activity in cortico-limbic pathways that support fear and vigilance and which, via limbic-hypothalamic connections, converge on the HPA axis to produce stress elevations in cortisol (e.g. Rosen & Schulkin, 1998). Children in poorer quality care settings would be expected to experience more frequent periods of fear and anxiety (see also Kryzer, Kovan, Phillips, Domagall, & Gunnar, 2007) and thus should produce greater rises in cortisol at child care. The model of angry, aggressive behavior, however, is less straight-forward. Here the assumption is that these behaviors, which involve negative social interactions, result in heightened experiences of social threat which also activate amygdalar outputs to the HPA axis and thus produce stress elevations in cortisol at child care (see Dickerson and Kemeny, 2004).

Models predicting moderation assume that children's behavior at child care at least partially reflects traits that may make them more or less vulnerable to stress as child care quality decreases. For example, Boyce and Ellis (2005) have argued that anxious, vigilant children are more sensitive to context, not only experiencing more stress and negative outcomes when conditions are poor but less stress and better outcomes than other children when rearing conditions are especially supportive. Similarly, Belsky, Hsieh and Crnic (1998) proposed that infant negative emotionality, which includes proneness to anger, increases children's susceptibility to externalizing problems as a function of differential rearing experiences. Extrapolating from these theoretical models, both anxious, vigilant and angry, aggressive dispositions may moderate associations between care quality and cortisol stress responses.

To summarize, the purpose of this study was to develop a better understanding of the factors associated with rise in cortisol over the child care day. There were five goals: 1) determine whether cortisol increases of the day in family daycare settings, 2) examine the extent to which structural and process measures of child care quality are associated with the magnitude of cortisol increase over the child care day, 3) explore whether anxious, vigilant or angry, aggressive behavior is related to the child care cortisol stress response, 4) examine whether gender moderates child behavior-cortisol rise associations, and 5) examine whether child behavior mediates or moderates associations between process measures of child care quality and the child care cortisol stress response.

In conducting this study, we focused on children in family daycare homes for several reasons. First, relative to center-based care settings, there are less data on whether and to what extent cortisol increases over the day in family-based daycare. Second, we were able to collect a large number of daycare homes for analysis, many more than had we tried to sample across different child care centers, thus broadening our ability to examine relations between care qualities and HPA axis activity. Third, family-based daycare settings range widely in the number of children in care, with some including only one or two children, whereas others operate more like small daycare centers with 10 or more children and several adults, giving us the ability to evaluate the effects of group size. Finally, although we only examined licensed daycare homes, the education levels, daycare experience of the providers, and observational measures of care quality were expected to vary considerably, again allowing a broad examination of quality-related factors that might help explain variation in cortisol increases among the children.

Method

Participants

The children (N = 151, 54.3% girls) were recruited from family-based daycare settings in a major metropolitan area. Selection criteria were: child age 3 to 4.5 years (M = 3.81, SD = .23), full-time care (≥ 20 hours per week; M = 40.61 hrs, SD = 8.26 hrs), and at least two months attending the present *daycare* home (M = 27.52 months, SD = 13.40 months, range 2 to 48 months). This last criterion was used to avoid assessing children who might still be adapting to the daycare arrangement. Of the children, 85.4% were White, 12% African American, 1% Asian and 1% Hispanic. Demographic data were collected on the families: 96% provided the requested information. Income before taxes was coded in $25,000 increments with mean income in the $51,000-$76,000 range. Parent education was coded as: (1) less than high school, (2) high school or GED, (3) some college, (4) associate's degree, (5) bachelor's degree, (6) master's degree, and (7) professional school or doctorate degree. Median level parental education was an associate's degree, with no parent reporting less than a high school or GED education. Only 4% of the children lived in single parent households. As such, we consider the sample to be low-risk.

Recruitment procedures

Children were recruited through licensed family-based child care providers who had responded to solicitations for participation mailed directly to them or placed in the licensing agencies' newsletter. Providers were contacted to determine if they had a child in their care that met the selection criteria. Eligible providers who agreed to participate approached the parent(s) of the child to request permission to release their contact information. Parents were then contacted to request consent for their child to participate; of the parents contacted, 59% agreed to participate. Eighty-one participants were from child care sites that contributed only a single child, whereas 70 were from 30 sites that contributed two or three participants each. Recruitment took place over multiple years, implying that children from the same site were not necessarily present at the same time in the child care setting. Of the 30 sites with multiple participants, 36 of the 70 participants were present together at the same time. Sites contributing more than one child to the analyses tended to be the larger child care sites, t = 1.71, df = 149, $p < .10$.

Daycare settings

Daycare providers completed a brief questionnaire about their setting and experience. All 120 daycare sites were licensed. The number of children present ranged from 2 to 12 (M = 5.25, SD = 2.25) and the number of adults at each site ranged from 1 to 3 (*Median* = 1, M = 1.14, SD = .44). The ratio of children to each adult who was physically present and within

sight of the children averaged at the time of our observations was 1:5.38 (*SD* = 2.25, range 1:1 to 1:25). Ninety-six percent of the providers classified themselves as Non-Hispanic White. Providers averaged 13.84 (*SD* = 7.69) years of work as a daycare provider, and 30.7% were providing care to a child of their own who was under the age of 5 years. We note that being the child of the care provider was an *exclusion* criterion. Using the same education scale described above, the median education of the providers was some college, including 2-year degree from a technical or community college. We also examined formal child development or early childhood education course work in high school or college; 11.30% reported no such formal coursework, 52% reported some formal training but not college level, whereas 37% reported college level training.

Procedures and measures

Care provider and child behavior measures—The children were observed on two mornings between 8:30 and 9:30 AM on days the provider deemed *typical* (i.e., no field trip, birthday parties, etc.). The observation inventory developed for this research project was a modification of the Observational Rating Scales of Care giving Environment (ORCE; see NICHD ECCRN, 2000; for a complete description of the modified or M-ORCE, see Kryzer et al., 2007). Like the ORCE, the M-ORCE involves two 44-minute observations (one per day), divided into four 10-minute observation periods. In each 10-min period, observers alternate between 30 second observe and record frames for coding frequency counts of behavior. At the end of each 10-minute period the observer makes brief notes and tentative qualitative ratings for 2 minutes. This process was repeated for three 10-minute periods. In the final 10-minute period the observer makes observations exclusively for the qualitative ratings. All qualitative ratings were 4-point scales. Coding of the M-ORCE was performed by six coders, one of whom was the reliability coder who trained the others. The reliability coder scored 41% of the children in the study, while the other coders contributed between 3% and 19% of the data each. Coders were trained through discussion of the codes and practice on videotapes. They then accompanied the reliability coder to practice coding sites and scored with her until they achieved Cohen's kappas of at least .80 on all scales. During data collection, coder agreement was re-assessed approximately every fifth session for each coder by having the reliability coder jointly code one of the target subjects with each coder. Approximately every 6 weeks the coders reviewed a master coding videotape and discussed discrepancies to reduce problems of coding drift.

For the present report, nine qualitative variables of care quality were examined as dimensions of the daycare environment expected to influence the stressfulness of the context. These variables were: Sensitivity, Intrusiveness, Detachment-Disengagement, Positive Regard for the Child, Negative Regard for the Child, Positive Community Building, Negative Community Building, Environmental Rating of Chaos, and Environmental Rating of Overcontrol. Sensitivity, Positive and Negative Regard, Detachment and Intrusiveness were rated based on the provider's behavior towards the target child. Overcontrol was a rating of the care provider's structuring of the care environment, whereas Chaos reflected the general degree of unpredictability of the setting. These measures were identical on the ORCE and M-ORCE. Positive Community Building was a measure of the care provider's use of strategies to help the children understand and relate positively to one another (e.g., “Johnny is feeling sad today because his mother is in the hospital, so that is why he wants to play alone right now.”). Negative Community Building was a measure of the care provider's use of strategies that created winners and losers and children pitted against one another as a discipline strategy (e.g., “Sam isn't doing what I asked, is he? We can't start the story until Sam is quiet.”). These measures were developed based on work by Lewis, Watson, and Schaps (2003). As would be expected, positive and negative community building were very

highly correlated ($r > .80$) and thus were combined into one scale by subtracting negative from positive community building scores.

To reduce the number of scale examined, the eight qualitative measures noted above were entered into a principal axis factor analysis. The results indicated two factors with eigenvalues > 1 that accounted together for 68% of the variance. The first factor, with an eigenvalue of 3.98, explained 49.7% of the variance and consisted of Sensitivity, Detachment (negative loading), Positive Regard, Chaos (negative loading), and the summary Community Building variable. The resulting measure was labeled Warm-Supportive Care. The second factor with an eigenvalue of 1.5 accounted for 19% of the variance and consisted of Intrusiveness, Negative Regard, and Overcontrol. We labeled the resulting measure Intrusive-Overcontrolling Care. After Varimax rotation, there were no variables that cross-loaded > .40.

Six measures of child functioning were examined. Three were qualitative measures: Vigilant-Anxious Mood, Angry-Irritable Mood and Belonging. Three were based on the frequency counts: Solitary Play, Positive Integration, and Negative Peer Interactions. All except Solitary Play were measures developed for the M-ORCE. Vigilant Mood (1 = no evidence of Vigilant-Anxious behavior, 4 = high level of Vigilant-Anxious behavior) described the extent to which the child engaged in such behaviors as flinching when others came near, moving quickly away from others, and continuously dividing attention between what they were doing and tracking events in the environment. Angry-Irritable Mood (1 = no evidence of Angry-Irritable behavior, 4 = high level of Angry-Irritable behavior) described the extent to which the child expressed anger and irritability in face, voice or gesture. While such behavior might involve interactions with others, it could also reflect behavior directed towards objects (e.g. throwing down a toy). Belonging was a qualitative rating reflecting the extent to which the child acted relaxed, confident, and assured (4 = acts highly relaxed, confident, and assured) as opposed to anxious, tentative, and cautious (1 = anxious, tentative, and cautious); that is, the extent to which the child seems to “fit in” in the setting as opposed to appearing like an outsider who is not integrated into the setting. Children did not have to be involved in play with other children in order to score high on this measure, as long as their behavior was relaxed and they appeared confidently engaged in what they were doing. Solitary play was a frequency count of the number of 10 sec periods when the child was playing alone computed as the percentage of intervals when the child was not engaged in physical care activities (i.e., was available for social interaction). Positive Integration assessed not only the frequency with which the child was positively engaged with others in the setting, but also the extent to which the child's role in the interaction was central to the group. A score of 4 indicated positive engagement where the child was a key player in the interactions, directing the interactions or being looked to by others while a lower score indicated positive engagement but in a role where the child was a subordinate or more peripheral actor in the activities. Thus this measure reflected not only the amount but the centrality of the child in positive social interactions. Negative Peer Interactions was a frequency count of the number of intervals in which the child was either the recipient or agent of aggressive behaviors towards other children. Aggression was scored as verbal, physical and relational aggressive acts. Verbal acts included verbal threats of physical harm, physical acts included pushing and shoving as well as hitting and forcibly attempting to take things away from other children, while relational aggression involved threats or actions directed at harming relationships with peers (e.g., “If you don't give me that, I won't be your friend”; see Crick, Casas, & Mosher, 1997).

These child measures were also submitted to a principal component analysis to permit data reduction. The initial results with Varimax rotation indicated that Solitary Play loaded greater than .40 on multiple factors and thus was removed. The resulting analysis yielded

two factors with eigenvalues > 1 which accounted for 73% of the variance. The first factor had an eigenvalue of 2.52 and accounted for 50% of the variance. It consisted of Belonging, Positive Integration and Vigilant-Anxious Mood (negative loading). The resulting measure was reverse scored to yield a measure of Anxious, Vigilant Behavior. The second factor with an eigenvalue of 1.13 accounted for 23% of the variance and consisted of Angry-Irritable Mood and Negative Peer Interactions. The resulting measure was labeled Angry-Aggressive. After Varimax rotation, none of the variables cross-loaded > .40.

To examine the stability or trait-like aspect of these measures over time, we examined data on a subset of the children (N = 108) who were seen in these same child care settings six months later. The test-retest correlations computed on these participants were: Warm, Supportive Care, r = .49, p < .001; Intrusive, Over controlling Care, r = .37, p < .001; Anxious, Vigilant, r = .29, p < .01; and Angry-Aggressive Behavior, r = .30, p < .01.

Salivary cortisol—Saliva was obtained for cortisol determination by having the children dip a 1.5″ cotton dental roll into approximately .025 g of cherry flavored Kool-Aid ™ mix and mouth the cotton to obtain the sweet taste. This small amount of Kool-Aid ™ has not been found to significantly affect the cortisol assay (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Once the cotton roll was saturated, it was placed in a needleless syringe and the saliva was expressed into a 1.50 ml Eppendorf Safe-Lock microtube and sealed. At daycare, samples were collected by the daycare provider on two days between 10:00 and 11:00 a.m. and 3:00 and 4:00 p.m. These times were chosen based on previous work showing that when 4 samples of cortisol are obtained (mid-morning, noon, immediately post nap, late afternoon), the rise in cortisol over the child care day can be reliably assessed at these two time points (Watamura, Sebanc, & Gunnar, 2002). Providers were asked to avoid sampling immediately before a meal, to not give the child caffeinated drinks or dairy products within an hour of sampling, and to wait until 30 minutes after the child got up from a nap to sample to avoid the decrease in cortisol typically seen over nap time (Watamura et al., 2002). To determine the actual time of sampling, the cotton dental rolls were supplied in a bottle with a MEMS V Track Cap ™ (Aardex, Zug, Switzerland) which automatically recorded the time when the container was opened. Use of such devices allows verification of compliance with sampling protocols and also increases compliance (Kudielka, Broderick, & Kirschbaum, 2003). The care provider also completed a brief diary on each day of sampling recording timing of sample, nap times, and meal times.

The data from the MEMs cap and diary were examined to determine if the care provider had complied with the instructions. Twenty children were removed prior to assaying saliva for cortisol because the care provider deviated by more than 1.5 hours in the timing of nearly all of their samples resulting in the child having no usable cortisol data. The 151 with usable samples are described in this report. Parents were also asked to collect samples using the same protocol on two days when the child did not attend daycare. These measures provided a home baseline against which to judge the impact of daycare on children's cortisol activity. Parents followed the same protocol as the daycare providers, including use of the MEMS V Track Cap™. There were 108 children with a sufficient number of home samples for analysis. Comparisons of child care cortisol for those with and without home cortisol measures yielded no significant (all p's > .10) differences for morning or afternoon levels or for change over the day.

Once collected, daycare and home samples were stored in the refrigerator and then mailed to the laboratory. These procedures have been shown not to affect cortisol data (Clements & Parker, 1998). Once in the laboratory, samples were frozen at -20° centigrade until assay. Assays were conducted in duplicate using a time-resolved fluorescence immunoassay

(DELFIA). Intra- and inter- assay coefficients of variation were at or less than 6.7% and 9.0%, respectively, and duplicates correlated highly, $r = .997$, $p < .001$.

Both home and daycare cortisol measures were averaged within time and context over the two days of collection. Within each context, children needed at least one data point at each time of day to obtain a final score. Four measures were computed: Home AM, Home PM, Daycare AM and Daycare PM. The distributions of these variables were examined and were found to be positively skewed and thus were $\log_{10}$ transformed for analysis. The raw AM values were subtracted from the raw PM values to produce Home Rise and Daycare Rise. These values had roughly normal distributions and thus were not $\log_{10}$ transformed. To examine stability in the rise in cortisol across the days, we also calculated the rise on each of the two days of assessment. Finally, to determine whether the levels at child care represented a biologically significant secretory response of the HPA axis for each child, we used the criteria of an increase of at least 0.10 μg per dl and 10% derived from work on previously used in adult studies (Kirschbaum, Pirke & Hallhammer, 1993; Kirschbaum et al., 1995; Van Cauter & Refetoff, 1985).

Analysis plan and preliminary analyses

In some instances, more than one child was obtained from the same daycare setting, so we accounted for this nesting in all analyses. This was accomplished in SAS using procedures for analyzing nested data (SAS Institute, 2004). In our regression analyses (SAS Proc Surveyreg), the daycare provider was declared to be the “primary sampling unit” or PSU, while in the ANOVA analyses (SAS Proc Genmod or Proc Mixed) the daycare provider was declared to be the subject. In addition to accounting for the variance associated with children nested within daycare provider, these procedures also adjust the degrees of freedom such that they are based upon the number of daycare providers ($N = 112$) rather than the number of children.

Because some studies of child behavior relations with cortisol activity at child care have yielded evidence of associations for one sex but not the other, in all of the analyses described below we included child sex and examined possible main and interaction effects. The first analyses were conducted to determine whether cortisol rose more over the day at child care than at home. This analysis was computed using a 2 (child sex) by 2 (place: home vs. child care) by 2 (time: morning vs. afternoon) doubly repeated measures ANOVA. We also examined the day-to-day stability of the rise at child care and relied on descriptive statistics to help interpret the percentage of children exhibiting biologically significant increases over the day.

We then attempted to identify background and child care structural quality factors that contributed to the rise at daycare. This analysis included child age and sex, family demographics (e.g., family income), daycare descriptors (e.g., ratio of providers to children), and provider variables (e.g., education, years of experience). Any significant associations were then included as covariates in subsequent analyses of the association of provider behavior measures of quality with the cortisol rise at child care. Next we examined the two care daycare observational quality measures and the two child behavior measures. Initial analyses examined possible age and sex effects for each variable. Subsequent analyses examined first the association of observed quality measures and then child behaviors with the rise in cortisol over the daycare day.

Mediation analyses require that care quality and child behavior measures were associated with the outcome (i.e., Daycare Rise) and, further, that the care quality and child behavior measures were significantly associated. To prepare for mediation analyses, we next examined whether the care quality measures were associated with the child behaviors.

Finally, for the measures meeting the requirements for meditational analysis, we constructed a hierarchical regression, entering first the care quality measure(s) associated with the cortisol rise and, in the next step, the child behavior measure(s) associated with the cortisol rise. The question addressed was whether the association with care quality decreased significantly once the child behavior measures were in the equation.

Moderation analyses were computed for child behaviors exhibiting significant associations with the Daycare Rise. For such measures, hierarchical regressions were computed entering each care quality measure and then, in the interaction step, the interaction between each care quality measure and the child behavior measure. Of interest was whether the interaction term produced a significant increase in the variance explained.

Results

Cortisol change at daycare and home

The 2×2×2 ANOVA analysis of the home and daycare cortisol measures revealed that neither the effect of child sex nor interactions with child sex was significant. However, the time-of-day by place interaction effect was significant, $F(1,107) = 5.12$, $p < .05$. Descriptive data are shown in Table 1. Tests for simple effects of time-of-day within place were computed to explicate the interaction. At home, we found no significant change from morning to afternoon, $F(1,107) = 1.12$, *ns*, while at child care, cortisol levels increased significantly from morning to afternoon, $F(1,107) = 29.42$, $p < .001$. In addition, cortisol levels were higher when the children were at family-based child care than home both in the morning, $F(1,107) = 9.65$, $p < .01$, and afternoon, $F(1,107) = 33.92$, $p < .001$, although the effect size was larger in the afternoon ($\eta^2_{AM} = .08$ vs. $\eta^2_{PM} = .24$ and $\omega^2_{AM} = .07$ vs. $\omega^2_{PM} = .23$).

To examine the day-to-day stability of the rise in cortisol at daycare, we examined each day separately. At child care, the rise over the day was stable from day 1 to day 2, $\beta = .43$, $p < .001$; but this was not the case for the rise at home, $\beta = .04$, *ns*. We then examined the individual values to provide a more descriptive interpretation of these effects. At daycare, 63% of the children showed positive (> 0) change from morning to afternoon cortisol levels; 52% showed this degree of rise on each of the two days of testing. For those with home data, we examined whether, relative to home baseline, the child's cortisol levels at child care met criteria for a stress response (for criteria see Kirschbaum et al., 1993). In the morning, 10% of the children were exhibiting an HPA stress response to daycare, while in the afternoon this increased to 40%. Examined across the two days of assessment, 20% exhibited an HPA stress level response on both of the days, while 56% exhibited such a rise on at least one of the two days.

Rise at daycare related to child demographics and structural aspects of child care quality

Our next step was to relate individual differences in the rise in cortisol over the daycare day to child and caregiver factors. A hierarchical regression predicting Daycare Rise was computed. In step one, we included child (i.e., age, sex) and family (i.e., parental education, family income) variables, but none of these measures significantly predicted the rise (*t*'s < 1.7 or > -1.7, *ns*). At step two we included structural characteristics of the daycare setting (i.e., number of months at that daycare home, hours per day, group size, number of care providers, ratio of adults to children, and whether a sibling was at that daycare home, with the last entered as a dummy variable). None of these descriptive daycare measures significantly predicted the rise (*t*'s < 1.0 or > -1.0, *ns*). At step three we examined daycare provider information (i.e., years of experience, highest level of education, formal training in child development or early childhood education, and whether the provider had their own

child under age 5 in the daycare, the last coded as a dummy variable). None of the provider variables significantly predicted the rise (*t*'s < 1.3 or > -1.3, *ns*). Thus, there was no evidence that any of these child, family, or daycare characteristics affected the magnitude of the rise in cortisol at daycare.

We were particularly struck by the lack of effect of the number of children in care as in some of these daycare homes there were only one or two children in addition to the target child, while in others there were 10 or more children. To be sure that there was not a threshold effect of daycare size, we identified 4 children who were in care with only one other child (small group) and compared their rise over the day with children in care with 2-5 (medium group) and 6+ other children (large group). Examination of the medians provided no evidence of a differential rise for these 3 group sizes (i.e., 0.07, 0.02, 0.04 μg per dl, *Mn SD* = *.03* μg per dl, respectively).

Sex and age differences in child behavior measures and observed care quality measures

Prior to examining associations between process measures of quality, child behavior and cortisol increases, we examined whether these measures differed by child sex or age. Descriptive data on scales that contributed to observed quality measures and child behaviors measures are shown in Table 2. To examine possible gender differences, ANOVAs were computed on the two process measures of quality and the two child behavior measures. The results indicated that girls received more warm, supportive care than did boys (girls: M = .70, n = 82; boys: M = -.83, n = 69; z = -2.25, p < .05), while girls exhibited less anxious, vigilant behavior (girls: M = -.47, n = 82; boys: M = .56, n = 69; z = 2.65, p < .01) and less angry, aggressive behavior (girls: M = -.49, n = 82; boys: M = .47, n = 69; z = 3.39, p < .001) than did boys. There were no significant child sex differences in negative, intrusive care (z = 1.48, *ns*). We also examined associations with age for the child behavior measures but none were found. The pattern of gender differences supported our concern with examining whether child sex moderated any of the associations in observed measures with the cortisol rise at daycare.

Daycare cortisol rise associated with process measures of care quality

We first examined whether process measures of child care quality predicted the rise in cortisol using a hierarchical regression; we first entered child sex, then the two measures of quality, and finally the interactions between each observed measure of quality and child sex. As expected, given the null results for gender differences in the ANOVA analysis of the cortisol child care rise, in the first step child sex was not significant (t = .25, *ns*, R^2 = .00). In the second step (ΔR^2 = .04), the regression coefficient for Intrusive, Over controlling Care was significant (β = .17, t = 2.17, p < .05), while Warm, Supportive Care was not (t = -.39, *ns*). In the final step, no interaction terms were significant (t's < 1.0, ΔR^2 = .00). Thus, cortisol rose more over the day in family daycare homes where children received more intrusive, over controlling care.

Associations between child behavior and cortisol rise

Using hierarchical regression, we entered child sex at step 1, each of the two child behavior measures on step 2, and the interaction between sex and each of these measures on step 3 to predict the cortisol rise. The results, shown in Table 3, indicated child sex moderated the associations between both child behavior measures and the cortisol rise. To visualize these interactions, Daycare Rise was plotted by sex for the bottom and top quartiles of the distributions for each child behavior. As shown in Figure 1a, for girls but not boys, Anxious, Vigilant Behavior was associated with larger rises in cortisol over the daycare day. As shown in Figure 1b, for boys, Angry, Aggressive Behavior was associated with the rise in cortisol.

Associations between provider quality measures and child behavior

To prepare for examinations of mediation and moderation, we next examined associations between observed care quality and child behavior. We computed two hierarchical regression models, one predicting Anxious, Vigilant Behavior and the other predicting Angry, Aggressive Behavior. In each we entered child sex on step 1, the observed care quality measures on step 2, and the interaction of child sex and the two observed quality measures on step 3. The results are shown in Table 4. As expected from the ANOVA analysis, step 1 (child sex) was significant in each equation. For Anxious, Vigilant Behavior (top of table 4), no significant interactions with sex were observed. However, the second step was highly significant and showed a significant negative association between Warm, Supportive Care and Anxious, Vigilant Behavior; no significant association with Intrusive, Over controlling Care was obtained. For Angry, Aggressive Behavior, the interaction step was significant. With the interactions in the equation, Warm, Supportive Care was associated with less Angry, Aggressive Behavior for both sexes, while Intrusive, Over controlling Care showed an interaction with child sex. Plotting the top and bottom quartile on Angry, Aggressive Behavior against Intrusive, Over controlling Care for each sex (see Figure 2) revealed that boys but not girls exhibited more Angry, Aggressive Behavior in association with higher Intrusive, Over controlling Care.

Mediational analysis

According to Baron and Kenny (1986), we have documented three of the four steps that are required to demonstrate mediation for Intrusive, Over controlling Care and Angry, Aggressive Behavior for boys, but not girls. Specifically, we have shown that (1) Intrusive, Over controlling Care was related to Daycare Rise; (2) Intrusive, Over controlling Care was related to the mediator, Angry, Aggressive Behavior for boys; and, (3) Angry, Aggressive Behavior for boys was related to the Daycare Rise. The interaction between Angry, Aggressive Behavior and sex was related to the outcome (i.e., the rise in cortisol). To examine mediation we examined only the data for boys and computed another hierarchical regression, entering child sex on step 1, Intrusive, Over controlling Care on step 2, and then Angry, Aggressive Behavior and its interaction with sex on step 3. Of interest was the decrease in the regression coefficient for Intrusive, Over controlling Care when the child behavior scores were in the equation. The results failed to indicate any decrease in the regression coefficient for Intrusive, Over controlling Care when Angry, Aggressive Behavior was in the equation; thus there was no basis for examining whether the decrease was significant (i.e., via a Sobel Test).

Moderation analyses

Because of the gender differences in the pattern of child behavior associations with the cortisol rise, we computed these moderation analyses within sex. For girls, because Anxious, Vigilant but not Angry, Aggressive Behavior was associated with the cortisol rise, we examined whether this behavior moderated associations between observed quality measures and the rise in cortisol. A hierarchical regression was computed entering Anxious, Vigilant Behavior on step 1, the two observational measures of care quality on step 2, and the interaction between Anxious, Vigilant Behavior and each of the care quality measures on step 3. In step 3 ($\Delta R^2 = .02$), neither the Vigilance by Warm, Supportive Care ($t = -.26$, *ns*) nor Vigilance by Intrusive, Over controlling Care ($t = 1.05$, *ns*) interactions were significant. Thus there was no evidence that Anxious, Vigilant Behavior moderated the effects of the measures of care quality for girls.

For boys, because Angry, Aggressive Behavior but not Anxious, Vigilant Behavior was associated with the cortisol rise, we examined whether Angry, Aggressive Behavior moderated the associations between observed care quality and cortisol. Again, a hierarchical

regression equation was computed, entering Angry, Aggressive Behavior on step 1, the two measures of care quality on step 2, and the interaction of Angry, Aggressive Behavior and each care quality measure on step 3. In step 3, the interaction between Intrusive, Over controlling Care and Angry, Aggressive Behavior was not significant (t = -.95, *ns*, ΔR^2 = .00); however, the interaction between Warm, Supportive Care and Angry, Aggressive Behavior was significant (t = 2.06, $p < .05$, ΔR^2 = .02). The interaction is depicted in Figure 3. Boys who were low in aggressive behavior appear to have been sensitive to variations in warm, supportive care, exhibiting larger cortisol increases when they received low levels of supportive care and smaller cortisol increases when they received more supportive care. In contrast, highly aggressive boys appear to exhibit comparable increases in cortisol regardless of the warmth and supportiveness of the provider.

Discussion

The results of the present study confirmed that even in family-based daycare settings, cortisol levels rise over the day. We also found that certain dimensions of care quality and child behavior were associated with the cortisol rise. Finally, although boys and girls did not differ in their cortisol responses to child care, the associations between child behavior, care quality, and the cortisol rise were moderated by child sex. Thus child sex appears to be a critical factor in understanding the psychosocial processes underlying cortisol stress reactions at child care. Each of these aspects of the results will be discussed below.

The majority of the children showed an increase in cortisol from mid-morning to mid-afternoon at daycare, while at-home levels remained relatively flat over the mid-section of the day. There was no evidence of a sex difference; boys and girls exhibited similar increases in cortisol over the child care day. Furthermore, the lack of a change from morning to afternoon in the home data was the typical diurnal basal pattern for children of this age (Watamura et al., 2004). Specifically, the basal decline from mid-morning to mid-afternoon has been shown to emerge at home as circadian sleep patterns become more adult-like (i.e., as the child gives up the afternoon nap). In addition, the rise at daycare was relatively stable across the two days of assessment with over half of the children producing a rise from morning levels on each day of assessment. Because even small changes in cortisol could be statistically but not necessarily *biologically* significant, we used criteria previously used in adult studies to identify HPA stress responders. This analysis indicated that only 10% of the children exhibited a stress response in the morning at daycare, while 40% could be classified as stress responders in the afternoon.

Taken together these data suggest that a substantial number of children exhibit a stress response of the HPA system over the day at daycare; further, as evidenced by the day-to-day stability data, this occurs on a regular basis for a number of children. It is telling that this response is seen more clearly in the afternoon than the morning, indicating a rising pattern of cortisol over the day. This suggests that the response is not a reaction to arriving at child care in the morning, but a response that emerges over the accumulation of the day's experiences. This may explain why in half-day, preschool programs, cortisol levels are not elevated over home levels for classes that meet in the morning or for those meeting in the afternoon (Gunnar, Tout, de Haan, Pierce, & Stansbury, 1997), while these elevations are observed by afternoon in full-day programs. The question, of course, is *why*. What is it about spending a full day at child care that produces a stress response of the HPA system by the afternoon in so many young children?

We found no significant associations between any of the structural measures of care quality and the rise in cortisol at daycare. Thus, there was no evidence that provider experience or training predicted this rise, nor was there evidence that the rise was related to the number of

children in care or the number of adults in the setting. Our failure to find an association with group size was particularly noteworthy. Not only was no linear association found, but when we examined settings with only one or two children in addition to our target child, increases in cortisol over the day averaged the same as increases in settings with 10 or more children.

Previously we have suggested that the complexity of the demands of managing interactions with large numbers of children might account for cortisol increases over the day among children in center-based care settings (Gunnar & Donzella, 2002; see also Belsky et al., 2007, regarding the possible importance of the peer group in daycare effects). This argument was predicated on the following findings. In comparing the cortisol response over the child care day for toddlers and preschoolers in center-based care, we did not observe elevations for children cared for in infant rooms (Watamura, Donzella, Alwin, & Gunnar, 2003). In infant rooms, activities are typically organized around the infant's schedule, which means that at any given time, only a few infants are awake and when awake, they are likely to be interacting with adults. In toddler rooms, however, the children eat, sleep and play at the same times and thus there is much more peer interaction in these rooms. Furthermore, with development over the toddler period, time spent interacting with peers increases (Hughes & Dunn, 2007). Second, when we examined changes in cortisol over nap time for preschoolers in center-based care, we noted significant decreases from before to immediately after the nap, even for children who did not appear to sleep over the nap period (Watamura et al., 2002). Because the nap period at child care is one during which children usually do *not* interact with other children, the removal of social interaction demands during this period seemed a reasonable hypothesis for why even those children who did not sleep showed decreases in cortisol over the nap period.

The fact that we observed the same cortisol rise in settings with only two or three children that we found in settings with 10 or more children strongly argues *against* the social complexity hypothesis, at least with regard to daycare settings. It still might be that center-based child care rooms with many children and adults evoke more of a cortisol response than those with fewer children (see Legendre, 2003), given that none of our settings were that large. However, the fact that the rise in cortisol by afternoon was observed in very small settings suggests that social complexity is not the primary factor accounting for the cortisol stress response. This conclusion also is consistent with the fact that the present findings are largely consistent with previous findings for children of this age studied in center-based care. The average number of children per each preschool-aged care room in daycare centers ranges between 13.0 (at 36 months; NICHD ECCRN, 1999) and 15.4 (at 54 months; NICHD SECCYD, 2008), while the mean number of children in the family daycare homes we studied was around five. One would have expected, therefore, that if the demands of negotiating complex social environments was the key factor, then many fewer children would have shown a cortisol stress response over the day in family-based daycare than has been observed in studies of center-based care. But that was not the case.

We did obtain evidence that our measures of care provider behavior, sometimes termed *process* measure of care quality, were related to the rise in cortisol at daycare. Principal factor analysis yielded evidence of two factors: warm and supportive care and intrusive, over controlling care. Child care studies typically identify one dimension of quality that includes both warmth and control dimensions (e.g., NICHD ECCRN, 2000). As in many studies of parenting (e.g., Barber, Stolz & Olsen, 2005), these dimensions were distinct in our data. It may be that the use of family daycare settings that were more home-like produced patterns of provider behavior that were more like those seen in parenting research.

Our results also showed that these two dimensions were differentially related to the rise in cortisol at daycare. Specifically, although warm, supportive care was related to our measures

of child behavior, it was not associated with the cortisol rise either for boys or girls (although see discussion of moderation by angry, aggressive behavior for boys, below). In contrast, intrusive, over controlling care was associated with larger rises in cortisol over the daycare day. This was true for both boys and girls as gender did not interact with this effect. It is not clear why intrusive, over controlling care was stress-provoking. It may be that this dimension of care reflected a daycare environment that was structured to provide fewer developmentally-appropriate experiences. Anecdotally, settings scoring high in intrusive, over controlling care were often ones in which the children were transitioned frequently between activities, were permitted relatively little time in free play, and spent long periods in provider-directed structured activities. In the Sims et al. (2006) study, developmentally-appropriate practices related to transitions and scheduling were facets of quality associated with the rise in cortisol for children in child care centers. It may be that when young children are required to manage a day with many structured activities and transitions, this overtaxes their coping capacities as the day progresses. Notably, however, although intrusive, over controlling care accounted for statistically significant variance in the cortisol rise, there was still a good deal of individual variation that was not explained by this process dimension of quality.

Some of this unexplained variance was associated with child behavior patterns at daycare. As expected, two dimensions of behavior were identified. The first dimension involved variation in anxiety and vigilance and in social integration. Children scoring high on this dimension were more anxious and vigilant and were less socially integrated in the setting. The second dimension involved variation in angry mood and negative interactions with peers. Both anxious vigilance and angry, aggressive behavior were observed more in boys than girls. Given the association with the cortisol rise, this would suggest that boys might have experienced more stress at child care than girls. However, as noted above, we found no gender difference in cortisol at daycare. Rather, we found that gender moderated the associations of behavior and rise in cortisol over the day. For boys, larger cortisol increases were associated with more angry, aggressive behavior, while for girls it was associated with more anxious, vigilant behavior.

Other studies of child care have also noted gender differences in associations between child behavioral dispositions and HPA activity. For example, Dettling and colleagues (1999) found that parent-reported surgency positively predicted the cortisol rise for boys but not girls, while parent-reported effortful control negatively predicted the rise for girls. Why associations might differ for boys and girls is unclear. By adulthood, there are clear differences in reactivity of the HPA axis to stressors, as well as evidence that the type of stressors that most strongly affect men differ from those that are most provocative for women (for review, see Dedovic, Wadiwalla, Engert, & Pruessner, 2009). For both men and women, threats to the social self stimulate the HPA axis, but for women the most salient threats to the social self appear to be ones that threaten relationships, while for men they appear to be ones that threaten social status and agency (see also Stroud, Salovey, & Epel, 2002).

While these gender differences in stress provocation appear to emerge most clearly after childhood (see Stroud et al., in press), the present results are consistent with the adult findings. That is, larger cortisol increases were observed for the girls who, in addition to being anxious and vigilant, were also less socially integrated into the daycare setting. Our scoring of positive social integration was based on whether the child was central to the action, such that if the child left the activity or decided to change the activity then the other children would follow or play would stop. When combined with anxious, vigilant behavior, our summary score may have reflected anxiety over threats to goals of having and maintaining relationships with peers. If by this young age social relationships are beginning

to emerge as a more central goal for girls than boys, then this might explain its greater association with the cortisol rise for girls at daycare. Note that this argument is consistent with evidence that by preschool age, girls use somewhat more relational aggression or actions which threaten relationships than do boys (Crick et al., 1997).

This argument does not explain why we obtained a significant association between angry, aggressive behavior and the rise in cortisol for boys but not girls. As noted, our finding for boys was consistent with the meta-analysis of aggressive, under-controlled behavior which provided evidence that while such behavior is associated with low cortisol for school-aged children, among preschool-aged children it is associated with higher cortisol levels (Alink et al., 2008). What was noteworthy was the gender difference in the association of care quality with angry, aggressive behavior. For both sexes, this behavior was observed more when children were receiving less warm and supportive care, but only for boys was angry, aggressive behavior associated with higher levels of intrusive, over controlling care. In fact, as suggested in figure 2, it was at high levels of intrusive, over controlling care that the gender differences in angry, aggressive behavior were most pronounced. This suggests that not only the frequency but the social meaning and function of angry, aggressive behavior may have differed for boys and girls. For boys it may have been a reflection of salient threats to agency, which, even at this age, may be a more central goal for boys than girls. Indeed, this would be consistent with evidence that as early as one year of age, loss of control (agency) over a loud, noise-making toy elicits more negative affectivity in boys than girls (e.g., Gunnar, 1980), as well as evidence of gendered differences in adulthood in the role of fighting and asserting dominance or control versus seeking relationships in coping with threatening situations (Taylor et al., 2000).

The data for angry, aggressive behavior and stress for boys, however, was even more nuanced than gendered association with intrusive, over controlling care would suggest. Specifically, in the moderation analyses we found that among boys, angry and aggressive behavior moderated the association between warm, supportive care and the cortisol rise. Specifically, we found that it was the boys who scored low on this measure whose cortisol rise at child care was associated with the degree of warm and supportive care they received. Under conditions of low warmth and support they showed large increases in cortisol over the day, while under conditions of high warmth they show small increases that were well within the norm for cortisol changes over the day at home. Boys scoring high on angry, aggressive behavior did not show any modulation of the cortisol response to child care in relation to care provider warmth and support. This moderation effect was the opposite of that predicted based on arguments that children who are more vulnerable to behavior problems (e.g., externalizing-type problems in this case) might be more sensitive to variations in care quality. However, as boys were more aggressive than girls, it may be that boys scoring low on angry, aggressive behavior may have had more difficulty managing play with other boys and thus may have been more dependent on the degree of warm, supportive care they were receiving from the care provider.

We recognize that our explanations for the gender differences in cortisol-behavior associations are speculative and that these findings will contribute to continued uncertainty about how behavior in peer settings is associated with activity of the HPA axis in young children. However, they should also encourage researchers to consider the role of gender differences in future studies of child care stress. Our findings suggest that even when boys and girls do not differ in their physiological stress reactions to complex social situations, they may differ in how emotional-behavioral dispositions and patterns of action relate to their stress responses in those contexts. Furthermore, it seems likely given our pattern of results that these gender differences reflect the gendered aspects of children's social worlds and socialization experiences. As noted above, by adulthood there is good evidence for

gender differences in stress reactivity and regulation (Dedovic et al., 2009; Taylor et al., 2000); thus, an increased focus on gendered patterns of stress-behavior-context associations during development is needed.

One of the challenges of interpreting the present findings is that the associations we found accounted for only modest amounts of the variance in the cortisol rise. For example, our measure of intrusive, over controlling behavior only accounted for 4% of the variance in the cortisol rise, while in combination the child behaviors and their interaction with sex explained 10% of the variance. It is likely that our methods limited the amount of variance that we could explain. First, cortisol and behavior were measured on different days, and there was likely error in our measures such that behavior on one the days of observation was not exactly the behaviors we would have observed had we measured on the days of cortisol assessment. Unfortunately, the M-ORCE, like the ORCE, requires summating over the two days of observation, thus we do not have a measure of day-to-day variability in either our two process measures of quality or our two measures of child behavior. For the cortisol rise, although we observed a significant correlation across the two days of assessment, the rise on day one explained only 16% of the variance in the rise on day 2. Thus, limitations in the method likely limited the magnitude of the associations we could hope to find. Nonetheless, the effect sizes we observed were consistent with others often reported in child care studies, and it has been argued that such effect sizes should not be dismissed in considering the import of findings for child care policy and practice (McCartney & Rosenthal, 2000).

Our findings provide added impetus to the effort to understand whether and how HPA stress responses at child care impact children's development. Although early life stress is typically viewed as a risk factor for healthy development, recent studies in both non-human primates and in human populations raise questions about this type of blanket assessment of early life stressors. For example, Lyons and Parker (2007) have examined the impact of repeated, one-hour separations in infant squirrel monkeys. As in the work on cortisol responses to full-day child care, these separations in squirrel monkey infants produced marked and repeated activations of the HPA axis. However, followed into the late juvenile and early adult age, animals exposed to this form of early life stress were found to be less fearful, to produce lower rather than higher cortisol responses to stressors, and to show more optimal development of prefrontal regulatory brain circuits; consistent with these findings, they also performed better on tests of executive functioning. Thus, at least for this animal model, repeated separation stress early in life fostered a form of resilience.

In studies of human populations, most of the work on early maltreatment and risky family patterns has been conducted on adults with various affective disorders. Here the evidence is that early life stress produces hyper-reactivity of the HPA axis, consistent with views that early life stress enhances the neural substrate underlying vulnerability to affective disorders (e.g., Heim, Plotsky, & Nemeroff, 2004). However, in recent studies, researchers have sought to examine the associations between early life stressors and HPA reactivity among adults who are free of significant internalizing pathology. These studies are showing hypo-activity of the HPA axis compared to healthy adults without early life stressors (Carpenter et al., 2007; Elzinga et al., 2008). These findings are also consistent with recently reported evidence that teenagers who experienced more full-time, center-based daycare under the age of three produce lower than average levels of cortisol early in the morning at the peak of the diurnal cycle (Roisman et al., in press). We clearly need to continue to be cautious about interpreting the implications of cortisol elevations in daycare. Nonetheless, evidence that this rise is associated with anxious, vigilance (i.e., internalizing) behavior for girls and anger, aggression (i.e., externalizing) behavior for boys should not make us sanguine about its potential implications for children's development.

Several limitations in this study also should be noted. First, although we included family demographic factors in our analyses, we did not examine the quality of parent-child relations and their associations with the daycare cortisol rise. As in other studies of child care effects, it is possible that issues in the children's families carry more of the weight in predicting how the child responds to child care than issues within the child care setting (Ahnert & Lamb, 2003; Phillips et al., 2006; Love et al., 2003). Although we did not attempt to pre-select for parents with fairly high educations, incomes, and marital status, the variation in family characteristics was quite narrow, reflecting fairly advantaged and low-risk segments of the U.S. population. It may well be the case, as has been found in prior research (Loeb, Fuller, Kagan, & Carrol, 2004; NICHD ECCRN, 2001), that quality of child care matters more for low-income children and may thus play a stronger role as an influence on cortisol elevations for children from disadvantaged backgrounds. We were not able to randomly assign children to daycare homes, and thus selection factors could not be controlled in our analyses. We only included licensed daycare homes in our analyses, and thus cannot generalize to effects in the many unlicensed settings used for daycare. Finally, although we worked to increase our racial-ethnic variation, the sample was still predominantly white and of European heritage. This again limits generalizability.

With these limitations, however, we have clearly documented that, similar to children in center-based care, many children in family daycare settings show a substantial rise in cortisol over the day, and they do not show this rise when at home. We have found that this rise is observed even in care settings with only two or three children, thus arguing against the hypothesis that social complexity is a major factor underlying increasing activation of the HPA stress response. We also noted associations between intrusive, over controlling care and the rise in cortisol for both boys and girls and between warm, supportive care and the cortisol rise for less angry, aggressive boys. These findings may help refine our attempts to understand aspects of child care that promote increases in cortisol in young children. Finally, the gendered differences in associations we noted should focus attention on understanding how boys and girls may differentially process and experience stressors in child care settings.

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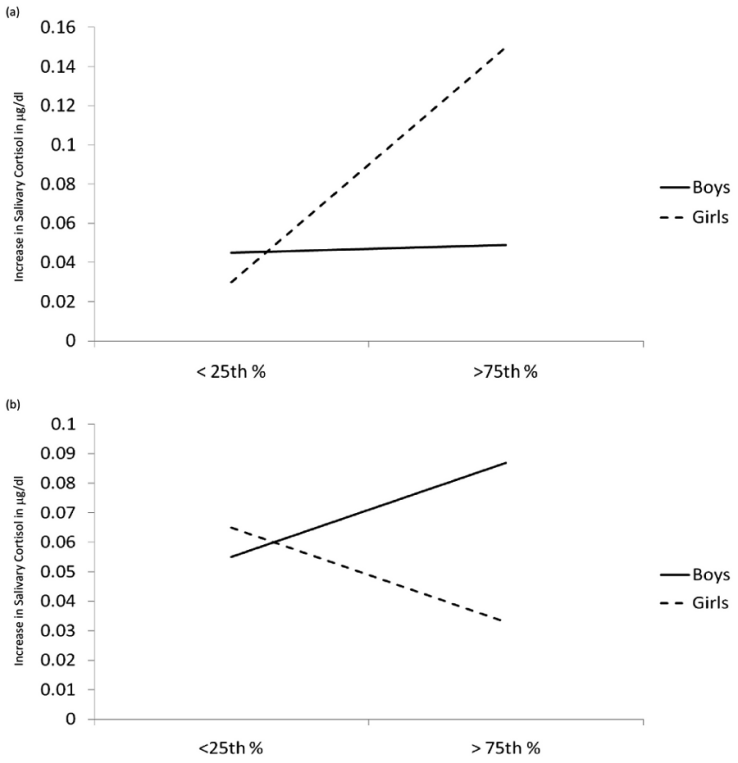

Figure 1.

Rise in cortisol over the daycare day as a function of child sex and child. (a) Cortisol rise for the 25th and 75 percentiles of anxious, vigilant behavior. (b) Cortisol rise for the 25th and 75 percentiles of angry, aggressive behavior.

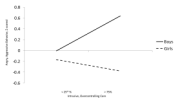

Figure 2.
Standardized measure measure of angry, aggressive behavior as a function of child sex and intrusive, overcontrolling care is plotted using the 25th and 75 percentiles of intrusive, overcontrolling care.

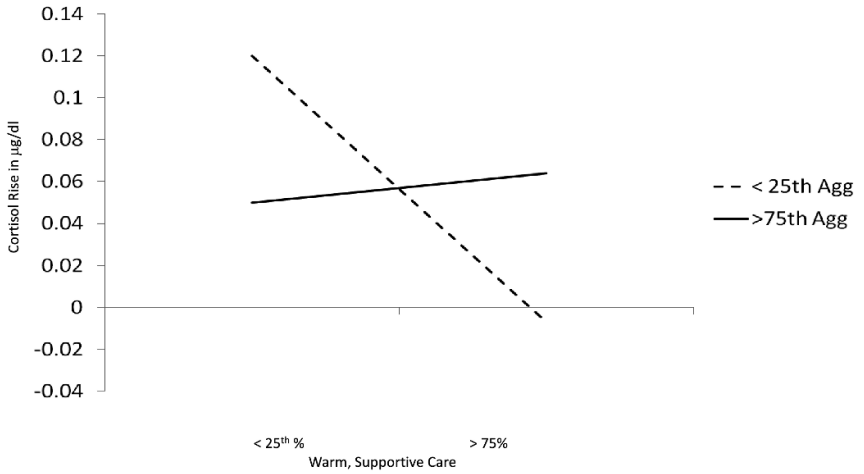

Figure 3.
For boys, the interaction between warm, supportive care and angry, aggressive behavior is plotted using the 25th and 75 percentiles of each measure.

Table 1
Means and Standard Deviations for Cortisol Data in μg per dl

Variables	*N*	*M*	*SD*
Home Cortisol (10am)	113	.12	.14
Home Cortisol (4pm)	112	.14	.15
Daycare Cortisol (10am)	151	.13	.11
Daycare Cortisol in (4pm)	151	.19	.18
Daycare Cortisol Rise in μg per dl	151	.06	.14

Note. Cortisol variables are presented in a linear scale, not log10.

Table 2
Descriptive Data on M-ORCE Measures (N = 151)

	M	*SD*
Care Provider Warm-Supportive Care Summary Score Measures		
Sensitivity	3.05	.39
Detachment (R)	1.58	.75
Positive Regard	2.68	.80
Chaos (R)	1.54	.74
Community Building	.43	1.27
Care Provider Intrusive-Overcontrolling Care Summary Score Measures		
Negative Regard	1.17	.45
Intrusiveness	1.51	.77
Overcontrol	1.55	.73
Child Anxious-Vigilant Summary Score Measures		
Vigilant, Anxious	1.42	.65
Belonging (R)	3.03	.76
Positive Social Integration (R)	31.49	10.88
Angry-Aggressive Summary Score Measures		
Angry-Irritable	1.44	.69
Negative Peer Int.	1.51	2.00

Note. R indicates the measure loaded negatively on summary scale.

Table 3

Regression Analysis Predicting the Rise in Cortisol as a Function of Child Sex, Child Behavior, and Their Interactions

	Step 1		Step 2		Step 3	
	B	***SE B***	***B***	***SE B***	***B***	***SE B***
Child Sex	.04	.14	.11	.15	.09	.15
Anxious-Vigilant	-	-	.19*	.09	-.01	.10
Angry-Aggressive	-	-	-.01	.12	.15	.15
Sex * Anxious-Vigilant	-	-	-	-	.39*	.18
Sex * Angry-Aggressive	-	-	-	-	-.41*	.20

Note. R^2 (Step 1) = .00. ΔR^2 (Step 2) = .04. ΔR^2 (Step 3) = .06. Total R^2 = .10. Only the highest-order terms in each equation are evaluated for significance.

* $p < .05$.

Table 4

Regression Predicting Child Behavior as a Function of Child Sex, Care Provider Behavior and Their Interactions

	Step 1		**Step 2**		**Step 3**	
Model 1: Anxious-Vigilant	***B***	***SE B***	***B***	***SE B***	***B***	***SE B***
Child Sex	-.42**	.16	-.27	.14	-.26	.14
Warm-Supportive	-	-	-.42***	.08	-.35	.12
Intrusive-Overcontrol	-	-	.06	.09	.11	.12
Sex * Warm-Supportive	-	-	-	-	-.14	.17
Sex * Instrusive-Overcontrol	-	-	-	-	-.11	.17
	Step 1		Step 2		Step 3	
Model 2: Angry-Aggressive	*B*	*SE B*	*B*	*SE B*	*B*	*SE B*
Child Sex	-.56***	.17	-.43**	.15	-.43	.14
Warm-Supportive	-	-	-.46***	.09	-.44	.12
Instrusive-Overcontrol	-	-	-.08	.09	.17	.12
Sex * Warm-Supportive	-	-	-	-	-.04	.17
Sex * Instrusive-Overcontrol	-	-	-	-	-.52**	.16

Note. Model 1: R^2 (Step 1) = .04. ΔR^2 (Step 2) = .20. ΔR^2 (Step 3) = .00. Total R^2 = .24. Model 2: R^2 (Step 1) = .08. ΔR^2 (Step 2) = .17. ΔR^2 (Step 3) = .07. Total R^2 = .32. Only the highest-order terms are evaluated for significance. When the Sex * Warm-Supportive interaction is removed, both individual factors are significant.

* $p < .05$.

** $p < .01$.

*** $p < .001$.