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Child care setting affects salivary cortisol and antibody secretion in young children

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Summary Elevated afternoon levels of cortisol have been found repeatedly in children during child care. However, it is unclear whether these elevations have any consequences. Because physiologic stress systems and the immune system are functionally linked, we examined the relationship between salivary cortisol concentration and antibody secretion across the day at home and in child care, and their relationships with parent-reported illnesses. Salivary antibody provides a critical line of defense against pathogens entering via the mouth, but little is known about its diurnal rhythm in young children or the effect of different environmental contexts. Saliva samples were taken at approximately 10:30 a.m., 3:30 p.m. and 8:00 p.m. on two child care and two home days in a sample of 65 3–5-year-old children attending very high quality, full time child care centers. Results indicated that (1) a rising cortisol profile at child care, driven by higher afternoon levels, predicted lower antibody levels on the subsequent weekend, (2) higher cortisol on weekend days was related to greater parent-reported illness, and (3) a declining daily pattern in sIgA was evident on weekend and child care days for older preschoolers, but only on weekend days for younger preschoolers. The results suggest that elevated cortisol in children during child care may be related to both lowered antibody levels and greater illness frequency.
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A decade ago Gunnar and colleagues first reported that cortisol levels increased in some children across the day at full-day child care (Tout et al., 1998). In the years since that

first report, this finding has been replicated and extended to include differences in cortisol levels between home and child care settings, with higher afternoon levels on child care days (Dettling et al., 1999, 2000; Watamura et al., 2002, 2003, 2009). Studies in the U.S. (Dettling et al., 1999, 2000; Tout et al., 1998; Watamura et al., 2002, 2003, 2009; Rappolt-Schlichtmann et al., 2009), France (Legendre, 2003), Germany (Ahnert et al., 2004), Australia (Sims et al., 2005, 2006), Sweden (Lundberg et al., 1993), and Korea (Park et al., 2007) have revealed many cross-cultural similarities in the cortisol profiles of children in care settings.

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A number of important results have emerged, including the consistent finding that in lower quality care environments a higher percentage of children exhibit a rise in cortisol from morning to afternoon (Tout et al., 1998; Dettling et al., 2000; Sims et al., 2005, 2006; Watamura et al., 2003), that older toddlers and younger preschoolers are the ones who most often show rising cortisol at child care (Dettling et al., 1999; Sims et al., 2006; Watamura et al., 2003), and that most children (approximately 70%) from relatively low-risk populations show the more typical decline in cortisol on weekend days at home (Dettling et al., 1999, 2000; Watamura et al., 2002, 2003, 2009). While moderate sample sizes have limited the full exploration of individual differences in these patterns, some studies have also reported correlations between cortisol levels and measures of temperament and social competence. For example, children who are particularly inhibited (Watamura et al., 2003), or who are high in externalizing behavior (Tout et al., 1998; Dettling et al., 1999), and those who are experiencing more peer rejection (Gunnar et al., 1997), or who play less well with peers (Watamura et al., 2003), are more likely to have higher cortisol levels than other children. The steadily growing body of evidence that a significant subset of children do exhibit afternoon cortisol elevations at child care raises the important question of whether these afternoon elevations have any consequences for the developing child.

1. Potential consequences of elevated cortisol at child care

In the absence of acute stress, cortisol follows a typical day and night rhythm. For adults, the highest values are typically seen at waking, followed by a sharp decline and then a more gradual decline across the day, reaching a nadir at approximately midnight (Kirschbaum et al., 1999). Infants also show a morning peak and evening nadir, with a decrease from mid-morning to mid-afternoon at home appearing by 36 months (Price et al., 1983; Larson et al., 1998; Watamura et al., 2004).

In children, a flattening of the diurnal rhythm (lower morning values or no drop from morning to afternoon) has been found for infants with colic (White et al., 2000), children with psychosocial dwarfism (Vazquez et al., 2000), and children living near the epicenter of a major earthquake (Goenjian et al., 1996). Some studies with animal models have also found similar effects on the daily cortisol rhythm. When infant monkeys are repeatedly separated from the mother at young age, they may subsequently show a flatter cortisol rhythm, with lower morning values and reduced decline from morning to afternoon (Sanchez et al., 2005).

The afternoon cortisol elevations observed when children are in care settings are typically modest and morning values typically do not differ between home and child care. However, the afternoon elevations often result in a flat or rising pattern across the day. It is unclear at this point whether a flattening of the rhythm across the mid-portion of the day due to modest afternoon elevations has any consequences.

Although all the studies to date are cross-sectional, taken together they suggest that these afternoon elevations are more likely to occur from the late toddler period (age 2) through most of the preschool period (age 5). An examination of the literature utilizing animal models and the smaller body

of work with humans suggests at least four domains that might be affected by repeated cortisol elevations during child care: (1) physical health, including changes in immune function (Sergerstrom and Miller, 2004), (2) an influence on attention and memory (Maheu et al., 2005; Tang et al., 2003), (3) alterations in sociality such as increased fearfulness (Vallee et al., 1997), and (4) increased stress reactivity and altered emotion regulation (Heim et al., 2002; Ladd et al., 2004).

The present study focused on physical health, specifically on one aspect of immune function. We elected to focus on immunity both because stress can influence immune responses and because exposure to infectious illness is greater in group care settings. These two factors could potentially challenge immune defenses as a consequence of out-of-home child care. A recent study of school-age children demonstrated consistent effects of psychosocial stress (in this case parental psychiatric symptoms) on the children's illness frequency (Caserta et al., 2008). Children experiencing greater psychosocial stress at home had more frequent illnesses and fevers, and also had increased white blood cell counts and higher natural killer cell activity. We were interested in whether child care in group settings might also influence illness frequency and levels of nonspecific immunoglobulin A (IgA) in the oral cavity. It is already known that infectious illness is elevated among young children attending child care (McCutcheon and Fitzgerald, 2001; NICHD ECCRN, 2003). This increased occurrence of illness is typically attributed to the greater exposure to pathogens and the contagion that is more likely to occur in group settings. The important question was whether children attending full-day child care also had different antibody levels on child care versus home days, and whether the levels of cortisol predicted antibody secretion and illness frequency.

2. Assessing immune function in children

For this project, we sought a measure of immunity that was feasible and ethical to assess repeatedly in children, and that would also be potentially sensitive to stress. In saliva, tears, and on other mucosal surfaces, the predominant type of secreted antibody is of the IgA class. In healthy adults, the release of secretory IgA (sIgA) into saliva generally follows a circadian rhythm: highest prior to wake-up and then declining to a flat pattern by about 4 h after wakeup (Hucklebridge et al., 1998). IgA is produced by B lymphocytes within the gums and released into saliva as a secretory dimer form where it helps to prevent the attachment and replication of invading viruses and bacteria. It also assists in neutralizing toxins and pathogens, and once bound to the pathogen facilitates the identification of these pathogens for other cells, which aids in pathogen killing by cytotoxic T cells (Winzer et al., 1999). Chronic stress in humans is associated with reduced total sIgA (e.g. Deinzer et al., 2000; Phillips et al., 2006), while acute stressors may be associated with either transient increases or decreases in sIgA (e.g. Ring et al., 2002). Some studies have found that reduced sIgA is associated with increased susceptibility to upper respiratory tract infections (Winzer et al., 1999), which occur at higher rates among children in child care. In the case of rare genetic

conditions where the individual cannot produce IgA at all, or under conditions of severe immune suppression, there is an increased likelihood of upper respiratory infections (Hierholzer, 1992). In a study of children with a history of recurrent colds and flu and matched healthy children, psychosocial stressors were greater and sIgA levels lower in children with more recurrent illness (Drummond and Hewson-Bower, 1997). Because sIgA can be assessed in saliva, it provides a noninvasive measure of immune function in children that is sensitive to stress.

The current study thus aimed to (1) assess whether higher cortisol levels at child care is associated with differences in antibody levels, (2) examine whether higher cortisol is associated with increased parent-reported illness frequency, (3) describe sIgA profiles across the day in a community sample of children attending full-day child care, and (4) explore whether antibody secretion across the day differs at home and child care.

3. Method

3.1. Participants

Participants were children attending one of seven classrooms in one of three settings in upstate New York. The classrooms were rated as good-to-excellent on the Early Childhood Environment Rating Scale-Revised (ECERS-R; Harms et al., 1998). The centers also had other indicators of high quality, for example, teachers with master's degrees and high levels of family involvement. Seventy-nine (36 female) of 120 eligible three-to-six-year-old children enrolled in the study. Criteria for exclusion were (a) in the current classroom for less than one 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 per week ($n = 13$). Full-time status was verified via sign-in sheets on sampling days, with median day length of 8 h 17 min (range: 5 h 30 min to 9 h 40 min). All children attended childcare 5 days per week. Of the enrolled children, four were siblings. Of the 79 enrolled children, child care cortisol data were obtained from 64, child care sIgA data were obtained for 62, evening cortisol and sIgA data were available for 59, weekend cortisol and sIgA data were available for 48, and 45 had completed questionnaire data. See Table 1 for descriptive data on each subsample.

Families completing the questionnaires 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 second 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. Families were asked "Has your family experienced any major stressors in the last 6 months (for example a move, a birth of

sibling, death in the family, divorce, separation, remarriage, and death of a pet)? If yes, please circle the number of stressors such as those indicated above that your family has experienced." Data on the number of stressors in the past six months were available for 41 children for whom cortisol and antibody 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 more than two stressors in the past six months. This study was approved by the Institutional Review Board of Cornell University.

Five families reported that their child was adopted, four internationally and one from within the United States. These children were adopted between 8.5 and 18 months of age. Cortisol levels averaged across all time points were lower for the adopted children (.07 $\mu\text{g}/\text{dL}$ vs. .13 $\mu\text{g}/\text{dL}$), Mann-Whitney $U = 26$, $p = .001$. Salivary cortisol was lower at each time point for the adopted children, although this difference was significant only for afternoon child care samples, $U = 47$, $p = .009$. The five adopted children also showed greater mean sIgA secretion rate than non-adopted children, (56 $\mu\text{g}/\text{mL}/\text{min}$ vs. 75 $\mu\text{g}/\text{mL}/\text{min}$), $U = 25$, $p = .04$.

3.2. Measures

3.2.1. Saliva samples for cortisol and antibody assays

Saliva samples were collected (see collection procedures below) from children in the child care setting and at home, with the goal of obtaining samples on two child care and two home days at 10:30 a.m., 3:30 p.m., and 8 p.m., for a total of 12 samples per child. Median sampling times at child care were 10:48 a.m. (range: 9:16–11:23 a.m.) and 3:48 p.m. (range: 3:09–4:40 p.m.). Child care sampling times were at least 30 min after snacks to avoid post-meal surges in cortisol (Ward et al., 1995; Gibson et al., 1999), at least 1 h before lunch to avoid anticipatory or midday surges (Follenius et al., 1982), before gym or outside time to avoid the effects of aerobic activity (Kirschbaum and Hellhammer, 1994), and at least 30 min after nap to avoid nap-time cortisol decreases (Watamura et al., 2002). The median reported evening sampling time on child care days was 8:01 p.m. (range: 7:00–10:35 p.m.). Median reported sampling times on weekend days were 10:07 a.m. (range: 8:50–11:55 a.m.), 3:44 p.m. (range: 1:51–6:00 p.m.), and 8:06 p.m. (range: 7:05–10:00 p.m.). The saliva was expressed into vials and stored at -80°C until data collection was completed. For assay, samples were organized into batches so that all samples from the same child were in the same analysis, and classroom and batch were not confounded.

Cortisol assays were performed by the Biochemical Laboratory, Department of Psychobiology, University of Trier, GE using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFI). Cortisol assays were performed in duplicate on whole saliva. For samples retained in the analyses described below, the mean intra-assay coefficient of variation (CV) was 4.93% and the inter-assay CV was 5.7–8.2%.

sIgA assays were performed using a commercial enzyme immunosorbent assay (EIA) for sIgA (Salimetrics, Inc.) on whole saliva. The mean intra-assay CV was 8.0% and the inter-assay CV was 7.1%. Saliva collection was timed and specimen volumes (.10–.25 ml) were determined prior to

Table 1 Sample characteristics and mean values for cortisol and slgA.

	Eligible	Family Enrolled	Child care cortisol data available	Child care slgA data available	Child care evening data available	Weekend data available	Questionnaires completed
<i>N</i>	120	79	64	62	59	48	45
Reason for sample loss	Family not interested (<i>n</i> = 41)	Excluded because refused sampling, illness, or medication (<i>n</i> = 15)	Insufficient volume after cortisol assay (<i>n</i> = 2)	Evening samples not returned (<i>n</i> = 3)	Weekend samples not returned (<i>n</i> = 11)	Questionnaires not returned (<i>n</i> = 3)	
Age (M, SD)		4 years 3 months 2 years 9 months–5 years 5 months	4 years 3.5 months 2 years 9 months–5 years 5 months	M4 years 3.5 months 2 years 9 months–5 years 5 months	4 years 4 months 2 years 9 months–5 years 5 months	4 years 3 months 2 years 9 months–5 years 5 months	4 years 2.9 months 2 years 9 months–5 years 5 months
Sex (% and number female)		46% (36)	48% (31)	50% (31)	51% (30)	55% (28)*	60% (27)**
Child care AM cortisol (M, SE)			.15 (.01)	.15 (.01)	.15 (.01)	.13 (.01)	.14 (.01)
Child care p.m. cortisol (M, SE)			.16 (.01)	.17 (.01)	.17 (.01)	.16 (.01)	.15 (.01)
Child care a.m. slgA (M, SE)				35.94 (2.54)	36.30 (2.60)	36.90 (2.97)	35.5 (3.17)
Child care p.m. slgA (M, SE)				37.67 (2.53)	38.47 (2.66)	37.20 (2.78)	34.34 (2.67)
Child care evening cortisol (M, SE)					.06 (.01)	.05 (.01)	.05 (.01)
Child care evening slgA (M, SE)					37.60 (4.00)	34.50 (3.21)	35.7 (3.67)
Weekend AM cortisol (M, SE)						.14 (.01)	.14 (.01)
Weekend p.m. cortisol (M, SE)						.11 (.01)	.11 (.01)
Weekend a.m. slgA (M, SE)						42.16 (3.71)	40.98 (3.68)
Weekend p.m. slgA (M, SE)						39.20 (3.97)	38.37 (4.09)
Weekend evening cortisol (M, SE)						.05 (.004)	.05 (.01)
Weekend evening slgA (M, SE)						32.44 (3.01)	32.04 (3.16)

There were more girls with available weekend samples, **p* < .05, and with completed questionnaires ***p* < .01, as compared to the full sample of enrolled families.

aliquoting. Due to the known influence of salivary flow rate on salivary antibody concentrations, all analyses included secretion rates rather than concentrations alone: (slgA concentration) \times (sample volume/collection time).

3.2.2. Illness

To assess illness frequency, families were asked to indicate how often [daily (5), weekly (4), every other week (3), monthly (2), less than monthly (1)] their child had the following symptoms: fever, runny nose, cough, stomachache, other. Two measures were derived from these data: (1) overall illness frequency (the sum of all reported symptoms) and (2) upper respiratory illness frequency (runny nose, and cough). Due to seasonal variation in illness frequency, the summer months were avoided. Saliva samples were collected in the winter months for upstate New York (November through March) for 40 children, in the spring months (April and May) for 20 children, and in the fall months (September and October) for 5 children. Analyses of seasonal effects are reported in the preliminary results section. Although we were interested in illness frequency, we wanted to avoid confounding current infection with our cortisol and antibody assessments. As a proxy for current infection, children's temperatures were taken with an ear thermometer on sampling days and children with ear temperatures above 99.5 °F were not sampled. If children were absent due to illness, we waited at least one week after their return and until symptoms were absent to collect samples. Height and weight measurements were taken on 56 of 65 children by research assistants in the classroom. Using CDC age and sex specific norms for body mass index (BMI), 14 children were in the bottom 25% for BMI, 27 were between the 25th and the 75th percentile and 15 were above the 75th percentile. There were no differences in cortisol, slgA secretion rate or illness frequency by BMI group, all $ps > .5$.

3.2.3. Procedure

3.2.3.1. Classroom data collection procedures. *Saliva sampling:* Group saliva sampling in a classroom generally occurred over 3–5 days. If any child had provided low volumes or had been absent or ill, they were sampled individually on subsequent days. Saliva samples were taken mid-morning and mid-afternoon, as close to 10:30 a.m. and 3:30 p.m. as possible without disrupting classroom routines and avoiding daily snack, lunch, nap, gym, and outside time. Saliva samples were collected via cotton dental rolls by research assistants using simple, well-established procedures (Gunnar and White, 2001; Watamura et al., 2002, 2003). However, in the present study no flavor or other substance, such as Kool-AidTM, was used to stimulate saliva flow, and samples were collected during timed 2-min intervals (exact times were recorded for each child). Saliva was expressed into plastic vials using a mechanical compression device and a needleless syringe. Cotton weights were individually recorded prior to sampling and wetted sample weights were recorded post-sampling. Child care samples were frozen until assayed. Although we and others have subsequently discontinued the use of cotton-based collection devices due to the fact that cotton fibers retain small amounts of cortisol and slgA (Strazdins et al., 2005), cotton collection devices were still prevalent at the time of this study. Thus, absolute levels of cortisol and slgA may be underestimated in these data. However, the main analyses are within-subject time and

context comparisons and thus should not be meaningfully compromised by the use of cotton.

3.3. Home data collection procedures

Sample collection: Parents were asked to collect saliva samples on two weekday evenings at 8 p.m., and on two weekend days at mid-morning, mid-afternoon, and 8 p.m. On the first full day of saliva sampling at child care, parents were given a kit containing sampling materials, instructions, and a daily checklist. They were asked to take the first evening 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 p.m. Parents were asked to sample their child at 8 p.m., when the watch alarm sounded, for a 2-min interval, using the watch to time the exact length of the sampling interval, to refrigerate the sample, and to return it the following day to child care. Subsequent sampling materials were distributed when the first sample was returned to collect a second 8 p.m. child care day sample and to collect two days of three samples per day at approximately 10 a.m., 3:30 p.m., and 8 p.m. on two separate (consecutive or non-consecutive) weekend days. Saliva samples were picked up daily from the child care centers; the saliva was expressed, weighed and frozen on the same day.

Sample aliquots: To aliquot samples for cortisol and slgA assays, during manual extraction from the cotton collection device, the initial 200 μ L was reserved for cortisol determination and the remaining saliva was collected and stored in a separate vial. Prior to slgA assay, 50 μ L of clear saliva were aliquoted from each vial after vortexing.

Compliance: As cortisol and slgA both follow diurnal rhythms, 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 et al., 2003). To encourage compliance, the importance of the time of sampling was emphasized in the directions, the digital child's watch was used as a reminder and encouragement of compliance, and for the weekend samples, 94% of families were given sampling materials with compliance caps (APREX Corp, Union City, CA) or compliance boxes (Cayuga Design, Ithaca, NY). 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: 0–153 min). Interestingly, less compliance (a greater discrepancy between reported and recorded times) was positively correlated with cortisol (but not slgA) both on weekend days $r = .35$, $p = .03$, and on child care days, $r = .32$, $p = .04$. This may reflect higher cortisol for children with families experiencing greater disorganization. Actual sampling time was controlled in our main analyses described below.

3.4. Data reduction

All cortisol assays were performed in duplicate and the results averaged. These values were then averaged within context by time point, creating six cortisol variables reflecting average

mid-morning, mid-afternoon, and evening cortisol on child care days, and mid-morning, mid-afternoon, and evening cortisol on weekend days. Cortisol values at each time point were examined for positive skew. No skew was evident, so non-transformed values were used in all analyses. Average Child Care Cortisol (morning, afternoon and evening) and Weekend Cortisol (morning, afternoon and evening) levels as well as Overall Mean Cortisol (the average of the six time point cortisol levels) were computed. Similar variables were created for slgA secretion rate, for each time point and averaged for child care, weekend, and overall slgA secretion rate across all 6 time points.

Children were also classified as having rising cortisol across the child care day (a.m. to p.m. average increase $\geq .05 \mu\text{g/dL}$), falling cortisol (a.m. to p.m. average decrease $\geq .05 \mu\text{g/dL}$), or flat cortisol. We have categorized children in this way in several previous studies of cortisol profiles at child care (e.g. Watamura et al., 2009). The .05 cutoff was chosen because it is above the error rate for duplicate assays of the same sample in this and previous work of child care cortisol. The benefit of this approach is that it distinguishes between children who are clearly showing a reactive profile (rising across the day), those clearly showing a non-reactive profile (falling across the day) and those with a flat profile, a heterogeneous group that may include children showing partial reactivity, children still exhibiting an immature rhythm across the mid-portion of the day, and children with absolute values of cortisol that are quite low.

4. Results

4.1. Preliminary analyses

Bivariate correlations between age and the six outcome variables representing cortisol values averaged within time for each context, Child Care Cortisol, Weekend Cortisol, and Overall Mean Cortisol were not significant, $ps > .19$. There were fairly consistent linear associations between age and the slgA variables, with older children having higher antibody secretion rates overall, $r = .31$, $p = .02$. This age effect was clearer for weekend slgA secretion rate, $r = .40$, $p = .01$, with a trend toward a similar pattern on child care days, $r = .23$, $p = .08$. There were no sex differences in cortisol results (all $ps > .22$), and only one sex difference in slgA secretion rate data. Weekend evening secretion rates of slgA were higher for boys, (boys $M = 39$, girls $M = 27$), $t(36) 2.07$, $p = .046$. Thus, age was statistically controlled in all analyses with the slgA variables and sex was controlled in the analyses of weekend evening slgA.

A series of one-way ANOVAs were conducted to examine whether parent-reported average illness frequency, upper respiratory illness frequency, mean slgA secretion rate and mean cortisol differed by season of data collection (winter, spring, and fall). There were no differences by season of data collection for reported total illness frequency, $F(2,42) = 2.06$, *ns*, or reported upper respiratory illness frequency, $F(2,42) = 1.36$, *ns*. Mean slgA secretion rate did not differ by season, $F(2,59) = .04$, *ns*. Mean cortisol was slightly higher in the fall (beginning of the school year) as compared to the winter and spring, $F(2,62) = 3.42$, $p = .04$, with higher afternoon cortisol for children sampled in the fall, $F(2,61) = 3.18$, $p = .049$.

4.2. Relations between cortisol, antibody, and illness

slgA and cortisol: There were negative correlations between child care cortisol levels and both child care slgA secretion rates controlling age, $partial r(59) = -.26$, $p = .04$, and weekend slgA secretion rates controlling age and sex, $partial r(37) = -.33$, $p = .04$, so that higher cortisol levels at child care (average of all three time points on child care days) were associated with lower nonspecific antibody secretion rates (slgA) both at child care (see Fig. 1) and at home. Afternoon cortisol levels at child care accounted for the relationship, with negative associations (controlling age), with weekend morning slgA secretion rates [$partial r(33) = -.52$, $p = .001$, for reported time of saliva sample; $partial r(23) = -.60$, $p = .002$, for recorded time of saliva sample], and weekend afternoon slgA secretion rates [$partial r(34) = -.37$, $p = .03$, for reported time of saliva sample; $partial r(25) = -.45$, $p = .02$, for recorded time of saliva sample]. There was a trend toward a negative correlation between afternoon child care cortisol and evening child care slgA secretion rate, $partial r(47) = -.26$, $p = .08$, for reported sample time. Comparing children who did or did not show rising cortisol values from morning to afternoon at child care indicated that children showing rising cortisol (a.m. to p.m. increase $\geq .05 \mu\text{g/dL}$) had lower antibody secretion rates ($28.6 \mu\text{g/mL/min}$ vs. $41.1 \mu\text{g/mL/min}$) on the subsequent weekend than did children showing flat or falling cortisol on child care days, $F(1,39) = 4.32$, $p = .04$, $d = .72$. This analysis controlled for age and sex. In addition, the length of time between a.m. and p.m. samples was entered into the model to insure that differences in the change from morning to afternoon were not due to sampling time.

There was also a trend for weekend cortisol levels to be negatively correlated with child care, $partial r(38) = -.29$, $p = .07$, and weekend, $partial r(59) = -.27$, $p = .10$, antibody secretion rates. However, cortisol values from individual time points did not predict slgA secretion rates.

Illness: Bivariate correlations between cortisol variables, antibody variables, and parent-reported illness indicated that mean weekend cortisol levels were positively related to parent reports of total illness frequency, $r = .44$, $p = .003$, specifically the frequency of upper respiratory illness, $r = .43$, $p = .004$. To estimate the clinical relevance of this relation, we calculated the odds ratio (OR) for illness frequency given elevated cortisol levels. Weekend cortisol above the median ($.095 \mu\text{g/dL}$) was associated with illness frequency above the median (daily), OR = 5.53, 95% confidence interval 1.46–20.9, indicating that children in the top half of the distribution on weekend cortisol levels were approximately 5 times more likely than those in the bottom half of the distribution to experience daily illness symptoms. Cortisol levels while at child care and slgA secretion rates were not correlated with parent-reported illness. Following Baron and Kenny (1986) we found no evidence that the effect of cortisol on parent-reported illness was mediated via slgA secretion rates. Although child care cortisol was associated with antibody secretion rates at child care and at home, and weekend cortisol was related to parent-reported illness, no measures of slgA were related to parent-reported illness controlling for cortisol. Therefore our data failed step 3

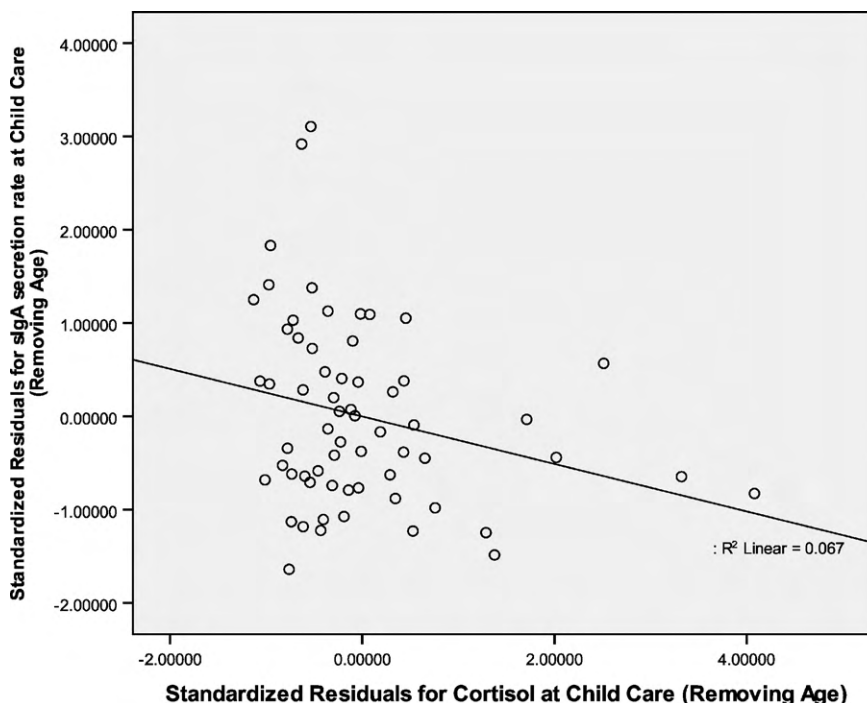


Figure 1 Partial correlation between sIgA secretion rates and cortisol on child care days, controlling for age of the child.

(the mediator did not predict the dependent variable controlling for the independent variable).

4.3. Cortisol and sIgA patterning on child care and weekend days

The above results demonstrate that higher cortisol levels were associated with lower antibody levels and more parent-reported illness. As these data may be the first to examine diurnal patterning of sIgA in relation to cortisol patterning across contexts in young children, the following analyses were designed to examine and fully describe the patterning results. For salivary cortisol, a Context (Child Care Day, Weekend Day) × Time (Morning, Afternoon, and Evening) repeated measures ANOVA indicated main effects for Time, $F(2,88) = 67.60, p < .001, \eta_p^2 = .61$, and Context, $F(1,44) = 4.05, p = .05, \eta_p^2 = .08$, and a Time × Context interaction, $F(2,88) = 3.08, p = .05, \eta_p^2 = .07$ (see Fig. 2). Tests of simple effects indicated that afternoon cortisol was higher at child care than at home, $t(48) = 3.2, p = .002, d = .61$, and that cortisol decreased from morning to afternoon, $t(47) = 2.13, p = .04, d = .5$, and from afternoon to evening on weekend days, $t(46) = 7.42, p < .001, d = 1.27$, and from afternoon to evening on child care days, $t(58) = 6.68, p < .001, d = 1.05$.

To test whether daily sIgA secretion rate patterns differed by time and across context, a Context (Child Care Day, Weekend Day) × Time (Morning, Afternoon, Evening) repeated measures ANOVA was conducted. Reported results reflect the Greenhouse–Geisser correction for non-sphericity or correction for unequal variances, where relevant. There were no main effects for Time or Context, and a trend for a Time × Context interaction, $F(1.51,45.36) = 2.69, p = .09$. Repeating these analyses within Context, there was no evidence of a diurnal rhythm on child care days,

$F(1.58,79.17) = .22, ns$. However, there was a clear diurnal rhythm at home on the weekend, $F(2,66) = 4.66, p = .013$ (see Fig. 2). Follow-up tests of simple effects indicated sIgA secretion rates dropped from weekend afternoon to weekend evening, $t(35) = 2.07, p = .046, d = .31$.

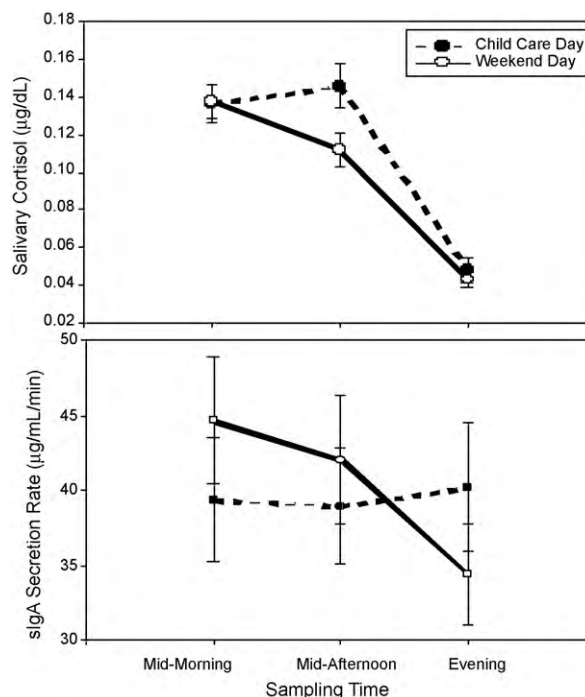


Figure 2 Mean (±SEM) salivary levels of cortisol and sIgA secretion rates during the morning, afternoon and evening on child care and weekend days.

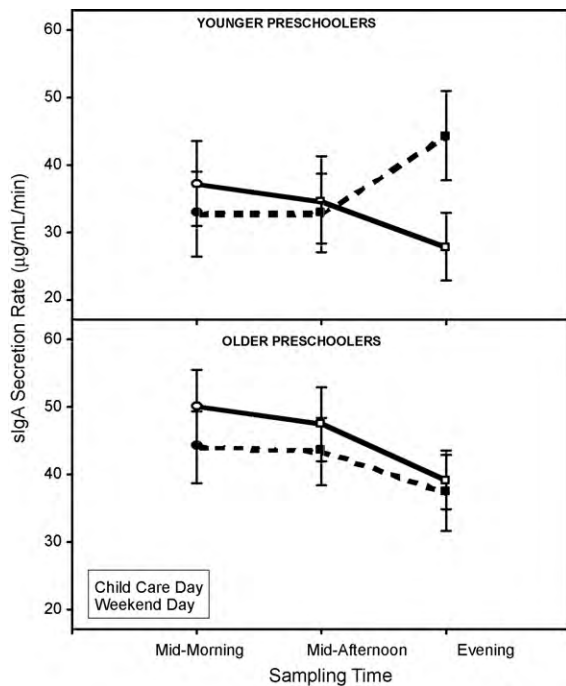


Figure 3 Mean (\pm SEM) sIgA secretion rates in younger (2 years 7.5 months to 4 years 6 months) and older (4 years 6.1 months to 5 years 4 months) preschoolers during the morning, afternoon, and evening on child care and weekend days.

Thus, comparing cortisol and sIgA patterns, the weekend sIgA pattern was similar to that for cortisol (falling across the day), while the pattern on child care days differed. On child care days, cortisol rose from morning to afternoon and then fell sharply by evening, whereas antibody secretion rates were relatively low and flat from morning to afternoon and rose in the evening (see Fig. 2).

Due to the age differences noted above, we repeated the Context (Child Care Day, Weekend Day) \times Time (Morning, Afternoon, Evening) repeated measures ANOVA separately for the younger and older children using a median split for age. As displayed in Fig. 3, there was a Time \times Context interaction for the younger children, $F(2,24) = 6.17$, $p = .007$, due to the fact that the younger preschoolers showed elevated sIgA on child care evenings. Older children exhibited the expected diurnal decline on both child care and weekend days, with a main effect for Time, $F(2,34) = 3.32$, $p = .048$, and no Time \times Context interaction.

5. Discussion

Our study is the first to investigate the potential health consequences of rising cortisol levels in the afternoon while at child care. It was designed to assess associations among cortisol profile, antibody secretion at home and at child care, and infectious illness in three to six-year-old children. The children were attending high quality full-day child care.

There were four key findings. First, cortisol predicted lower total sIgA secretion rates. This effect was accounted for by higher afternoon cortisol at child care predicting lower antibody secretion rates, particularly on weekend mornings.

Delayed effects of stress on sIgA levels are typically manifested as decreases (Tsujita and Morimoto, 1999), so these results may reflect a delayed effect of weekday cortisol elevations. This interpretation is supported by the finding that children who exhibited a rising cortisol profile across the day at child care had lower weekend morning sIgA secretion rates. That is, children who showed HPA activation (cortisol elevations) to full-day child care were more likely to show lower antibody secretion rates than children who did not. Higher cortisol on weekend days was also marginally related to lower antibody secretion rates at home and child care. In a study of Spanish preschoolers (Sanchez-Martin et al., 2001), no correlations were found between cortisol and sIgA concentrations. However, they were not able to control for salivary flow rate, a critical factor in this measure as stress can result in reduced saliva flow and thus can confound sIgA assessments if not controlled (Kugler et al., 1993). Furthermore, these authors examined cortisol and sIgA only in the morning at preschool, although the body of work on cortisol differences between child care and home consistently reports afternoon (and typically not morning) differences. Other authors have reported sIgA decreases in children to time-limited psychosocial stressors, and sIgA increases in children who have experienced chronic stressors. For example, Nozaki et al. (2007) reported decreased sIgA after dental treatment in a sample of Japanese children. In contrast, by adolescence, Shirtcliff et al. (2009), report higher total sIgA in those who had experienced the early deprivation of institutionalized care or physical abuse.

Second, higher cortisol was associated with greater parent-reported illness, accounted for largely by upper respiratory symptoms. This could reflect stress-related increases in susceptibility to infectious illness. It has been shown that psychosocial stress can predispose individuals to increased upper respiratory tract infections (e.g. Turner Cobb and Steptoe, 1996). Furthermore, in a recent study higher basal cortisol and lower basal sIgA predicted poorer health outcomes during the stress of an academic exam in young adults (Volkman and Weekes, 2006). Similarly, in a study of 5th grade Canadian children, lower sIgA was correlated with more frequent upper respiratory infections (Cieslak et al., 2003). Thus, the children in our study who had both higher cortisol levels and lower nonspecific antibody levels may be more susceptible to illness under subsequent stress conditions. The reverse is also possible. That is, children who have more frequent infections may show elevated cortisol because frequent illness itself can be a stressor. In particular, it is known that febrile illness is associated with a rise in cortisol (Nickels and Moore, 1989). However, the effects of fever are acute, and ear temperatures were taken on all children prior to each saliva sample in this study. Thus, although these children were reported to have more frequent illnesses in the past, no children had active illnesses with fever or other symptoms on or near sampling days. A third possibility is that families experiencing more stress or parents with particular characteristics are both more likely to report that their children are ill and to have children with elevated cortisol at home.

In our study of young children, the higher frequency of parent-reported illness in the children with elevated afternoon cortisol levels was more indicative of a stress-health link than were relations between sIgA and reported illness.

Children in the upper half of the distribution for weekend cortisol were 5 times more likely to have daily illness symptoms than children in the lower half of the distribution. While elevated cortisol predicted illness, and cortisol and sIgA were related, sIgA did not by itself predict parent-reported illness. The absence of apparent mediation by sIgA may be due to the fact that in this low risk sample total sIgA was still in the normal range. Within the normal range, lower total nonspecific salivary antibody levels may be a more sensitive biomarker of stress than a direct correlate of the frequency of infection. We considered the use of specific antibody to the Herpes simplex virus, a useful measure of immune function in exposed populations; however, the exposure rate in this young, low-risk sample (approximately 40% using an OD cutoff of .20) was inadequate to determine if lower antibody levels resulted from lack of exposure or failure to mount an antibody response (unpublished data).

Third, our weekend sIgA data indicate that a diurnal rhythm is apparent by three years of age. By 4-and-a-half years of age a rhythm was manifest both on weekend and on child care days. Our sIgA and cortisol concentrations were similar to those found in the morning at child care by Sanchez-Martin et al. (2001) in their sample of Spanish preschoolers [cortisol: Spanish sample, $M(SD) = .15 (.07)$, current sample $M(SD) = .15 (.09)$; sIgA: Spanish sample $M(SD) = 61 (81)$, current sample $M(SD) = 60 (27)$]. We were not able to find published data on sIgA secretion patterns across the day in preschoolers. However, our sIgA values are within the range of what might be expected in comparison to a healthy young adult sample, (Hucklebridge et al., 1998); Hucklebridge reported square root-transformed sIgA concentrations between 10 and 25, (highest at wakeup), and our transformed sIgA concentrations were between 6.8 and 8.2. Thus it seems that our sIgA values are reasonable (for young children) as compared to the body of published work using similar (cotton-based) collection devices with adults.

Lastly, we found that although sIgA patterns were similar to those found in healthy adults for older preschoolers in both contexts and for younger preschoolers on the weekend, sIgA secretion rates were elevated in the evening of child care days in younger children. The elevation in younger children may have reflected a response to the challenges presented by child care (Tsujita and Morimoto, 1999).

It is noteworthy that this sample of children should be considered as particularly low risk (the children of high SES, university-affiliated families who attended very high quality child care centers), and exhibited the lowest cortisol elevations we have observed in child care settings (see Watamura et al., 2009). The fact that we observed relations between cortisol elevations and both sIgA secretion rates and illness frequency in this population with a relatively small sample size suggests that further work addressing the stress-health associations in higher-risk families and children attending lower quality centers is needed.

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Conflict of interest: The authors have no conflicts of interest financial or otherwise in these results. Full IRB approval was obtained for data collection (Cornell University) and assay and analysis (University of Denver).

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