Children's Cortisol in Preschool and Aggression One Year Later in Kindergarten

by

Diana Michelle Reinecke

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 9, 2011

Keywords: preschool, aggression, cortisol

Approved by

Jacquelyn Mize, Chair, Professor of Human Development and Family Studies
Gregory S. Pettit, Professor of Human Development and Family Studies
Margaret Keiley, Professor of Human Development and Family Studies

Abstract

Previous research has examined associations between aggression and cortisol throughout the lifespan, with most studies concluding that individuals with lower basal cortisol exhibit more aggressive and antisocial behavior. However, studies investigating links between aggression and cortisol in young children are unclear. The purpose of this study was to examine basal and reactive cortisol in preschool and whether it was predictive of concurrent and later aggression. Preschool children (N = 189) enrolled in community child care programs served as participants. Cortisol was measured in saliva samples collected from children at child care. Samples collected on two mornings were used as an index of basal cortisol. Samples collected over the course of a series of challenging tasks and teacher-child interaction served as indices of reactive cortisol. Teachers provided ratings of child aggression in preschool and in kindergarten. Multiple models were fit using path analysis examining associations among cortisol measures, aggression during preschool, and aggression a year later in kindergarten. In a model examining basal (i.e., morning) cortisol, controlling for preschool aggression and time of saliva collection, lower morning cortisol predicted higher aggression in kindergarten a year later. In a model examining reactive cortisol, declines in cortisol over the challenging tasks was associated with higher aggression ratings concurrently, whereas increasing cortisol during teacher-child interaction predicted higher aggression ratings a year later in kindergarten. When both basal and reactive cortisol were included in the same model, only basal cortisol predicted kindergarten aggression.

Acknowledgements

This article is based on work supported by the National Science Foundation under Grant 0126584 and the Agricultural Experiment Station (ALA010-008) to Jacquelyn Mize and by Graduate Dissertation Research Awards from the Auburn University Graduate School to Jared A. Lisonbee and Amie Lapp Payne.

The author would like to thank her committee members, Dr. Greg Pettit and Dr. Margaret Keiley for their guidance and support throughout this process and for their willingness to serve on this committee. She would also like to extend her greatest appreciation to her mentor, Dr. Jacquelyn Mize, for her love, encouragement, and dedication to the author's learning for the past four years. A special thank you to her family, especially her parents, A.J and Gail, and her amazing siblings, Michael, Angela, and Mark, for always serving as positive role models throughout her life. Thank you to Maria Ramage, for her constant positive outlook on life and for the encouragement she provided during our graduate career, personally and professionally. Finally, the author would like to thank her fiancé, Zack Blackwell, for his continuous love and for believing she was capable of conquering this challenge as well as any other challenge that may come her way.

Table of Contents

List of Tables

List of Figures

I. INTRODUCTION

Most violent adult offenders began life as aggressive preschoolers. Coming from the other perspective, a high proportion of aggressive preschoolers, around 50% to 70%, depending on diagnostic criteria (Campbell, Shaw, & Gilliom, 2000; Moffitt, 2007), show continuity in externalizing behavior problems from toddlerhood or preschool into middle childhood. On the other hand, virtually all toddlers and preschoolers exhibit aggressive behavior and, even among those showing severe early problems, many do not grow up to be violent offenders (Campbell et al., 2000; Moffitt, 2007). These facts have important implications for social policy, intervention, and basic research priorities. An important goal for current and future research is to identify factors that differentiate children whose early aggression reflects transient adjustment difficulties from those who will follow a path toward more persistent, life-course behavior problems (Campbell, Spieker, Vandergrift, Belsky, Burchinal, and the NICHD Early Child Care Research Network, 2010).

Historically, most empirical efforts to identify predictors of aggression and other forms of antisocial behavior have focused on psychosocial risk factors (Dodge, Coie, & Lynam, 2006). Young aggressive children living in poverty, in single-parent homes, and with harsh, insensitive parents (Campbell et al., 2010) are more likely to have adjustment problems in elementary and high school. Within the past decade, however, there has been a revolution in how developmentalists view the origins of externalizing problems. Many authorities now argue that, because individual differences in aggression and antisocial behavior appear so early and are so stable, they must have roots in biological or constitutional vulnerabilities (Raine, 2002; Scarpa & Raine, 1997). Identifying easily measured biological factors that predict aggressive problems could help intervention specialists and policy makers develop programs that more accurately target children at greatest risk for persistent aggression. This study will examine one such biological marker, salivary cortisol, measured in preschool as a predictor of aggression one year later in kindergarten.

Persistence of aggression from early childhood. Twenty years ago, parents and teachers who complained about a preschool child's aggression or non-compliance might well be told that the behaviors were developmentally normative and that the child would outgrow them by school age (Campbell et al., 2000). Fortunately, for many children this is the case. But recent longitudinal studies prompted by the developmental psychopathology perspective indicate that for many children, early behavior problems persist into middle childhood and even into adulthood (Campbell et al., 2000; Moffitt, 2007). A recent person-centered analysis of children in the National Institute of Child Health and Development Study Early Child Care and Youth Development (SECCYD) found that early behavior problems are strong predictors of later significant externalizing behaviors. More than half of the children in the sample had at least somewhat elevated levels of aggression at age 2. Of these, 75% continued to show problematic aggression at age 9 (Campbell, Spieker, Burchinal, Poe, and the NICHD Early Child Care Research Network, 2006). A review of longitudinal studies of antisocial behavior concludes more generally that a substantial proportion of children will follow a life-course persistent pattern of antisocial or aggressive behavior from early childhood into adulthood (Moffitt, 2007).

Predicting childhood aggression. Decades of research indicate that children growing up in adverse circumstances are at risk for developing behavior problems. Ecological risk factors for youth violence include low socioeconomic status (SES), parental harsh discipline and poor supervision, living in a large family, having a single parent, associating with delinquent peers, and living in a high crime neighborhood (Dodge et al., 2006; Farrington, 2007).

Recent research reflects what may be a paradigm shift in how the causes of antisocial behavior are viewed, however (Dodge & Sherrill, 2007). The last decade has witnessed a dramatic increase in research on biological processes underlying individual differences in social-emotional development (Eisenberg, Fabes, & Spinrad, 2006). In the study of antisocial behavior, findings from biologically based research are sufficiently robust for knowledgeable observers to conclude that serious problems of aggression derive partly from biological dysfunction (Scarpa & Raine, 1997). Researchers have identified risk factors for aggression in genetic, endocrine (van Goozen, 1998), neurotransmitter, and autonomic and central nervous system functioning of animals and humans (Nelson & Trainor, 2007).

Perhaps the most consistent finding in psychophysiological research on aggression is that a low level of autonomic arousal, as indexed, for instance, by a low resting heart rate, is associated with problem aggression (Raine, 2002; Scarpa & Raine, 1997). Numerous studies have reported links between low resting heart rate and violence and aggression both concurrently and predictively and meta-analyses report medium to large effect sizes in samples of children and youth (Lorber, 2004; Oritz & Raine, 2004). Results for measures of reactivity (change from baseline as a result of a stressor) have been less consistent; some studies report that high reactivity is associated with aggression (Kobak, Zajac, & Levine, 2009), whereas others report that low reactivity is associated with aggression (Van Goozen, Matthys, Cohen-Kettenis, Buitelarr, & van Engeland, 2000; Snoek, van Goozen, Matthys, Buitelaar, & van Engeland, 2004).

Other indices that seem to reflect low autonomic arousal, such as low resting and task electrodermal activity (Lorber, 2004; van Bokhoven, van Goozen, van Engeland, Schaal, Arseneault, Seguin, Nagin, Vitaro, & Tremblay, 2004) and low baseline cortisol (Loney, Butler, Lima, Counts, & Eckel, 2006; McBurnett, Lahey, Rathouz, & Loeber, 2000; Smider, Essex, Kalin, Buss, Klein, Davidson, & Goldsmith, 2002; van Goosen et al. 1998; Shirtcliff, Granger, Booth, & Johnson, 2005) also have been linked to aggression. Children’s cortisol provides another index of basal and reactive physiological response. Cortisol is the primary end product of the hypothalamic-pituitary-adrencortical (HPA) axis and as part of the body’s normal regulatory functioning, cortisol secretion follows a circadian rhythm. In addition, cortisol is released during stressful situations (Kirschbaum, Wust, & Hellhammer, 1990). Until recently, cortisol was measured in blood or urine; however, now it can be measured in saliva and is relatively simple to obtain (Kirschbaum & Hellhammer, 1994).

The purpose of this study is to examine children's cortisol, collected while at child care when they were age 4, as a predictor of aggression in kindergarten the following year. Cortisol was collected in several different contexts and at different times of the day while children were in full-day child care at age 4. Teachers completed questionnaires in preschool and one year later in kindergarten about children’s behavior.

II. LITERATURE REVIEW

This literature review is divided into three major sections. The first will describe the function of cortisol, how it is measured, and factors that appear to influence cortisol levels in children. In this study, cortisol was measured at child care, and so research on the effects of child care on children's cortisol will be summarized. The second section will focus on early childhood aggression, stability of early aggression, and factors associated with children's aggression. The final section will review evidence of links between children's aggressive behavior and cortisol and unresolved issues will be highlighted.

Throughout this review, it will be important to keep in mind that over the past decade, research on cortisol in children has exploded. Models guiding this research change frequently in response to new studies whose findings are inconsistent with past research and with previous models. It is not possible to summarize the hundreds of studies on children's cortisol; this review attempts to reflect a judicious review of important, representative studies.

Cortisol

In response to stress, hormones known as glucocorticoids are released. When a stressful situation occurs, part of the brain called the hypothalamus emits hormones into the hypothalamic-pituitary-adrenocortical (HPA) axis. The primary hormone secreted is corticotrophin releasing hormone (CRH), which supports the pituitary in releasing adrenocorticotropic hormone (ACTH), also known as corticotropin. ACTH then travels through the bloodstream until it makes contact with the adrenal gland, where glucocorticoids are secreted within minutes. In conjunction with the emissions of catecholamine's (epinephrine and norepinephrine) in the sympathetic nervous system, glucocorticoids represent a large percentage of what is happening in a person's body during stress. Whereas catecholemines are released within seconds, glucocorticoids support the catecholemines over the course of minutes or hours (Sapolsky, 2004). It is important to know that production of glucocorticoids is the result of a series of steps because any variation in these steps can alter the amount of cortisol that is released.

In humans, the primary end-product of the HPA axis is cortisol. Cortisol production follows a circadian rhythm as part of the body's normal regulatory functioning. The highest level of cortisol normally occurs approximately 30 min after awakening. This is followed by a quick decrease over the next one to two hours, and a slower decline throughout the day and evening hours. Cortisol normally reaches its lowest level around midnight (Kirschbaum et al., 1990; Gunnar & Cheatham, 2003). Because of this circadian pattern, measurement time typically is statistically or methodologically controlled in studies of cortisol.

Cortisol is essential to life. Its primary function is to regulate energy metabolism. The complicated HPA network also facilitates regulation of a person's response to stressful situations (Gunnar & Donzella, 2002). An increase in stress usually results in higher levels of cortisol in the body. This increase prepares the body for "fight or flight" by increasing blood levels of glucose, relaxing bronchial tubes, reducing inflammation, and suppressing maintenance functions, such as digestion and immune activity (Sapolsky, 2004). However, prolonged cortisol elevation can cause dysregulated diurnal rhythms and can be dangerous to human health (Shirtcliff & Essex, 2008). Health problems associated with long-term elevations in cortisol include heart disease, diabetes, and osteoporosis (Sapolsky, 2004).

Conversely, low levels of cortisol also are maladaptive. Extremely low levels of cortisol are symptomatic of disease states such as Addison's disease. People with Addison's disease cannot secrete a sufficient amount of glucocorticoids, which results in an inability to mobilize the body during energetic demands. People with untreated Addison's disease suffer from what is called an "Addisonian" crisis when a major stressor occurs. The crisis will cause a drop in blood pressure, inability to maintain circulation, and eventually shock. Consequently, it can be difficult for people with Addison's disease to meet the demands of everyday life (Sapolsky, 2004). More subtle disorders linked to undersecretion of stress hormones include chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, a subtype of depression, and perhaps post-traumatic stress disorder (Sapolsky, 2004). As will be described subsequently, some research suggests that violent or aggressive individuals also have low levels of cortisol.

Influences on Cortisol Levels in Children

Recent analyses suggest that most of the variance in cortisol levels among children is attributable to transient contextual factors, with a smaller proportion of variance being attributable to more stable individual differences. Shirtcliff and colleagues (2005) report that about 70% of the variance of cortisol levels in children is attributable to changeable, state-like sources, whereas only about 30% is attributable to more stable trait-like sources. In this section, following Shirtcliff, we will distinguish between stable, trait-like influences on cortisol and more transient, state-like influences. Shirtcliff does not specify all the sources of trait and state variablility in cortisol, but an examination of the research literature reveals several factors within each category that have been studied in children.

Stable trait-like influences on cortisol. Several important factors appear to be associated reliably, though perhaps not robustly, with cortisol in a trait-like way. Among the more frequently studied are child temperament and socioeconomic status (SES). Several studies suggest that young children from low-income homes show higher cortisol levels (Chen, Cohen, & Miller, 2010). For instance, Chen and her colleagues found that 9- to 18-year-old children from low-SES homes showed increases in daily cortisol output over a two-year period in comparison to children from high-SES homes. Evidence that SES is causal in this association comes from a study in which families' incomes were supplemented. Children whose families were given income supplementation showed lower cortisol levels after the supplementation compared to children whose families were not supplemented (Fernald & Gunnar, 2009).

It is not clear, however, how long effects of SES on cortisol persist. At least one study found that these differences persist into adulthood in that adults who experienced low SES early in life had higher cortisol output across the day as adults (Miller, Chen, Fok, Walker, Lim, Nicholls, Cole, & Kobor, 2009). Other studies, however, report that differences as a function of SES wash out by the time children transition to high school (Lupien, King, Meaney, & McEwan, 2001).

Moreover, the question of why low SES is associated with higher cortisol among young children remains an open one. Some researchers have speculated that trauma common in low-income families is responsible for the link (Bevans, Cerbone, & Overstreet, 2008), but Flinn (2009), in a 10-year study of children growing up on a Caribbean island, found no connection between trauma and subsequent cortisol levels. An alternative hypothesis is that maternal stress, which covaries with low SES, is the proximal influences on children's cortisol. Essex, Klein, Cho, and Kalin (2002) report that 4.5-year-old children whose mothers were highly stressed had cortisol levels that were significantly higher than 4.5-year-old children whose mothers were only moderately stressed.

Regardless of the reason SES is associated with cortisol levels, it is usually advisable to examine potential associations with children's SES when assessing links between cortisol and children's adjustment. This would be particularly true when examining factors, such as aggression, that are correlated with SES.

Temperament also is thought to be a fairly stable correlate of children's cortisol levels. However, patterns of association are not consistent and can be difficult to discern (Gunnar, Sebanc, Tout, Donzella, & van Duhlmen, 2003) because different contexts elicit different responses as a function of temperament. Two temperament dimensions for which there is considerable evidence for associations with cortisol are shyness – inhibition and anger – frustration proneness.

Shyness is a particularly salient temperament dimension when children are faced with social tasks such as negotiating peer relationships or meeting strangers. Schmidt, Santesso, Schulkin, and Segalowitz (2007) report that mother ratings of shyness were positively correlated with basal cortisol levels during a visit to the research laboratory (during which children presumably met new adults). Even shyness rated in infancy is associated with basal cortisol during middle childhood (Pérez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008).

Proneness to anger also is associated with higher cortisol among children, particularly with cortisol change over the day or cortisol reactivity to a stressor. Toddlers who are rated as more prone to anger displayed higher cortisol reactivity to potentially stressful situations, such as confronting a stranger or a scary robot (van Bakel & Riksen-Walraven, 2004). More anger-prone children also exhibit higher cortisol in the first week of school (de Haan, Gunnar, Tout, Hart, & Stansbury, 1998) and during competitive games when they are losing (Donzella, Gunnar, Krueger, & Alwin, 2000).

As noted previously, evidence for temperament-cortisol associations is far from consistent, and for every study reporting substantial links, others can be identified in which no significant correlations were obtained. One source of the inconsistency is that temperament effects often are qualified by context. For instance, a shy child may show cortisol increases in a novel social situation, such as meeting a stranger, but not when interacting with a familiar adult. Such interaction effects involve both trait-like and state-like factors and will be considered after research on state-like influences on cortisol is summarized.

Transient state-like influences on cortisol. State-like factors known to influence cortisol levels include the social context preceding sampling and any experimental or natural stressor. Stressors, such as performing before an audience and fear- and frustration-inducing activities tend to increase cortisol levels (Gunnar, Talge, & Herrera, 2008). In contrast, interacting with a comforting adult can lower cortisol levels in children (Gunnar, Brodersen, Nachimias, Buss, & Rigatuso, 1996; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002). Moreover, some contexts, such as group child care, seem to be particularly stressful for preschool-age children, but not necessarily for children of other ages.

In experimental studies with children, social-anxiety or fear-producing activities are often used to elicit cortisol responses. For instance, Buske-Kirschbaum, Wustmans, Kirschbaum, and Hellhammer (1997) used a variation of the Trier Stress Test, in which the children were asked to give a speech and perform mental arithmetic in front of an audience who evaluated the child's performance. Children showed a marked increase in cortisol after this activity.

Lopez-Duran, Hajal, Olson, Felt, and Vasquez (2009) exposed children to both a fear-inducing stimulus (a snake that the researcher quickly pulled from a terrarium prior to informing the child that the snake was not real) and a frustration task (an impossible task that was described as easy). The two tasks resulted in approximately equal increases in cortisol.

The presence of an adult with whom the child has a secure attachment relationship, or who is warm and comforting to the child, can lower cortisol, or keep cortisol from rising as much as might be expected. For instance, Gunnar, et al. (1996) suggest that for young children, a secure attachment with their caregiver can serve as a protective factor during stressful situations. They report that 63% of young children who were insecurely attached displayed higher cortisol levels after a 15-month inoculation exam with their mothers present. The adult does not have to be a parent. Lisonbee, Mize, Payne, & Granger (2009) report that children with closes relationships with their teachers experienced lower cortisol after a teacher-child interaction session than did children with less close, more conflictual relationships with their teachers.

Considerable attention has focused on child care as a context that influences children's cortisol levels (Geoffroy, Cote, Parent, & Seguin, 2006). Because in the present study cortisol was measured while children were at child care, literature on cortisol in child care will be considered in some detail. Evidence indicates that the association between child care and cortisol is not due to selection factors, but is probably causal (Geoffroy et al., 2006; Ouellet-Morin, Tremblay, Boivin, Meaney, Kramer, & Sylvana, 2010; Watamura, Donzella, Alwin, & Gunnar, 2003). Comparing the same children at home and at child care, Watamura and colleagues (2003) found that many young children displayed increases in cortisol from morning to afternoon when at child care, whereas the same children did not show these increases, and most showed decreases, in cortisol over the course of the day at home. Similarly, Dettling, Parker, Lane, Sebanc, and Gunnar (2000) found that 55% of children showed increases in cortisol from morning to afternoon at child care, whereas 68% of the children displayed decreasing levels of cortisol over the course of the day at home. Likewise, Ahnert, Gunnar, Lamb, and Barthel (2004) found that during the first two weeks of child care, children's cortisol levels were higher even when their mothers were present. When the children were left alone, their cortisol levels were 75% to 100% higher than levels at home. Furthermore, five months after the children began child care, their cortisol levels were still significantly higher than their home levels, even though they appeared to adapt to the school environment (Ahnert et al., 2004).

Preschool-age children appear to be particularly susceptible to cortisol increases over the day at child care. Dettling, Gunner, and Donzella (1999) found that preschool children's cortisol levels increased from mid-morning to mid-afternoon while at school for over 80% of 3-year olds, for over 60% of 4-year olds, 50% of 5- to 6-year olds, and 30% of 7 to 8 year olds. Preschoolers tended to exhibit greatest increases in levels of cortisol over the course of the day compared to infants or school-aged children (Geoffroy et al., 2006). What is unclear is the cause of this increase in cortisol levels over the course of the day for preschool children at childcare. It has been suggested that negotiating peer relationships is novel and challenging for young children (Gunnar & Donzella, 2002). Infants, in contrast, are not as interactive with peers and so the child care context may be less socially stressful. As for school-aged children, it could be that they are more socially competent and thus less challenged by peer interactions.

Children in poorer quality care also seem especially susceptible to cortisol increases over the day at child care. In a review of research on cortisol and child care, Geoffroy et al. (2006) conclude that children who attend a high-quality child care facility on average display decreasing levels of cortisol over the day, whereas children attending low-quality daycare facilities tend to display increasing levels of cortisol over the course of the day. These findings have been replicated using several different measures of quality. For instance, Legendre (2003) found that children displayed increasing levels of cortisol if their class contained more than 15 children, there was not enough space per child, and if there were more than four adult caregivers in the classroom. Using a more global measure of child-care quality, Sims, Guilfoyle, and Parry (2005) found that children attending a high-quality preschool displayed decreasing levels of cortisol over the course of the day whereas children attending a poor-quality preschool displayed increasing levels of cortisol over the course of the day. However, as will be seen in the following section, the extent to which child care influences cortisol varies as a function of children’s age, temperament, and behavioral profiles (Geoffroy et al., 2006; Gunnar & Cheatham, 2003; Watamura et al., 2003). In fact, although there are undoubtedly some situations that would cause cortisol increases in virtually everyone, most situations invoke somewhat different responses across individuals. The next section, therefore, will consider some of the interactions between state- and trait-level factors and children’s cortisol. Because such potential interactions are almost limitless, only a few illustrative cases will be provided.

Interactions of trait and state influences on cortisol. One case of state-by-trait interactions in predicting cortisol has already been considered: Preschool-age children experience greater increases in cortisol at child care than do younger or older children. Perhaps the most replicated pattern of interactions between state- and trait-level influences on cortisol is that cortisol responses to a given context vary as a function of child temperament. Geoffroy et al. (2006), for instance, summarize several studies indicating that children with more difficult temperaments experience greater increases in cortisol at child care than do more easy-going children. Moreover, seemingly small variations in context can result in large differences in cortisol-temperament links. For instance, and perhaps counterintuitively, some studies have shown that shy children do not show elevated levels of cortisol during the first weeks of school (de Haan et al., 1998; Poggi Davis, Donzella, Krueger, & Gunnar, 1999), but do show higher levels of cortisol later in the year, presumably after they have begun interacting with other children (Stansbury & Harris, 2000). In contrast, outgoing children show cortisol elevations during the first weeks of school (de Haan et al., 1998).

The current study will examine children's cortisol as a predictor of aggression in kindergarten. Because both state-level (changeable factors, such as context) and trait-level (more stable factors such as child age, SES, and temperament) variables influence cortisol, it is important to examine such potential associations in any study of children's cortisol. The next section will consider other issues in measuring cortisol. The variables to be considered in this next section are usually conceptualized as measurement-level factors of no theoretical interest, in contrast to the state- and trait-level influences considered in this section.

Measuring Cortisol

Traditionally, cortisol was measured in blood or urine (Kirschbaum & Hellhammer, 1994). However, recent advances in technology have allowed cortisol to be measured in saliva (Kirschbaum & Hellhammer, 1994; Schwartz, Granger, Susman, Gunnar, & Laird, 1998). These advances have led to a tremendous increase in the number of studies with children that incorporate measures of cortisol. Collecting saliva samples, even with young children, is relatively simple and non-invasive (Salimetrics, 2000) and salivary cortisol correlates highly with serum cortisol (r = .71 to r = .91; Kirschbaum & Hellhammer, 1994). Saliva samples are often obtained by passive drool into a cup, in dental cotton held in the mouth until it is saturated, or with Sorbettes, small absorbent-tipped devises held under the tongue. All of these methods are considered acceptable.

Although collecting saliva samples is relatively simple, it is important to adhere to specific guidelines in order to obtain valid measures of cortisol because a number of factors can introduce variability that would contribute to measurement error if they are not controlled. These are factors that would be of no theoretical interest in most studies of human development, such as acidity of saliva, time of day, and the presence of blood or particulate matter in saliva. In well-done studies, these factors are controlled methodologically, for instance, by collecting saliva samples at the same time every day and not collecting samples immediately after children have eaten, or statistically, for instance, by controlling the time at which saliva was collected. Additional guidelines include insuring that participants are adequately hydrated so they can produce enough saliva to conduct assays, checking to see that saliva samples do not contain blood (because cortisol concentrations are higher in blood) or particulate matter, not scheduling saliva collection immediately after a meal, snack, or nap, and excluding children with recent exposure to nicotine (Salimetrics, 2000).

There is debate about the use of oral stimulants, such as sugar-sweetened cherry Kool-Aid® mix, candy, or gum to increase saliva production. Some researchers report that the use of stimulants can change the pH of saliva and thus bias cortisol assays (Schwartz et al., 1998). For this reason, use of unstimulated saliva for cortisol assays has become more common in recent years.

This section has focused on factors that presumably influence cortisol. It has not dealt with individual characteristics that are associated with cortisol levels but are presumed to reflect outcomes of HPA-axis activity or the effects of third variables that influence both the factor in question and HPA-axis activity. Specifically, and particularly relevant for this study, a number of health and behavioral problems, such as internalizing and externalizing problems, are correlated with cortisol levels, but these problems typically are not thought of as influences on cortisol. Rather, behavior problems are usually conceptualized as consequences of HPA-axis activity or as stemming from one or more third variables common to both the behavior problem and HPA-axis activity. This important research – links between cortisol and behavior problems – will be considered in the final section after a discussion of aggression in preschool children.

Aggression

Persistently aggressive children tend to grow into aggressive adults. It has been estimated that the stability of aggressive behavior approximates that of measures of intelligence (Olweus, 1979). Most longitudinal studies of aggression focus on children of school age or older. For example, in a 22-year-longitudinal study, Huesmann, Eron, Lefkowitz, and Waler (1984) examined behavior in participants from approximately 8-years old to about 30-years old. They found that children who were aggressive at an early age tended to be aggressive in adulthood (*r*s for males and females over the 22-year period were .50 and .35, respectively). Moreover, for both males and females, early aggression predicted subsequent difficulties in multiple domains, including criminalility, physical aggression, and child abuse. Additionally, early aggression predicted spouse abuse and driving violations for males.

Although several decades of research has demonstrated that aggressive problems become stable by middle childhood, until recently, aggression in preschool children was usually considered only an annoyance that children would eventually outgrow (Campbell, 1991). This view was probably based on the facts that virtually all preschool children exhibit at least some aggression and that in most children aggression does, in fact, decrease dramatically by school age (Coie & Dodge, 1998). Aggressive behavior is a characteristic of the human species and is apparent in infants before one year of age (Alink, Mesman, van Zeijl, Stolk, Juffer, Koot, Bakersman-Kranenburg, & van Ijzendoorn, 2006). Developmentally, the frequency of aggressive acts increases up to age 2, but then aggression decreases throughout childhood (Coie & Dodge, 1998).

Recent studies, however, suggest that persistent early aggression is predictive of later externalizing behaviors in childhood (Campbell & Ewing, 1990) and even into adulthood (Asendorpf, Denissen, & van Aken, 2008; Huesmann et al., 1984). This section will focus on studies of young children and the ability of early aggression to forecast subsequent aggression and antisocial behavior.

Campbell et al., (2006) followed children from 24 months through 12 years of age, charting trajectories of aggressive behavior from 24 months to 9 years. The children were rated on aggressiveness by their mothers and the researchers classified the children into five trajectories of physical aggression. Almost half of the children (about 45%) were in a group that showed low rates of aggression up to age 4, then virtually no aggression throughout childhood (the very low-aggression group). A second group (making up about 12% of the sample) showed moderate levels of aggression at age 2 that decreased to virtually no aggression by age 9 (the moderate-decreasing group). Only these two groups whose members became non-aggressive during childhood were functioning well on measures of adjustment at 9 to 12 years of age. All other children showed fairly stable aggression from age 2 to age 9 and displayed at least some adjustment problems across middle childhood. Children with the most significant adjustment problems in middle childhood had high and stable aggression from age 2 to age 9 (the high-stable group); these children made up only 3% of the sample. Teachers reported that children in the high-stable-aggressive group had poorer social skills and higher externalizing problems than children in other groups. Children in this trajectory group also self reported more symptoms of depression and more difficulties with peers. However, even children with less severe aggression showed signs of maladjustment. In middle childhood, children in the moderate-stable-aggression trajectory (15% of the sample) showed more ADHD symptoms, bullying, depression, and risky behavior, and poorer social skills. To the surprise of the investigators, even children in the low-stable-aggression trajectory (26% of the sample) displayed social and behavioral difficulties, including more risk-taking behavior, bullying, loneliness, and ADHD symptoms than children in the never-aggressive group. This study is important for at least two reasons. First, over half of the sample (549 children) showed at least modestly elevated aggression at age 2; of these, over 75% (434) continued to show elevated aggression at age 9. Second, all children who showed elevated aggression throughout childhood (55% of the sample) also displayed evidence of other adjustment problems in middle childhood.

Other studies have reported similar proportions of children who show severe, persistent aggression from toddlerhood to middle childhood. Shaw and his colleagues (Shaw, Gilliom, Ingoldsby, & Nagin, 2003) report that in a sample of low-income boys, 6% showed high, persistent levels of conduct problems from age 2 to age 8. Other trajectory groups also appear to be similar to those identified in the Campbell et al. (2006) study of the NICHD sample, although the proportions of children in each of the trajectory groups differ. Specifically, these authors report that 14% of boys were in a low problem group throughout childhood, with 80% being in one of two groups that showed moderate to high aggression over the whole period. The larger proportion of children with problem behavior in the Shaw study may reflect the higher risk status of the sample.

At least one study followed preschool children into adulthood to chart the course of aggressive behavior problems. Asendorpf and collegues (2008) compared outcomes at age 23 of the most aggressive preschool children (top 15%) with other children in the sample. The most aggressive children in preschool were less likely to have finished high school or have full-time employment, were more likely to have been charged with a criminal offense, and reported having more conflict in close relationships.

The research reported in this section suggests that aggressive behavior in early childhood is a serious issue that warrants attention by parents, educators, researchers, and policy makers. Identifying factors that discriminate young children who will continue to be aggressive from those who will desist should be an important research agenda. In subsequent sections, it will be argued that cortisol levels and cortisol reactivity should be examined as factors that predict future aggression, particularly in combination with level of early aggression.

Measuring Aggression

There are many approaches to assessing aggression in young children. Observations are often considered one of the best measures of social behavior (Nock & Kurtz, 2005). It is argued that an objective measure, such as observations, can assess a broad range of behaviors as they take place in the classroom and reflect an unbiased measurement of child behavior (Nock & Kurtz, 2005).

There also are disadvantages of using observational techniques, however. On a practical level, observations are extremely costly, both in terms of time and money. Perhaps more important, observational measures may not capture events that occur rarely, but have large impacts. For instance, a single act of intense, hostile aggression may carry a lot of weight in influencing perceptions that a child is aggressive, but be missed by an observer. Perhaps because of this, some studies suggest that observational measures are not stable as reports of informants who know the child well, at least for young children (Mesman, Alink, Zeijl, Stok, Bakermans-

Kranenburg, IJzendoorn, Juffer, & Koot, 2008). For these and other reasons (Kazdin, 1978; Skinner, Dittmer, & Howell, 2000), the most commonly used approach to assessing aggression in young children is not observation, but rather relies on judgments of people who know the child well.

The most widely used measure of parent and teacher perceptions of child behavior problems is probably the Child Behavior Checklist (CBCL). A limitation of the CBCL is that it focuses on extreme behavior problems, hyperactivity, conduct problems, violence, and defiance.

A number of other measures that assess aggression, but also assess more positive forms of behavior, such as sharing, have been developed for preschool children. One of the most widely used, the Teacher Checklist of Peer Relationships (TCPR) has the advantage of being brief, in addition to assessing social competence and peer relationships. This measure will be described in some detail because it is used in the current research. (TCPR; Dodge & Somberg, 1987). The TCPR was developed to assess social competence and aggression and has been shown to be highly correlated with the CBCL (r = .72; Dodge & Somberg, 1987).

Cortisol and Aggression

Based on research with older children (McBurnett, et al., 2000; Pajer, Gardner, Rubin, Perel, & Neal, 2001), adults (Gunnar & Vazquez, 2001), and animals (Dettling, Feldon, & Pryce, 2002; Haller, van de Schraaf, & Kruk, 2001; Kruk, Halasz, & Meelis, Haller, 2004) it would be expected that low levels of cortisol would be associated with higher levels of aggression in young children (Brennan & Raine, 1997). Some (but not all) studies also point to lower HPA reactivity among aggressive individuals. Impressively, there is experimental evidence with animals that the association between low cortisol and aggression is causal. In a series of studies with adrenalectomized rats, Haller and colleagues (2001) implanted, under the rats' skin, pellets that released small doses of corticosterone (the primary version of rat glucocorticoids). These rats displayed high levels of vicious aggression toward intruder rats. Specifically, the rats with low levels of corticosterone ferociously attacked the intruders' heads, an atypical behavior aimed at killing the opponent. This was in contrast to the typical response to intruders, which consists of mild attacks on parts of the body that would not result in lethal injuries. As an aside, it is interesting that these researchers describe the adrenalectomized rats as displaying hostile attributional bias.

However, the pattern of cortisol – aggression correlations has not been found consistently, especially with young children. To complicate the matter further, cortisol is measured in many different situations: at home, at child care, in the morning, in the afternoon or evening, as change over the day, as basal (while relaxed or resting), under stress, and as reactivity (change from basal value to the value after a stressor). It is not clear whether the inconsistent pattern in studies of young children reflects an HPA axis that is not yet mature or whether it reflects the wide variation in measurement approaches. In this section, this complex literature will be summarized. Illustrative studies of older children will be presented prior to describing the few studies with preschool children.

Cortisol and aggression among school-age children and adolescents. McBurnett, et al. (2000) examined cortisol and aggression longitudinally in a sample of boys clinically referred for problem behavior. A single cortisol sample was collected on arrival at a clinic in each of years two and four of the study. Substantial, negative correlations were found between cortisol and conduct disorder and aggressive symptoms (especially when the two values of cortisol were averaged). Specifically, for every unit of decrease in logged-transformed values of cortisol, aggressive conduct-disorder symptoms increased by a factor of 2.13, covert conduct-disorder symptoms increased by a factor of 1.44, and oppositional-defiant symptoms increased by a factor of 1.28. Although the cortisol values in this study were treated as basal levels, it is possible they reflected reactivity to the arrival at the clinic. However, the study is important because it suggests that even within the population of seriously aggressive children, those with lower cortisol fare worse.

In a recent study, van Goozen et al. (2000) examined the activity of the HPA axis in children with disruptive behavior disorder (DBD) and normal controls (NC). This was investigated during stressful and non-stressful situations. A total of 52 children between the ages of 8 and 12 participated in the study. Nine saliva samples were collected from the participants during the experiment between 1:00 P.M. and 4:00 P.M. During the non-stress phase, participants spent 30 min completing questionnaires and watching videos. The stress phase involved 80 min of frustration, provocation, and aggression during a competitive session between the participant and a simulated opponent of similar age and sex. Children were subjected to frustration through a timed difficult computer task, while the simulated opponent supposedly watched them. The performance of the participant was criticized in a competitive and derogatory way through standardized provocation by the peer. The participant was then asked to give feedback on the simulated opponent's performance on a difficult task by pressing buttons that would either give a reward signal or an unpleasant white noise. Aggression intensity was indexed by the number of times the participant pressed the button for white noise. The stress phase was followed by a nonstressful condition for 30 min consisting of questionnaires as well as videos and the child was told he or she was the winner of the competition.

On average, the NC and DBD groups had approximately equal initial cortisol levels, and in both groups, cortisol levels decreased prior to the stressors. For the NC group, cortisol increased following the frustration and provocation sessions. As for the DBD group, cortisol levels appeared to remain relatively constant during the stress session. Furthermore, children in the DBD group punished the simulated opponent more severely than did children in the NC group. This study supports the hypothesis that aggressive children tend to have lower HPA reactivity than do nonaggressive children.

Using very similar procedures, Snoek et al. (2004) examined cortisol reactivity among groups of behavior-disordered 7- to 12-year-old children. However, children were classified into one of four groups: Normal Control (NC), Oppositional Defiant Disorder (ODD), Attention-deficit/Hyperactivity Disorder (ADHD), or Oppositional Defiant/Attention Deficit (OD/AD). Snoek and collegues (2004) found no significant differences for cortisol as a function of group levels prior to the stress phase. However, there were significant differences for the groups across time. The NC, ODD, and OD/AD groups displayed declines in cortisol prior to the stressful periods. The ODD and OD/AD group displayed declines in cortisol levels throughout the stress phase, whereas the NC and ADHD groups displayed increasing levels of cortisol. Furthermore, the ODD and OD/AD groups displayed the highest level of aggression toward a simulated opponent. For the purpose of the current study, it is important to note that ODD group displayed decreasing levels of cortisol throughout the stress session and exhibited the highest levels of aggression toward their opponent when presented with the opportunity to do so (Snoek et al., 2004).

There are also studies suggesting that lower basal cortisol levels and high reactive cortisol levels are associated with antisocial behaviors in early adolescence. Kobak and colleagues (2009) examined the relationship between 116 economically disadvantaged adolescent boys and girls and antisocial behaviors. Adolescents' cortisol levels were sampled at three time periods. The first sample was collected 5 minutes after arrival at the laboratory. The second sample was taken after the adolescent's interview and the third was taken at the end of the interactions (20 min after the conflict discussion). A conflict discussion took place between the parent and adolescent to examine their ability to sustain positive conversation about a disagreement.

Kobak and collegues (2009) found that decreased pretask cortisol levels were associated with adolescents' antisocial behavior. When including gender as a moderator, there was a significant main effect for the association between increased cortisol response to the conflict discussion and antisocial behavior.

Only one study of reactivity and aggression conducted with preschool-age children could be located. Blair et al. (2005) explored the relationships among cortisol reactivity and children's cognitive functioning and social behavior. The participants included 169 children who were 4 to 5 years of age and were attending Head Start. There were 80 girls and 89 boys. The children came from families in which the household income was below the poverty line. Two 45-min interviews took place in a quiet testing area with the children either in the morning or in the afternoon at the Head Start Center; the interviews were considered to be mildly stressful. Three saliva samples were collected from about two thirds of the children during one of the sessions, but the time of cortisol collection could not be standardized. Saliva samples were collected at the beginning of the session (Time 1), approximately 20 min later (Time 2), and again about 15 min after that (Time 3). Effects of time of day on cortisol levels were controlled (Blair et al., 2005).

Blair and colleagues (2005) used multivariate analysis predicting teacher-reported aggressive behavior from cortisol at the three time points and found there was a positive relationship between aggressive behavior and cortisol at Time 1, but not at Times 2 and 3. High initial cortisol levels were seen in children with higher levels of teacher-reported aggressive behavior, but cortisol levels of the aggressive children decreased throughout the session. Moreover, children rated as low aggressive by their teachers tended to display low levels of cortisol at Time 1 with increasing levels at Time 2 and a slight decrease at Time 3. This evidence suggests that aggressive preschool children are less reactive to mild stressors, on average, than non-aggressive children. It is possible that aggressive children were involved in more conflicts in the classroom prior to the first saliva collection, resulting in initially higher cortisol levels. Alternatively, it is possible that during preschool, in contrast to the situation at older ages, high arousal (including high cortisol) is associated with greater aggression. In conclusion, the main finding Blair and colleagues (2005) presented was that children with higher levels of teacher-reported aggression also had higher levels of cortisol at Time 1 with decreasing levels of cortisol at Time 2 and Time 3.

Smider et al. (2002) investigated the relationship between afternoon salivary cortisol levels measured at home and children's later socioemotional adjustment. A subsample of children from a large-scale longitudinal study of families, work, and child development participated in this study (*n* = 172). To be included in the analyses children had to provide at least two of the three home cortisol samples. Furthermore, the samples needed to be collected within a 2-week window and all samples had to be collected within a 90-min window across days. All the samples had to be collected in the afternoon or early evening and data from the three adult reporters were necessary to be included. At the age of 4.5, cortisol samples were collected three consecutive days prior to a home assessment. Socioemotional adjustment was reported by mothers, fathers, and kindergarten teachers approximately a year and a half after the home cortisol collection. This took place at the end of kindergarten.

There was an inverse relationship between cortisol levels at age 4.5 and father-reported aggression in boys the next year in kindergarten, but no associations of cortisol with mother or teacher reports. This suggests that boys with low afternoon basal cortisol levels tended to display higher externalizing behaviors, such as aggression, a year later. Though girls' cortisol did not appear to be associated with externalizing behaviors, there was a positive association with internalizing behaviors, as reported by mothers.

Other studies, however, have found no associations between children's cortisol and aggressive behavior. The null findings by Snoek and colleagues (2004) and van Goozen and colleagues (2000) with elementary-age children already have been described. Failure to find cortisol-aggression links also have been reported for samples of young children. Ouellet-Morin et al. (2010), for instance, in a study of 2- to 3-year olds, report that, although children with higher internalizing problems at age 2 had lower cortisol levels, cortisol was not associated with externalizing at age 2 or age 3.

There is obviously considerable inconsistency among these studies. Some studies report positive associations between cortisol and aggression, others report negative associations, and still others find no significant correlations. Similarly, some studies report positive associations between cortisol reactivity and aggression, whereas others report negative associations. Meta-analyses also have noted these inconsistencies in the research literature, but conclude that among preschool children, in contrast to older children, higher cortisol is associated with higher aggression (Alink et al., 2006; Vermeer & van Ijzendoorn, 2006). However, in the only other longitudinal study of preschool cortisol and aggression, lower basal cortisol predicted higher aggression one year later in kindergarten, although these findings pertained only to boys and only to fathers' ratings of externalizing (Smider et al., 2002).

The sources of the inconsistencies among study findings are probably numerous. Sampling times as well as sampling conditions affect cortisol levels. It is sometimes impossible to determine whether a cortisol value reflects basal level or reactivity, as when a single sample is obtained on arrival at a research site or clinic, because, although the studies usually treat these as basal levels, the child could be reacting to the unfamiliarity or the anticipation of the visit. Afternoon cortisol levels reflect a more quiescent period of the circadian cycle of cortisol release compared with morning hours (Smider et al., 2002), and so samples collected in the morning and afternoon may show divergent associations with behavior. A wide variety of stressors have been used in studies of children, many of which fail to produce increases in cortisol, or produce increases in cortisol only at certain ages (Gunnar et al., 2009; Gunnar & Vasquez, 2001). It is possible that responses to some stressors are associated with behavior disorders, whereas responses to other stressors are not. It is easy to imagine that virtually all children would respond with increases in cortisol to stimuli that produced intense fear, whereas responses might be variable to frustration or peer provocation situations. In order to illuminate sources of inconsistency in studies of cortisol and children's behavior, it would be beneficial to examine cortisol in different circumstances and at different times for the same children.

Moreover, it is possible that associations among cortisol and aggression are more complex than examination of simple correlations would reveal. In particular, it is possible that the interaction of cortisol and aggression conspire to predict the child's future development. The fact that McBurnett and colleagues (2000) report that, even in a sample of highly behaviorally disturbed children, cortisol levels contribute to higher levels of problem behavior is consistent with this hypothesis. The prediction also is consistent with the view that multiple risk factors compound the development of problem behaviors (Dodge & Pettit, 2003).

The Present Study

The current study examined links among measures of cortisol collected in child care centers when children were 4 years old and measures of aggression collected the following year from the children's kindergarten teachers. Based on findings of Smider and colleagues (2002) and other studies of adults and older children, it was predicted that children with lower basal levels of cortisol in preschool would be judged as more aggressive kindergarteners. It also was predicted that the interaction of basal cortisol and aggression would contribute to the prediction of kindergarten aggression even after controlling for preschool aggression. Cortisol reactivity measures also were available in the current data set. Based on findings of Blair and colleagues (2005), it was predicted that lower cortisol reactivity in preschool would predict higher aggressiveness in kindergarten. It also was predicted that the interaction of cortisol reactivity and preschool aggression would contribute to the prediction of kindergarten aggression even after controlling for preschool cortisol reactivity and aggression. In these analyses, to control for the diurnal variation in cortisol production, the average length of time since each child had awakened in the morning and saliva collection were statistically controlled.

In this study morning cortisol level collected at preschool serves as the index of basal cortisol. Two measures of cortisol reactivity were examined.

The following hypotheses were examined.

H1: Lower basal cortisol in preschool will be associated with higher teacher-rated aggression in kindergarten.

H2: The interaction of preschool basal cortisol and preschool aggression will predict kindergarten aggression after controlling for preschool aggression and preschool cortisol.

Specifically, children with lower cortisol and higher aggression in preschool will show the highest levels of aggression in kindergarten.

H3: Lower cortisol reactivity in preschool will predict higher aggression in kindergarten.

H4: The interaction of preschool cortisol reactivity and preschool aggression will predict aggression in kindergarten. Specifically, children with lower cortisol reactivity and higher preschool aggression will show the highest levels of aggression in kindergarten.

Figure 1. Hypothesized model: Preschool aggression and morning cortisol predict kindergarten aggression, controlling for wake to morning collection.

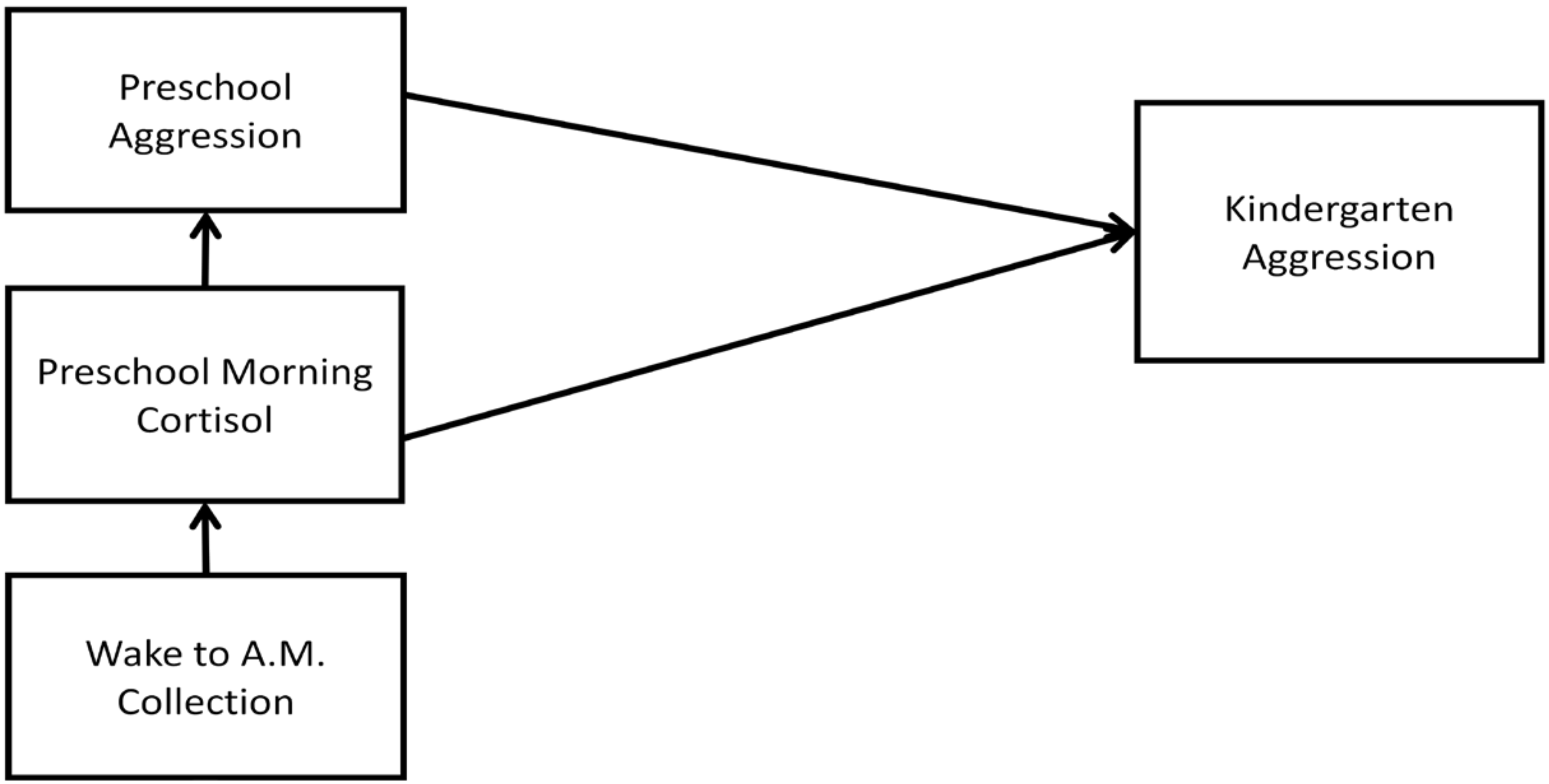


Figure 2. Hypothesized model: Preschool aggression, morning cortisol, and the interaction of preschool aggression and morning cortisol predict kindergarten aggression, controlling for wake to morning saliva collection.

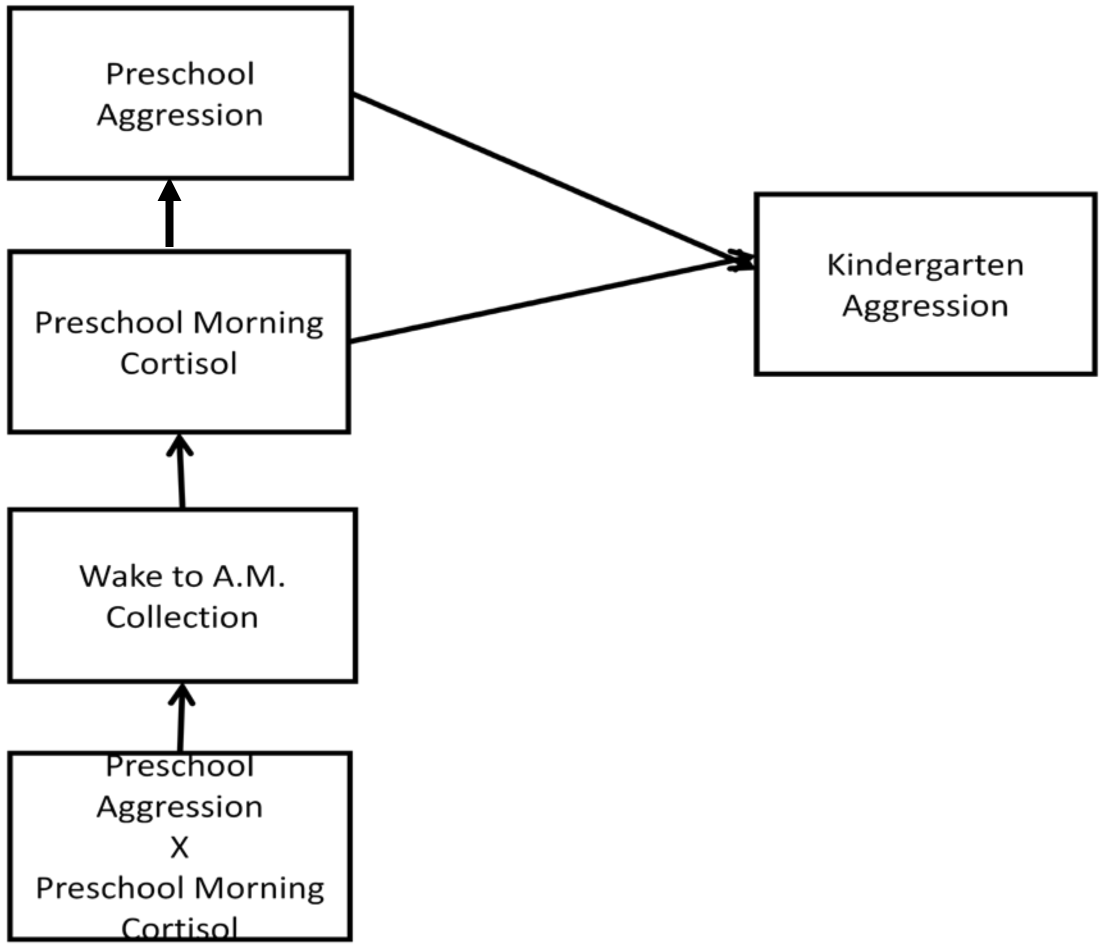


Figure 3. Hypothesized model: Preschool aggression, follow-up cortisol, and post-cortisol predict kindergarten aggression, controlling for pre-cortisol and wake to pre-challenge collection

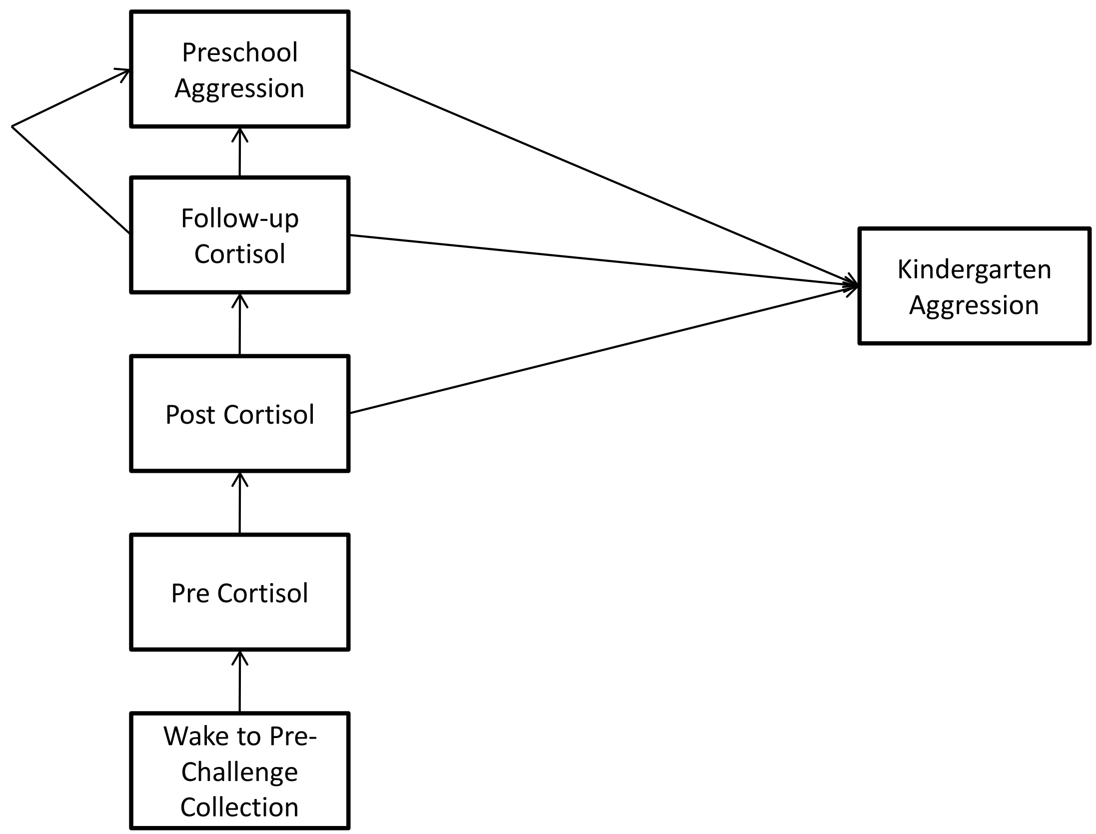
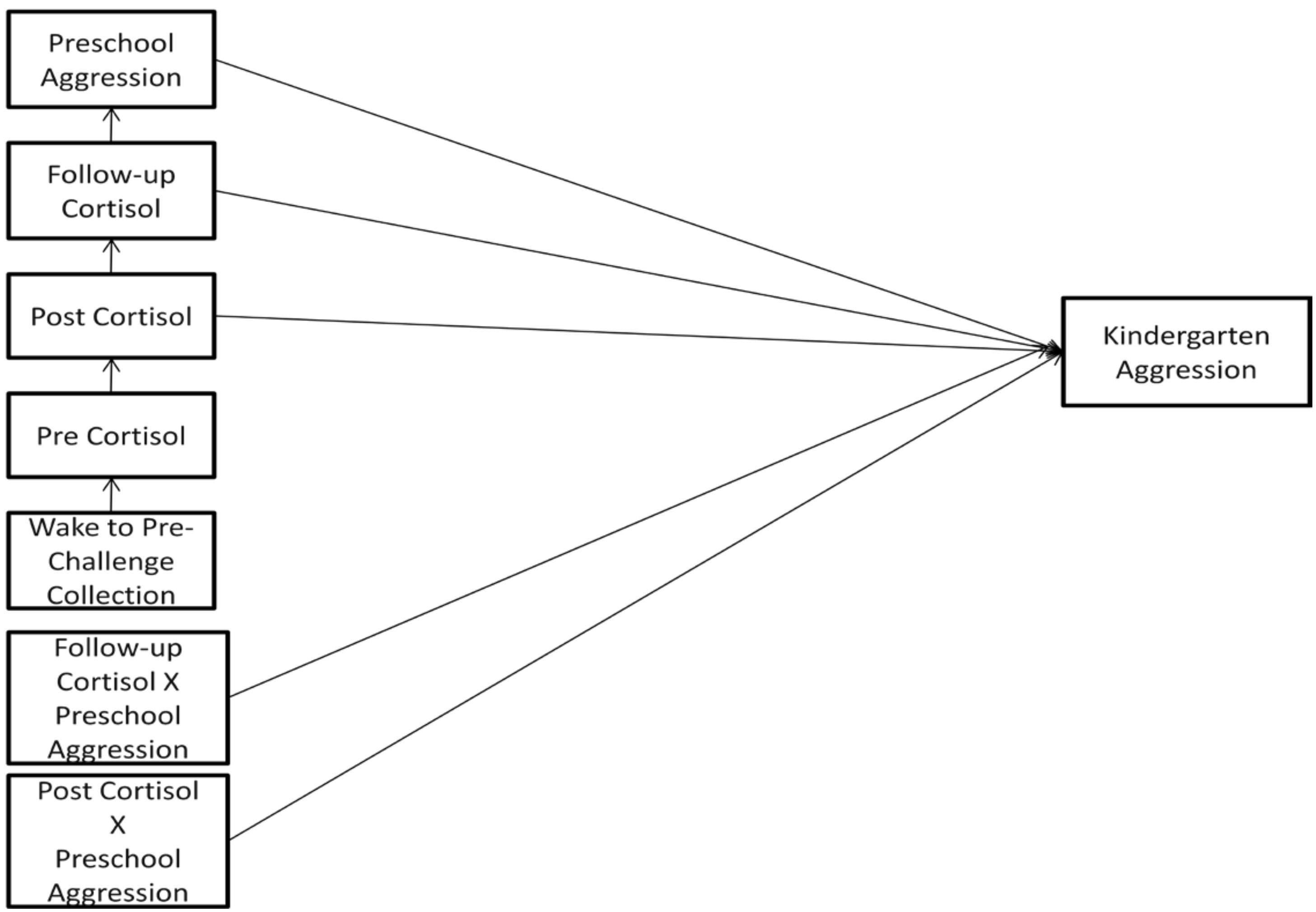


Figure 4. Hypothesized Model: Preschool aggression, follow-up cortisol, post-cortisol, the interaction of follow-up cortisol and preschool aggression, and the interaction of post cortisol and preschool aggression predict kindergarten aggression, controlling for pre-challenge cortisol and wake to pre-challenge cortisol.



III. METHOD

Data for this study were collected from children participating in the Child Care Quality Enhancement Project (CQEP), which was funded by a grant from the National Science Foundation (NSF #0126584 to J. Mize). The CQEP was a short-term longitudinal study that investigated child care and family experience and how both influence the development of social competence. In particular, the main goals of the project were to investigate children's cognitive, social, and physiological experiences in childcare and the relationships of each to competence and adjustment in kindergarten. The data were collected from three cohorts of children in childcare settings. Follow-up data were collected when the participants entered kindergarten. The university Institutional Review Board and the Office of Human Subjects Research approved all procedures for the CQEP study and the secondary data analyses reported here (10-158 EX 1006; Appendix A).

Physiological data are in the form of salivary cortisol obtained from the participating children while at child care. The purpose of this study is to examine associations between children's aggressive behavior and both basal cortisol levels and cortisol change in response to a stimulus, that is, cortisol reactivity. Morning and afternoon samples were collected from the children on the same day on two separate days to measure cortisol levels and daily change. Saliva samples also were collected from children prior to and after a series of mildly stressful activities (challenge task) and again after a teacher-child interaction session; these were used to assess children's reactions to stressful challenges and interacting with their primary teacher, respectively.

Teachers reported on children's aggression in preschool. Similarly, kindergarten teachers reported on children's aggression one year later.

Participants

Twelve centers in the Auburn-Opelika, Alabama area served as sites for this study. Participants were recruited from 4-year-old classrooms in these centers. There were a total of 17 classrooms in Year 1, 16 classrooms in Year 2, and 14 classrooms in Year 3. Informed consent letters were signed by center directors, teachers, and parents of the children who participated in the study. In order to encourage participation, child care centers were offered $5 for every family and child that participated in the study. For classrooms with participation rates greater than 75%, additional monetary incentive was offered. For completing questionnaires about their own background, education, experience, and teaching philosophy, teachers received $20. In addition, teachers received $5 for every videotaped interaction task they participated in with children in their classrooms. Across all classrooms and years, the average rate of participation was 81% (186/229 in Year 1, 167/205 in Year 2, and 154/195 in Year 3). There were no differences as a function of race or sex between the 507 children who received permission to participate and those who did not.

Focus sample. Budget constraints did not allow administration of the challenge task, collection of saliva, and conducting cortisol assays for all 507 children who received parental permission to participate. Thus, in each year, a subset of classrooms was identified in which to collect these more time- and cost-intensive data. The focus sample was comprised of children enrolled in six classrooms during the first year and children in five classrooms during each of the second and third years. This was approximately one-third of the total sample of classes participating each year. Because one purpose of the larger study was to examine physiology and adjustment as a function of child-care quality, a range of high-, medium-, and low-quality classrooms were represented in the focus sample.

From the 16 focus classrooms, 203 children had permission to participate over the three years. However, several children moved, attended part-time, or were unable to produce sufficient saliva to conduct assays. Therefore, 189 children (102 boys; 87 girls) had cortisol data and are included in this study. There were a total of 127 Caucasian, 48 African American, and 14 children of other ethnic or racial backgrounds, mostly Asian. On average, children were 53.22 months of age (range 36 months – 67 months). Procedures recommended by Entwisle and Astone (1994) were used to estimate family socioeconomic status (SES). Children represented a wide range of family SES, from unskilled laborers (25) to professional (93), with a median of 68 (managers). Children in the focus sample differed on age and race from children who had parental permission to participate but were not in the focus sample. Children in the focus sample were slightly older, 53.22 months versus 51.8 months for the focus and non-focus groups, respectively, F (1, 502) = 10.99, $p < .01$), and the focus sample had relatively fewer African American children (25% vs. 38% for the focus and non-focus children, respectively; Π^2 (2) = 9.02, $p < .05$).

Measures

*Aggressive behavior***.** Teachers were given questionnaires to complete about each participating child in preschool and in kindergarten. These questionnaires included a modified version of the Teacher Checklist of Peer Relationship (TCPR; Dodge & Somberg, 1987). The TCCPR is comprised of 17 items rated on 5-point scales ranging from (1) never to (5) always. Ten aggression items (e.g., “starts fights with other children” and “this child says mean things to peers, in teasing or name calling;” items 7 through 17 in the current version; see Appendix B) were used to form composite measures of aggression in preschool and kindergarten (both α = .91), with higher scores reflecting more aggressiveness.

Temperament. Parents completed the short form of the Child Behavior Questionnaire (CBQ; Rothbart, Ahadi, Hershey, & Fisher, 2001) to provide estimates of children's temperamental characteristics. The short form of the CBQ is made up of 94 items rated on 7-point scales. A copy is provided in Appendix C. Scales for shyness and anger-proneness were computed by averaging items in each scale. Shyness was made up of 6 items, including , "Acts shy around people she or he has known for a long time," and "Sometimes seems nervous when talking to adult he or she has just met," and had adequate internal consistency (α = .77). Higher scores reflect greater shyness. Anger also was composed of 6 items, including, "Gets angry when she or he has to go to bed," and "Has temper tantrums when he or she doesn't get what he or she wants," and had adequate internal consistency (α = .76). Higher scores reflect greater tendency to get angry.

Saliva collection, storage, and assay procedures. Cortisol levels in saliva samples were examined to assess children's HPA-axis activity. Children provided saliva samples in their classrooms during the mornings and afternoons of two days and also before, during, and after the challenge task session. Details of these saliva collection contexts will be provided in a subsequent section. Participating children in the focus classrooms were taught to spit into a cup during the fall of each academic year, but these first samples were treated as practice and were not analyzed with the exception of six cases in which the child could not produce enough saliva for the later samples. However, there were no differences in the mean values of the six substituted samples (see Lisonbee, 2004). By practicing, children were able to become more familiar with the procedures used in the study, limiting the potential influence of novelty on cortisol level (see Flinn, 1999) during later saliva collections. For a detailed description of saliva collection procedures see Lisonbee, 2004. The Saliva Collection Protocol (Lisonbee, Mize, Lapp, & Reeves, 2002) is provided in Appendix D.

Morning cortisol, afternoon cortisol, and classroom cortisol change. Using similar saliva collection procedures, baseline samples were collected from the children in their classrooms in the winter (February) and spring (May) to assess morning to afternoon cortisol change. Cortisol collection took place at approximately 10:00 A.M. and 2:00 P.M. on average. Only morning cortisol values will be used in this study; morning cortisol are used as an index of basal cortisol.

Challenge task. Participating children from the focus classrooms were individually brought from their classrooms to a designated area in the center to participate in the challenge task. Approximately 20 min before the challenge task took place, a child was selected from the classroom. The researcher then explained that it was the child's turn to play the games and was given a glass of water to cleanse his or her mouth. No child from the participating classrooms declined participation in this part of the assessment. Challenge task activities were recorded using a digital video recording camera.

After the child was brought into the assessment room, the researcher explained that he or she would be "making a movie" of the child playing games. The researcher then collected a saliva sample from the child. The child participated in the challenge task after the saliva was collected. The purpose of the challenge task was to elicit mild frustration or disappointment and was comprised of five tasks and lasted about 30 min. The tasks included the bear drop game which was a difficult coordination game, a disappointment experience (Cole, Zahn-Waxler & Smith, 1994), an impossible puzzle task (Smiley & Dweck, 1994; see also, Ziegert, Kistner, Castro, & Robertson, 2001), a delay of gratification task (Kochanska & Aksan, 1995; Kochanska, Murray, & Harlen, 2000), and an inhibitory motor activity task (Kochanska et al., 2000). Following completion of the challenge task the child was asked to provide another saliva sample. This sample was intended as an assessment of the change in cortisol after stress, and is referred to as the p*ost-challenge cortisol*. Next, the child's teacher was brought in to the same room in which the child had just completed the challenge task to participate in two non-challenging interaction tasks with the child. First, the teacher was asked to help the child reproduce a pattern with blocks with a 5-min time limit. There was no time-limit for the second activity in which the teacher and child "read" a book with no printed text together (*One Frog Too Many*; Mayer & Mayer, 1975). A third, saliva sample, referred to as *follow-up cortisol*, was collected after the teacher-child interactions. The change in cortisol values from pre-challenge to post-challenge and from post-challenge to followup are referred to as *challenge change cortisol* and *teacher-child interaction cortisol*, respectively. Table 1 summarizes the times and context for saliva sample collection for each cortisol value.

Immediately following sample collection, saliva samples were stored on ice in a cooler and were later taken to a freezer in the research offices and stored until they were shipped for assaying to the Salimetrics Laboratory (College Station, PA). To validate the concentration of the cortisol value, each sample was assayed in duplicate. The mean for each duplicate sample is represented and is expressed as a concentration of micrograms cortisol per deciliter of saliva (µg/dl; for storage, shipping, and assay detail see, Lisonbee, 2004; Lisonbee et al., 2008).

Table 1. Cortisol variable names and contexts and times of assessment

Variable Name	**Context and Time of Saliva Collection**
A.M.(or basal)	Average of winter and spring morning cortisol in classroom

cortisol	
Pre-challenge	Prior to challenge task, outside classroom, in afternoon
Post-challenge	Immediately after challenge task, outside classroom
Follow-up	Immediately after teacher-child interaction, outside classroom
Challenge change	post-challenge Cortisol – pre-challenge cortisol
Teacher-child interaction change	follow-up – post-challenge cortisol

Time since wake-up. Parents reported their children's wake time on a parent "sign-in" sheet on days saliva samples were collected and the minutes children had been awake at the morning saliva collections were computed.

Other potential influences. Children with serious health symptoms such as vomiting or fever and those who had participated in events that could possibly contaminate saliva samples with blood (such as brushing their teeth, visiting the dentist, or suffering a blow to the mouth) did not provide saliva samples. Excessive outliers were removed before analyses because such high values almost always reflect the presence of blood, illness, or drugs containing glucocorticoids, rather than valid cortisol values. Furthermore, Salimetrics technicians examined samples for possible blood contamination as part of the assay procedure.

Children's age, sex, and SES were obtained from parent reports. Group size was computed as the total number of children in each classroom. Control variables with significant associations with the outcome of interest were retained in subsequent analyses.

IV. RESULTS

The results of this study are presented in three sections. First, descriptive statistics for all study variables and results of analyses examining potential control variables will be summarized. The next section will present bivariate associations among cortisol and aggression measures. The third section contains results of path analyses addressing the research questions.

Descriptive statistics and analysis of control variables

Table 2: Univariate statistics for cortisol and aggression variables

Statistic	*N*	Mean	SD	Low value	High value
Kindergarten aggression[1]	128	1.61	.70	1.00	3.91
Preschool aggression[1]	161	2.08	.83	1.00	4.73
Raw morning cortisol [2]	184	.19	.12	.06	.93
Raw pre-challenge task cortisol[2]	182	.16	.11	.04	.87
Raw post-challenge cortisol[2]	183	.12	.09	.03	.72
Raw follow-up cortisol[2]	181	.11	.06	.03	.47

[1] Aggression was rated on scales of 1 to 5; higher scores indicate greater aggressiveness.[2] Raw (untransformed) cortisol values are presented (extremely high values reflecting blood contamination or illness were deleted prior to analyses). Values reflect a concentration of micrograms cortisol per deciliter of saliva (μg/dl).

Table 3: Univariate statistics for possible control variables

Statistic	*N*	Mean (Median)	SD	Low value	High value
Group size	189	(17)	3.41	11	24
Wake to a.m. collection[1]	167	2.41	.72	.20	4.23
Wake to pre-challenge collection[2]	182	2.99	1.13	.33	8.47
Classroom quality[3]	189	.82	.14	.55	.99
SES[4]	144	(68)	21.08	23	93
Angry temperament	152	4.26	1.09	1.67	6.83
Shy temperament	152	3.70	1.33	1	6.17

1. Time since child awoke to morning saliva collection; time is expressed in hours and proportions of hours.

2. Time since child awoke to pre-challenge saliva collection; time is expressed in hours and proportions of hours.

3. Classroom quality is based on NAEYC standards of classroom quality. Values reflect proportion of items scored as fully or partially meets criterion.

4. Higher values reflect higher occupational prestige.

Scatter plots, stem and leaf plots, and box plots were examined. Only cortisol variables showed substantial deviations from normality. As is common practice, extremely high cortisol values that appeared to be the result of child illness or contaminated saliva samples were deleted. One child had consistently high cortisol values, suggesting that the values should be treated as valid, therefore these cortisol values were retained. Because of extreme positive skew, cortisol values were log transformed for all analyses with other variables.

Means, standard deviations, and minimum and maximum values for the cortisol and aggression variables are presented in Table 2 and for the potential control variables in Table 3. As can be seen in Tables 2 and 3, there was considerable variation in all study variables. Examination of variance for the study variables in MPlus confirmed impressions from visual inspection (all *p*s < .001). In preschool, some children were rated as virtually nonaggressive, whereas other children were viewed as highly aggressive. The same was true for children in kindergarten. The time since children awoke to morning saliva collection ranged from less than 30 mins to more than four hours; the time since children awoke to pre-challenge cortisol ranged from less than 30 mins to more than eight hours. Raw cortisol values are presented in Table 2, but log transformed cortisol values are used for all other analyses.

Because a substantial number of children had missing kindergarten data, an attrition analysis was conducted to determine whether children whose kindergarten data were missing differed significantly from children who had kindergarten data. A series of t-tests were conducted for all study variables. Children with kindergarten data were significantly older than children who did not have kindergarten data (*M*s = 53.79 m and 52.03 m, respectively; t (187) = 2.73, $p < .01$). There were no other significant differences between children with kindergarten data and children without kindergarten data. This difference suggests that children without kindergarten data may have not progressed to kindergarten in the second year of the study, even though they had been in 4-year-old class rooms in the first year of the study.

Time since child awoke to saliva collection, group size, NAEYC, SES, race, sex, shy temperament, and angry temperament were examined as predictors of kindergarten aggression.

The only significant association with kindergarten aggression was the time since child awoke to morning cortisol collection ($r = .17$, $p < .05$, one-tailed). Children who had been awake longer before the morning saliva collections had higher levels of cortisol in kindergarten. Therefore, the time since the child awoke to morning saliva collection was controlled in all models using morning cortisol. Although time since child awoke to pre-challenge saliva collection was not associated with kindergarten aggression, for consistency it was controlled in all models using challenge-task cortisol values ($r = .04$, $p < .33$). The other control variables were not considered further.

Bivariate Associations Among Aggression and Cortisol Variables

The bivariate correlations among the main study variables are reported in Table 4. As would be expected, there was a tendency for cortisol values to be significantly inter-correlated and change scores were correlated with the cortisol values used to compute them. Preschool morning cortisol was significantly negatively associated with kindergarten aggression. That is, children with lower morning cortisol in preschool had higher ratings of aggression from kindergarten teachers. There also was a significant negative association between pre-challenge-task cortisol and preschool aggression. This indicates that children with lower cortisol before the challenge task had higher ratings of aggression in preschool. Furthermore, post-challenge task cortisol was significantly negatively correlated with preschool aggression, suggesting that children with lower cortisol levels following the challenge task had higher ratings of aggression in preschool. Follow-up challenge-task cortisol also was marginally significantly associated with kindergarten aggression. That is, children with higher levels of cortisol following the teacher-child interaction task (i.e., at followup) had marginally higher ratings of aggression in kindergarten. However, it should be noted that these are bivariate correlations and do not control for important covariates such as wake time. Path analysis will present a truer picture of associations among study variables.

Table 4. Correlations among main study variables

	2	3	4	5	6	7	8	9	10
1. Morning cortisol	.40**	.27**	.21**	-.14	-.12	-.06	-.22*	-.38**	-.29**
2. Pre-challenge cortisol		.73**	.48**	-.49**	-.42**	-.20*	-.01	-.19*	-.25**
3. Post-challenge cortisol			.77**	.23**	-.42**	-.17*	-.03	-.19*	-.11
4. Follow-up cortisol				.29**	.25**	-.07	.12+	-.11	-.06
5. Cortisol change during challenge					.06	.07	-.04	.03	.20**
6. Cortisol change during teacher-child interaction						.12	.17	.15	.11
7. Preschool aggression							.35**	.03	-.01
8. Kindergarten aggression								.17*	.04
9. Wake to morning									.50

collection	
10. Wake to pre-challenge collection	1.00

[1.] Time since child awoke to morning saliva collection; time is expressed in hours and proportions of hours.

[2.] Time since child awoke to pre-challenge saliva collection; time is expressed in hours and proportions of hours.

** $p \leq .01$ (2-tailed)

*$p \leq .05$ (2-tailed)

$^{+}p \leq .10$ (2-tailed)

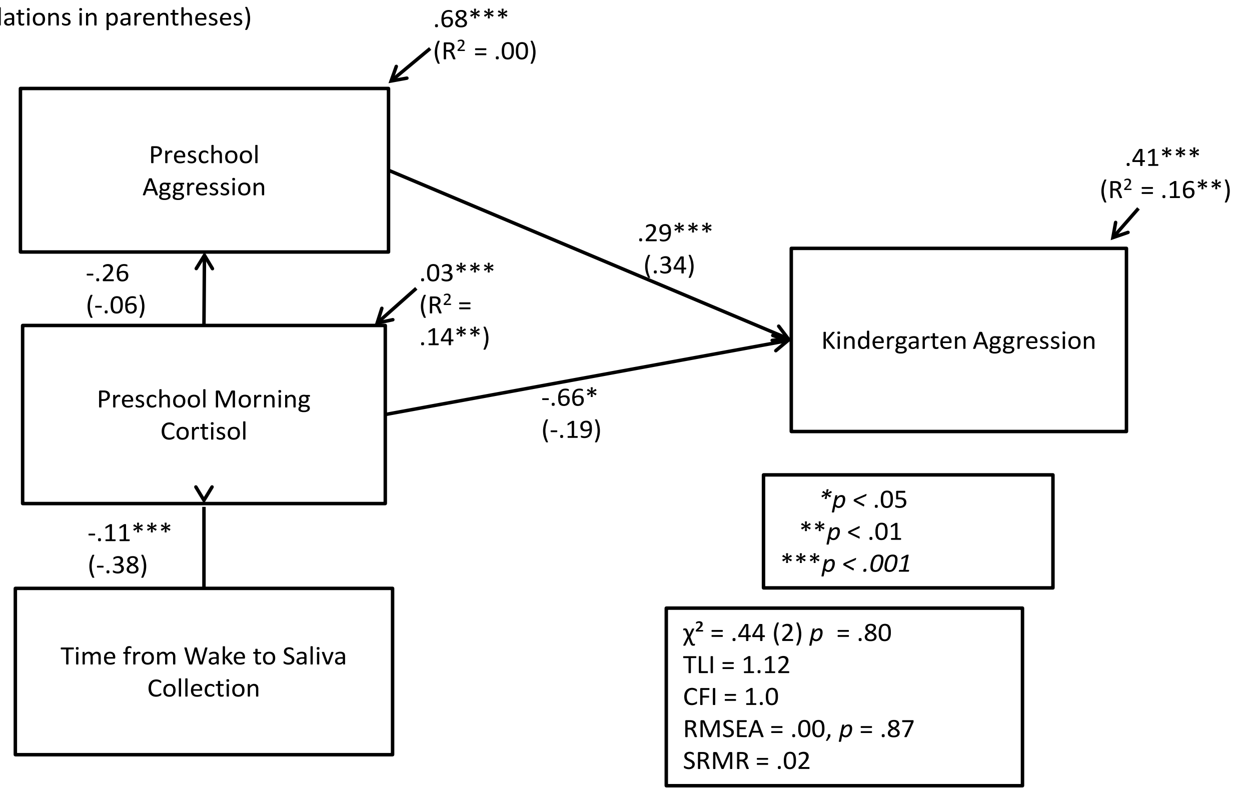
Examination of Study Hypotheses

To examine the hypotheses proposed in the literature review section of this paper, five models were fit using path analysis. Full Information Maximum Likelihood Estimation was used to estimate missing values. The proportion of data present for variables used in path analysis ranged from 68% to 97% with a median of 96%.

The first hypothesis stated that lower morning cortisol in preschool would be associated with higher teacher-rated aggression in kindergarten, controlling for preschool aggression and time from wake to morning cortisol collection. The first model used preschool aggression, morning cortisol, and time from waking to morning saliva collection to predict aggression in kindergarten one year later (Figure 5). The model was a good fit. The chi-square test of model fit was not significant ($\chi^2 = .44$, $df = 2$, $p = .80$). The Tucker Lewis Index (TLI) and the Comparative Fit Index (CFI) demonstrate how much better the model fits compared to a baseline model. A value between .90 and 1 indicates the model is a good fit. For the first model, the TLI was 1.12 and the CFI was 1.0, indicating good fit. Next, the Root Mean Square Error of Approximation (RMSEA) was examined, which tests a null hypothesis that the RMSEA is zero in the population. The RMSEA for this model also indicated that the model was a good fit (RMSEA = .00, $p = .87$). Finally, we examined the Standardized Root Mean Square Residual (SRMR), which should be less than .05 to indicate the model is a good fit. The value for the SRMR was .02, which again suggests the model was a good fit.

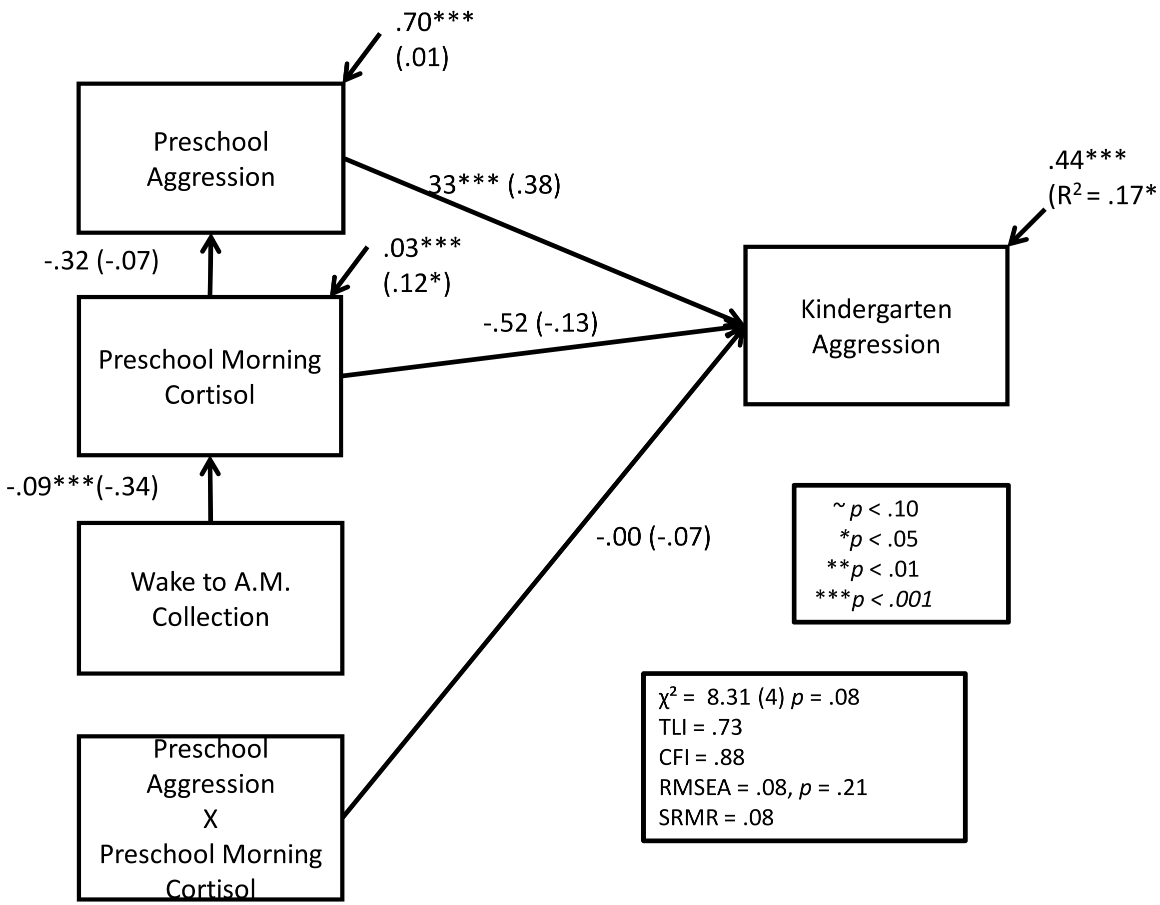
As can be seen from Figure 5, there are several significant paths. In the unstandardized solution, the estimated residual variance of the outcome variable, kindergarten aggression, is .41, and 16% of the variance predicted. The regression of kindergarten aggression on preschool aggression was examined and was statistically significant. That is, children with higher levels of aggression in preschool had higher levels of aggression in kindergarten one year later, controlling for morning cortisol and time from waking to saliva collection. Furthermore, there was a negative association between preschool morning cortisol and kindergarten aggression, controlling for preschool aggression and time from waking to morning saliva collection. This indicates that preschool children with lower levels of cortisol in the morning had higher levels of aggression in kindergarten, even controlling for preschool aggression.

Figure 5. Fitted path diagram (unstandardized solution): The regression of kindergarten aggression on preschool aggression and preschool morning cortisol, controlling for time of morning saliva collection (with estimated correlations in parentheses)



Hypothesis 2 stated that preschool aggression would interact with morning cortisol to predict kindergarten aggression. Specifically, it was expected that children with lower morning cortisol and higher preschool aggression would be more aggressive in kindergarten than children with lower morning cortisol and lower preschool aggression, or children who were high on one of these measures but low on the other. An interaction term was created by multiplying morning cortisol times preschool aggression. A model was fit (Figure 6) in which preschool aggression, morning cortisol, and the aggression by cortisol interaction term were used to predict kindergarten aggression, controlling for time since child awoke to morning saliva collection. The interaction term was not significant, and this model will not be considered further.

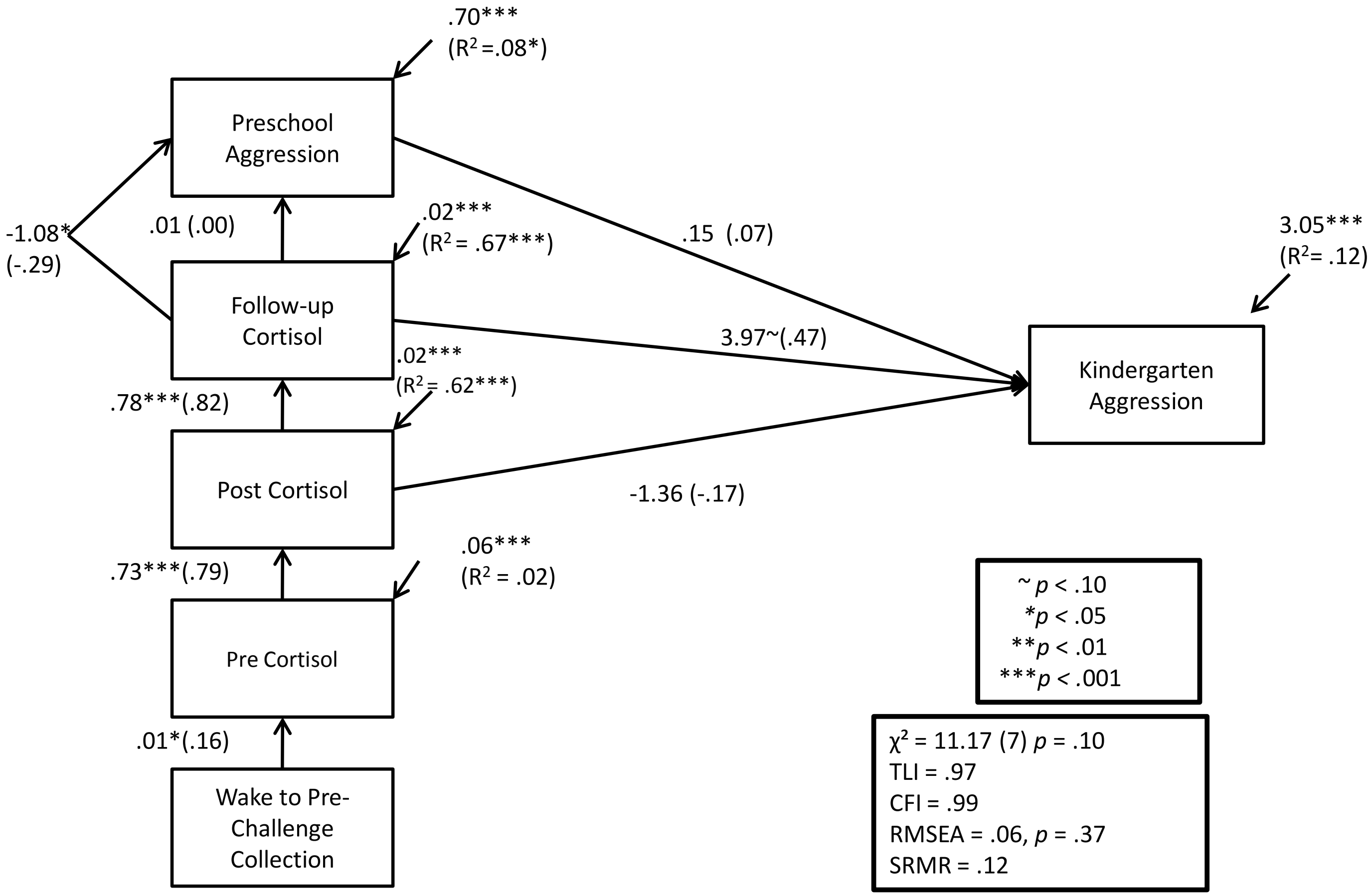
Figure 6. Fitted Path Diagram: The regression of kindergarten aggression on preschool aggression, morning cortisol, and the interaction of preschool aggression, controlling for wake to morning saliva collection.



In the second model (Figure 7), path analysis was conducted examining the associations between kindergarten aggression and follow-up cortisol as well as the association between kindergarten aggression and post-challenge task cortisol, controlling for pre-challenge task cortisol and time since the child awoke. After fitting the model, goodness of fit statistics were examined. The chi-square test indicated the model was a good fit ($\chi^2 = 11.17$, $df = 7$, $p = .10$). The TLI was .97 and the CFI was .99, both indicating the model was a good fit. The estimate for the RMSEA was .06 with a *p*-value of .37 and the SRMR was .12, suggesting this model was a good fit.

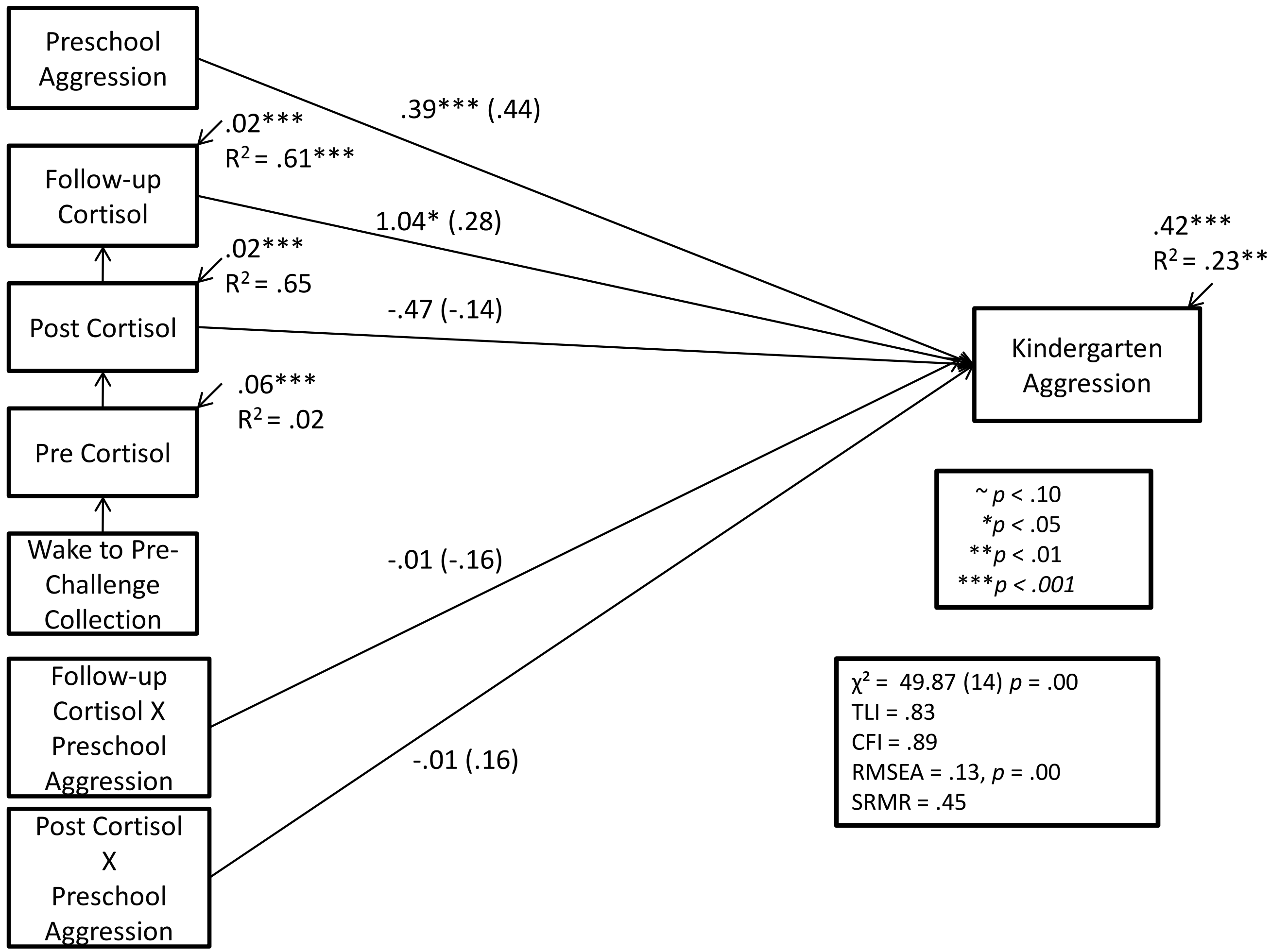
The estimated residual variance of the outcome variable, kindergarten aggression, was 3.05 in the unstandardized solution and 12% of the variance in the outcome was predicted. The path from follow-up cortisol to kindergarten aggression was positive and marginally significant. This indicated that children with higher levels of cortisol following the teacher-child interaction task had higher levels of aggression one year later in kindergarten, controlling for all other variables in the model. The path from post-challenge task cortisol to kindergarten aggression was not significant.

Figure 7. Fitted path diagram (unstandardized solution): The regression of kindergarten aggression on preschool aggression, follow-up cortisol, and post-cortisol, controlling for pre-challenge cortisol and wake to pre-challenge cortisol (with estimated correlations in parentheses)



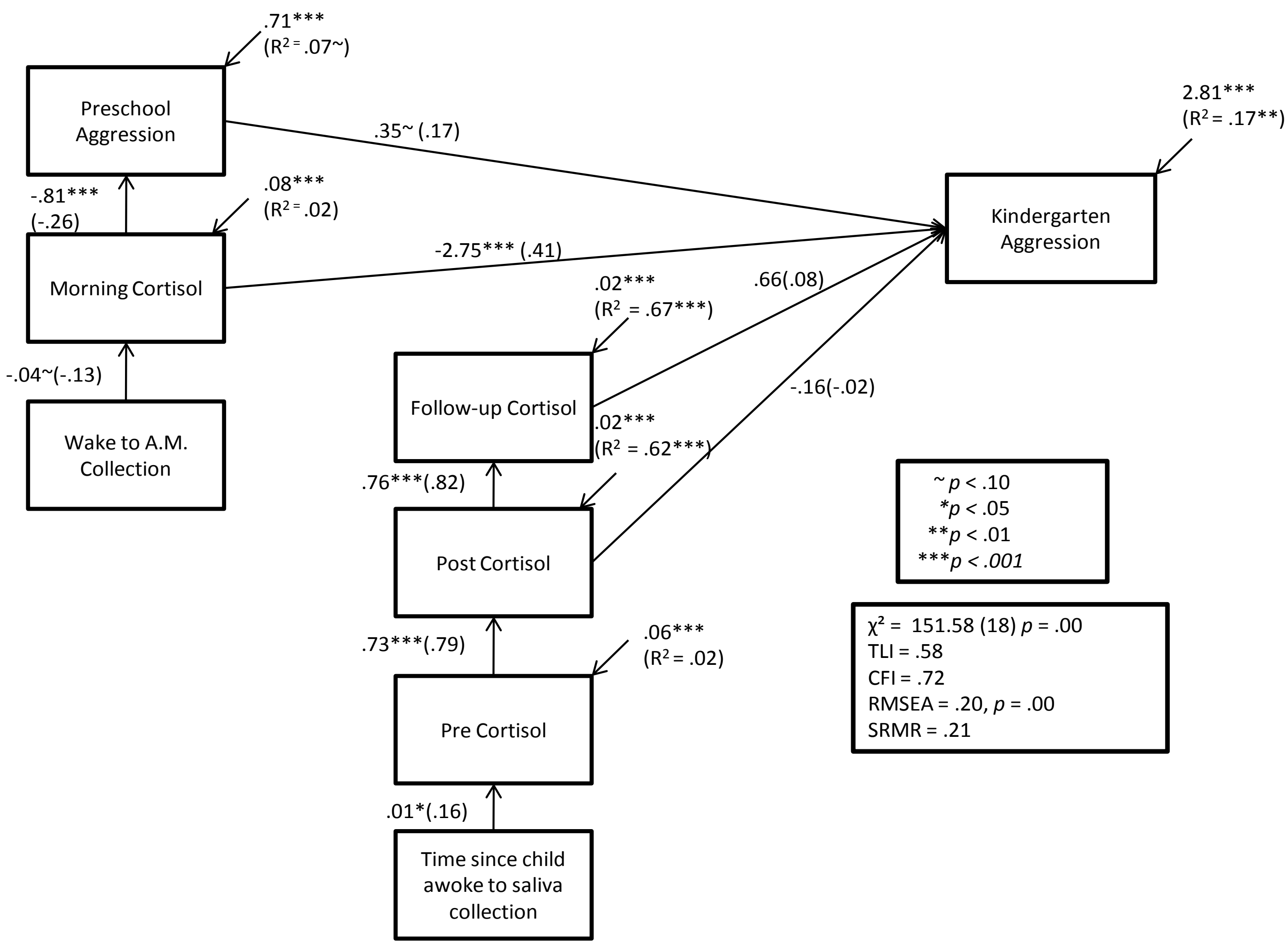
Hypothesis 4 stated that the interaction of reactive cortisol and preschool aggression would predict kindergarten aggression, after controlling for the main effects of reactive cortisol and the other control variables. A model was fit in which (Figure 8) two interaction terms were created by multiplying preschool aggression by post-challenge cortisol and follow-up cortisol. These two terms were added to Model 3. Neither term significantly predicted kindergarten aggression. This model will not be considered further.

Figure 8. Fitted path diagram: The regression of kindergarten aggression on preschool aggression, follow-up cortisol, post-cortisol, the interaction of follow-up cortisol and preschool aggression, and the interaction of post cortisol and preschool aggression predict kindergarten aggression, controlling for pre-challenge cortisol and wake to pre-challenge cortisol.



Because both basal and reactive cortisol had predicted kindergarten aggression in separate models, it was of interest to determine whether both basal and reactive cortisol made independent contributions to the prediction of kindergarten aggression. Thus, in the final fitted model (Figure 9), all paths from Figure 5 and Figure 6 were included. The chi-square test was 151.58 with 18 degrees of freedom and *p*-value of .000. Although the chi-square test was significant, indicating the model might not be a good fit, the ratio of the chi-square to degrees of freedom suggests the model was an adequate fit ($\chi^2/df = 8.42(1)$). The RMSEA also indicated the model was a good fit (RMSEA = .20, $p = .000$). In the unstandardized solution, the residual variance of the outcome variable, kindergarten aggression, was 2.81 ($p = .000$), the predictor variables accounted for 17% of the variance in kindergarten aggression. The path from preschool aggression to kindergarten aggression was statistically significant after controlling for everything else in the model. There was a statistically significant association between preschool morning cortisol and kindergarten aggression, after controlling for all other variables in the model. This implied that children with lower levels of cortisol in the morning were likely to be more aggressive one year later in kindergarten, even after controlling for all else in the model. Neither post-challenge nor follow-up cortisol predicted aggression one year later in kindergarten, after controlling for all other variables in the model.

Figure 9. Fitted path diagram (unstandardized solution): The regression of kindergarten aggression on preschool aggression, preschool morning cortisol, follow-up cortisol, and post challenge task cortisol, controlling for pre-challenge cortisol, wake to A.M. collection and wake to pre-challenge collection (with estimated correlations in parentheses)



IV. DISCUSSION

This study is the first to examine both basal and reactive cortisol in preschool as predictors of later aggression. Preschool children who had lower basal HPA axis functioning, as indexed by cortisol measured in the morning at childcare, were more aggressive at preschool and also showed increases in aggression from preschool to kindergarten. Results for associations between reactive cortisol and aggression are more complex. Children who were less reactive to a series of frustrating tasks were more aggressive in preschool. In contrast, children who experienced greater neuroendocrine responses to interactions with their teacher were marginally more aggressive in kindergarten. This study goes some way toward resolving the controversy regarding whether and how reactive cortisol is associated with aggression by suggesting that the nature of the stressor is critically important: Aggressive children may react more than nonaggressive children in some situations, but less than nonaggressive children in other situations. However, when both basal and reactive cortisol values were included in the same model, only morning cortisol predicted increases in aggression one year later in kindergarten, suggesting that, among preschool children, basal HPA axis function may be a more robust correlate of aggression than is HPA axis reactivity.

The longitudinal data are consistent with most of the evidence on basal cortisol and aggression in children and adults in showing that lower basal cortisol is associated with higher aggression (Kobac et al., 2009; McBurnett et al. 2000; Smider et al., 2002). However, this study contributes to the literature because it is the first to document links between low cortisol and later aggression in preschoolers while controlling for earlier aggression. This suggests that the link between basal cortisol and aggression emerges early in development and is fairly robust. Together, basal cortisol and preschool aggression predicted 16% of the variance in kindergarten aggression a year later.

Somewhat paradoxically, basal cortisol was not associated with aggression measured concurrently at age four. It is not clear why this is the case, particularly given that a handful of previous studies showing such a link. Perhaps preschool teachers are less accurate at rating aggression than are kindergarten teachers; this would have made it more difficult to detect associations with preschool aggression than with kindergarten aggression. However, the reliability estimates do not support this interpretation. It also is possible that basal cortisol in preschool serves as a marker for risk factors that predispose children to developing aggressive behaviors later. For instance, low basal cortisol could indicate the existence of subtle abnormalities in information processing or sensitivity to environmental contingencies. Precedence for the identification of child factors that predict future aggression problems can be found in studies of temperament. For example, disregulated temperament in children at age two predicts more aggressive behavior toward peers months later (Rubin, Hastings, Chen, Stewart & McNichol, 1998).

The question of why low autonomic arousal is associated with aggression and other antisocial behavior problems is unresolved. A number of theories have been proposed. The stimulation-seeking hypothesis states that individuals with low autonomic arousal find this physiological state uncomfortable and engage in risk-taking behaviors in order to raise their arousal (Zuckerman, 1979). A related notion is that individuals with low autonomic arousal and low autonomic reactivity are relatively fearless and so are not constrained by thoughts of potential negative consequences of antisocial behavior (Raine, 1993).

More recent models are grounded in cognitive neuroscience and posit neural and information processing mechanisms as explanations of the low arousal – aggression link. One intriguing model suggests that low basal arousal is a marker of callous, unemotional traits and insensitivity to punishment (poor avoidance learning), including the distress cues of victims (Blaire, 2003). This model is consistent with data showing that low cortisol is characteristic of psychopathic adults (van Goozen et al., 2007) and children with psychopathic traits (Loney et al., 2006) and with evidence from animal studies that sufficient cortisol is necessary for appropriate development of amygdala responsiveness and avoidance learning (Dadds & Rhodes, 2008; Moriceau & Sullivan, 2005). According to this model, low autonomic arousal should be associated only with "cold," proactive aggression (also called predatory or instrumental aggression) but not with "hot," reactive, aggression (Blaire, 1993). However, findings of differential associations between cortisol and proactive versus reactive aggression are mixed (Kempes, de Vries, Matthys, van Engeland, & van Hoof, 2008; Loney et al., 2006; Lopez-Duran, Olson, Hajal, Felt, & Vazquez, 2009). Thus, the low basal cortisol – aggression link remains a common finding in developmental research that is still in search of an explanation.

Results for reactive cortisol were less straightforward than the results for basal cortisol, partly because different associations were found for the two measures of cortisol reactivity. Yet this complexity lends strength to this study. Results of previous studies examining cortisol reactivity and aggression have been very inconsistent. Mirroring this inconsistency across studies, in this study, associations between reactivity and aggression differed for the two reactivity situations (the challenge task and teacher-child interaction) and cortisol changes over the two situations were not correlated. This suggests that the two situations elicited responses from different children and children who reacted to one did not necessarily react to the other. It is helpful to consider the nature of the challenge tasks and teacher-child interaction to make sense of this pattern. The challenge tasks used in the current study were designed to elicit disappointment (at receiving an unwanted object rather than a desired toy as a prize), frustration (at being required to wait for a prize and attempting a nearly impossible task), and frustration or embarrassment (being unable to complete a timed task while an adult observed). The activities during teacher-child interaction were not intended to be stressful, but apparently, for some children, interacting with the teacher was itself a stressor.

In a model examining only reactive cortisol, children with lower reactivity to the frustrating challenges were more aggressive in preschool. In contrast, children who reacted more to interacting with their teacher were marginally more aggressive in kindergarten. These data may help resolve some of the conflicting evidence regarding physiological reactivity and aggression. Some previous studies report that children with greater levels of physiological reactivity are more aggressive (Kobac et al., 2009), whereas others report that children with lower levels of physiological reactivity are more aggressive (Blair et al., 2005). If the challenge tasks are viewed as a series of punishing events, the current data may be consistent with a body of research showing that some children are relatively unperturbed by socialization efforts that involve application of negative contingencies and could, therefore, be difficult to socialize (Kochanska, 1997). Perhaps children in preschool who show low cortisol reactivity to disappointment and frustration are less responsive to aversive stimuli (which could include events in the challenge task as well as adults' socialization efforts) and therefore fail to learn that aggression is usually counterproductive. Indeed, temperamentally fearless infants and children tend to have low resting heart rates (Kagan, 1994; Scarpa, Raine, Venables, Mednick, 1997) and lack of fear is hypothesized to undermine socialization efforts by contributing to poor fear conditioning. Kochanska (1997) describes fearless children as those who are relatively unperturbed by adult socialization efforts and thus at risk for developing antisocial behavior. Children who are less responsive to socialization efforts may become more aggressive with the added pressures of kindergarten.

In contrast to the situation with the challenge task, children who had more intense reactivity to interacting with their classroom teachers were marginally more aggressive in kindergarten but were not more aggressive in preschool. Children on trajectories of increasing aggression may have felt uncomfortable with their teachers and so experienced greater cortisol reactivity. Alternatively, children with less positive relationships with teachers may have become more aggressive over time, particularly in kindergarten. In fact, previous analyses with this dataset indicate that children with more conflictual relationships with their preschool teachers had greater increases in cortisol across the teacher-child interaction task (Lisonbee et al., 2008).

When both basal and reactive cortisol were included in the same model, reactive cortisol no longer predicted kindergarten aggression, but the association between morning cortisol and kindergarten aggression remained highly significant. This suggests that low basal cortisol is a more robust predictor of later aggression, at least in preschool children.

We had expected that the interaction of preschool aggression and cortisol would predict aggression in kindergarten. However, there was no support for this in either the basal cortisol-by-aggression or the reactive-cortisol-by-aggression models. Interactions were expected based on arguments that low resting cortisol levels are characteristic only of severe and persistent conduct disorder (Loney et al., 2006). It is possible that in preschool-age children, low cortisol is a general marker of aggression, rather than being unique to the most severe cases. On the other hand, lack of evidence for an interaction between aggression and cortisol may reflect some peculiarity of this study. Future studies should examine this hypothesis further because, if support for such an interaction is found, it could aid in developing more targeted interventions.

The sample used in this study was a community based sample of normally developing preschool children enrolled in community child care programs. This is important because many studies examining cortisol and aggression use clinically referred adolescents or adults. In models with basal cortisol, there was no interaction between preschool aggression and cortisol, however, there was an additive effect. These findings suggest that biological markers such as cortisol may be a useful tool in identifying children at risk for the development of behavior problems at an early age. The fact that this study was able to obtain cortisol measures from a relatively large number of children in groups at childcare suggests that screening with cortisol may be practical. Moreover, obtaining saliva samples in fairly standardized contexts offers advantages over collection at home, in physician's offices, or in laboratory settings. In this study, researchers spent sufficient time in each classroom so that children were comfortable with them and practice sessions of saliva collection were conducted before the collections that were actually used in the study. Therefore, the situation should have been familiar and comfortable, rather than novel. In addition, in this study, saliva was not collected from children who recently had experienced some emotionally arousing event (e.g., falling down, scolding from teacher). These steps offer greater assurance that morning cortisol levels actually reflected basal activity and not reactivity to a novel or stressful event.

It is not clear why SES was not associated with aggression in this study. It is possible that the sample did not contain a sufficient number of very low-income, at-risk families, or that the measure of SES used in this study was not sensitive to family risk factors. The measure used in this study (Entwisle & Astone, 1994) relied solely on occupational prestige as determined by the U.S. Census Bureau. Occupational prestige may be a less sensitive reflection of children's actual living conditions that mediate the link between SES and child adjustment (McLoyd, 1998; Mistry et al., 2002) than are more common indices of SES, such as family income and income-to-needs ratio, housing conditions, and parental education.

Limitations

A number of factors limit conclusions that can be drawn from this study. Perhaps most obviously, nothing is known about children's HPA axis functioning during kindergarten. Many of the kindergartens that children attended in the second year of the study objected to children spitting while at school, and so these data are not available. Having cortisol measures in kindergarten might shed even more light on the role of cortisol in the development of behavior problems.

Having more measures of cortisol in preschool also would have strengthened the study. Most of the variance in cortisol is determined by the immediate context or recent events, rather than by stable individual differences (Shirtcliff et al., 2005). Having more samples allows for a more reliable assessment of individual differences. The high cost of salivary assays limited the number of assays that could be conducted, however.

Although the challenge tasks were designed to provoke a mild stress response, an examination of the mean values of cortisol before and after the challenge task suggests that for most children, the tasks were not particularly stressful. In future research, it would be useful to employ tasks that are universally (or near universally) stressful to preschool children. Whether such tasks exist, however, is not clear. In fact, numerous studies have come to similar conclusions – most efforts to stress children in research studies do not, in fact, produce elevations in cortisol (Gunnar, Talge, & Herrara, 2009). Moreover, even for children who did experience cortisol increases during the challenging tasks or during teacher-child interactions, it is not clear which aspects of the situations may have prompted stress. Future research should examine children's responses to discrete stressors, rather than to a series of stressors.

Future Directions

This study was not designed to answer questions about the origins of individual differences in cortisol, but it is useful to consider what might have led to the observed differences among children. Research to date does not provide a definitive answer. Low autonomic arousal as indexed by heart rate is heritable (Bloomsma & Plomin, 1986) and children of criminals tend to have low resting heart rate, supporting genetic influences (Farrington, 1987; Venables, 1987). There also appears to be some genetic basis to HPA axis functioning (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003). However, animal and human studies document experiential influences on individual differences in HPA axis functioning, especially early in development, and there has been a special interest in the role of adversity in shaping children's HPA axis functioning. The specific effects of childhood adversity on the HPA axis are far from clear or consistent, however (Gustafsson, Anckarsäter, Lichtenstein, Nelson, & Gustafsson, 2010). Although most models of the stress system and a number of studies (Lupien, King, Meany, & McEwen, 2000; Lupien et al., 2001; Tarullo & Gunnar, 2006) suggest that childhood adversity is associated with higher cortisol levels and greater stress reactivity, emerging evidence indicates that children reared in adverse conditions also can show hypocorticolism and a flattened diurnal pattern of cortisol during childhood (Gunnar & Vazquez, 2001) and adulthood (Miller et al., 2009). The fact that, in this study, children who woke earlier had lower morning cortisol values probably largely reflects the normal diurnal pattern of cortisol secretion (Sapolsky, 2004). However, particularly because children who had been awake longer at morning saliva collection were more aggressive in kindergarten, it also is possible that arising very early (or conditions associated with arising very early, such as parents working at low status jobs) influences daily cortisol patterns in children. Adding to evidence that HPA axis functioning is influenced by experiences, recent studies indicate that patterns of HPA axis functioning can be altered through early intervention (Fernald & Gunnar, 2009; Fisher, Stoolmiller, Gunnar, & Burraston, 2007; Raine et al., 2001). Particularly relevant for the topic here, changes in children's cortisol can mediate the effects of a family intervention on reductions in aggressive behavior (O'Neal et al., 2010). Thus, understanding mechanisms that link early experience, cortisol, and behavior problems could be important for the development of interventions to reduce aggression and violence.

REFERENCES

Ahnert, L., Gunnar, M.R., Lamb, M.E., & Barthel, M. (2004). Transition to child care: Associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75*, 639-650.

Alink, L.R.A., Mesman, J., van Zeijl, J., Stolk, M.N., Juffer, F., Koot, H.M., & Bakermans-Kranenburg, M.J., & van IJzendoorn, M.H. (2006). The early childhood aggression curve: Development of physical aggression in 10- to 50-month-old children. *Child Development, 77*, 954-966.

Asendorpf, J.B., Denissen, J.J.A., & van Aken, M.A.G. (2008). Inhibited and aggressive preschool children at 23 years of age: Personality and transitions into adulthood. *Developmental Psychology, 44*, 997-1011.

Bartels, M., de Geus, E., Kirschbaum, C., Sluyter, F., & Boomsma, D. (2003). Heritability of daytime cortisol levels in children. *Behavior Genetics*, *33*, 421-433.

Bevans, K., Cerbone, A., & Overstreet, S. (2008). Relations between recurrent trauma exposure and recent life stress and salivary cortisol among children. *Development and Psychopathology, 20,* 257-272.

Blair, C., Granger, D., & Razza, R.P. (2005). Cortisol reactivity is positively related to executive function in preschool children attending head start. *Child Development, 76,* 554-567.

Brennan, P., & Raine, A. (1997). Biosocial bases of antisocial behavior: Psychophysiological, neurological, and cognitive factors. *Clinical Psychology Review*, *17*, 589– 604.

Buske-Kirschbaum, A., Jobst, S., Psych, D., Wustmans, A., Kirschbaum, C., Rauh, W., & Hellhammer, D. (1997). Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosomatic Medicine, 59,* 419-426.

Campbell, S.B. (1991). Longitudinal studies of active and aggressive: Individual differences in early behavior and in outcome. In D. Cicchetti & S.L. Toth (Eds.), *Internalizing and externalizing expressions of dysfunction* (pp. 57-90). Hillsdale, New Jersey: Lawrence Erlbaum Associates, Inc.

Campbell, S.B. & Ewing, L.J. (1990). Follow-up of hard-to-manage preschoolers: Adjustment at age 9 and predictors of continuing symptoms. *Journal of Child Psychology and Psychiatry, 31*, 871-889.

Campbell, S.B., Shaw, D.S., & Gilliom, M. (2000). Early externalizing behavior problems: Toddlers and preschoolers at risk for later maladjustment. *Development and Psychopathology, 12,* 467-488.

Campbell, S.B., Spieker, S., Burchinal, M., Poe, M.D., & The NICHD Early Child Care Research Network. (2006). Trajectories of aggression from toddlerhood to age 9 predict academic and social functioning through age 12. *Journal Child Psychology and Psychiatry, 47,* 791-600.

Campbell, S.B., Spieker, S., Vandergrift, N., Belsky, J., Burchinal, M., & the NICHD Early Child Care Research Network. (2010). Predictors and sequelae of trajectories of physical aggression in school-age boys and girls. *Development and Psychopathology, 22,* 133-150.

Chen, E., Cohen, S., & Miller, G.E. (2010). How low socioeconomic status affects 2-year hormonal trajectories in children. *Psychological Science, 21,* 31-37.

Coie, J.D., & Dodge, K.A. (1998). Aggression and antisocial behavior. In W. Damon &N. Eisenberg (Eds.), *Handbook of child psychology: Vol. 3: Social, emotional, and personality development* (6th ed. (pp. 779–862). Toronto: Wiley.

Cole, P. M., Zahn-Waxler, C., & Smith, K. D. (1994). Expressive control during a disappointment: Variations related to preschoolers' behavior problems. *Developmental Psychology, 30,* 835 – 846.

de Haan, M., Gunnar, M.R., Tout, K., Hart, J., & Stansbury, K. (1998). Familiar and novel contexts yield different associations between cortisol and behavior among 2-year-old children. *Developmental Psychobiology, 33,* 93-101.

Dettling, A.C., Feldon, J., Pryce, C.R. (2002). Early deprivation and behavioral and physiological responses to social separation/novelty in the marmoset. *Pharmacology,73,* 259-269.

Dettling, A.C., Gunnar, M.R., & Donzella, B. (1999). Cortisol levels of young children in full-day childcare centers: relations with age and temperament. *Psychoneuroendocrinology, 24,* 519-536.

Dodge, K.A., Coie, J., & Lyman, D.R. (2006). Aggression and antisocial behavior in youth. In W.Damon (Ed.), *Handbook of child psychology, Vol. 3: Social, emotional, and personality development* (6th ed., pp. 719-788). New York: Wiley.

Dodge, K.A. & Pettit, G.S. (2003). A biopsychosocial model of the development of chronic conduct problems in adolescence. *Developmental Psychology, 39,* 349-371.

Dodge, K.A., & Sherrill, M.R.(2007). The interaction of nature and nurture in antisocial behavior. In D.J. Flannery, A.T. Vazsonyi, & I.D. Waldman (Eds.), *The Cambridge* *handbook of violent behavior and aggression* (pp. 215-242). New York: Cambridge University Press.

Dodge, K.A., & Somberg, D.R. (1987). Hostile attributional biases among aggressive boys are exacerbated under conditions of threats to the self. *Child Development, 58,* 213-224.

Donzella, B., Gunnar, M.R., Krueger, W.K., & Alwin, J. (2000). Cortisol and vagal tone responses to competitive challenge in preschoolers: Associations with temperament. *Developmental Psychobiology, 37,* 209-220.

Entwisle, D. R., & Astone, N. M. (1994). Some practical guidelines for measuring youth's race/ethnicity and socioeconomic status. *Child Development, 65,* 1521 – 1540.

Eisenberg, N., Fabes, R.A., & Spinrad, T.L. (2006). Prosocial development. In N. Eisenberg, W. Damon, & R.M., Lerner (Eds.), *Handbook of child psychology: Vol. 3, social, emotional, and personality development* (pp. 646-718). Hoboken, NJ: John Wiley & Sons, Inc.

Essex, M.J., Klein, M.H., Cho,E., & Kalin, N.H. (2002). Maternal stress beginning in infancy may sensitize children to later stress exposure: Effects on cortisol and behavior. *Society of Biological Psychiatry, 52,* 776-784.

Farrington, D.P. (2007). Origins of violent behavior over the life span. In D.J. Flannery, A.T. Vazsonyi, & I.D. Waldman (Eds.), *The Cambridge handbook of violent behavior and aggression* (pp. 19-49). New York: Cambridge University Press.

Fernald, L.C.H., & Gunnar, M.R. (2009). Poverty-alleviation program anticipation and salivary cortisol in very low-income children. *Social Science & Medicine, 68,* 2180-2189.

Fisher, P., Stoolmiller, M., Gunnar, M., & Burraston, B. (2007). Effects of a therapeutic intervention for foster preschoolers on diurnal cortisol activity. *Psychoneuroendocrinology, 32*, 892-905.

Flinn, M. V. (1999). Family environment, stress, and health during childhood. In C. Panter-Brick & C. M. Worthman (Eds.), *Hormones, health, and behavior* (pp. 105-138). Cambridge, UK: Cambridge University Press.

Flinn, M.V. (2009). Are cortisol profiles a stable trait during child development? *American Journal of Human Biology, 21,* 769-771.

Geoffroy, M.C., Cote, S.M., Parent, S., & Sequin, J.R. (2006). Datcare attendance, stress, and mental health. *Canadian Journal of Psychiatry, 51,* 607-612.

Gunnar, M.R., Brodersen, L., Nachmias, M., Buss, K., Rigatuso, J. (1996). Stress reactivity and attachment security. *Developmental Psychobiology, 29,* 191-204.

Gunnar, M.R. & Cheatham, C.L. (2003). Brain and behavior interface: Stress and the developing brain. *Infant Mental Health Journal, 24*, 195-211.

Gunnar, M.R. & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27,* 199-220.

Gunnar, M.R., Sebanc, A.M., Tout, K., Donzella, B., van Dulmen, M.M.H. (2003). Peer rejection, temperament, and cortisol activity in preschoolers. *Developmental Psychobiology, 43,* 346-358.

Gunnar, M.R., Talge, N.M., & Herrera, A. (2009). Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology, 34,* 953-967.

Gunnar, M.R. & Vasquez, D.M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology, 13,* 515-538.

Gustafsson, P., Anckarsäter, H., Lichtenstein, P., Nelson, N., & Gustafsson, P. (2010). Does quantity have a quality all its own? Cumulative adversity and up- and down-regulation of circadian salivary cortisol levels in healthy children. *Psychoneuroendocrinology, 35,* 1410-1415.

Haller, J., van de Schraaf, J., Kruk, M.R. (2001). Deviant froms of aggression in glucocorticoid hyporeactive rats: A model for 'pathological' aggression? *Journal of Neuroendocriniology, 13,* 102-107.

Huesmann, L.R., Eron, L.D., Lefkowitz, M.M., & Walder, L.O. (1984). Stability of aggression over time and generations. *Developmental Psychology, 20,* 1120-1134.

Kazdin, A. E. (1978). Artifact, bias, and complexity of assessment: The ABCs of reliability. *Journal of Applied Behavior Analysis, 10,* 141-150.

Kirschbaum, C., Wust, S., & Hellhammer, D. (1990). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine, 54,* 648-657.

Kirschbaum, C. & Hellhammer, D.H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology, 19,* 313-333.

Kobak, R., Zajac, K., & Levine, S. (2009). Cortisol and antisocial behavior in early adolescence: The role of gender in an economically disadvantaged sample. *Development and Psychopathology, 21,* 579-591.

Kochanska, G. (1997). Multiple pathways to conscience for children with different temperaments: From toddlerhood to age five. *Developmental Psychology, 33,* 228–240.

Kochanska, G., & Aksan, N. (1995). Mother-child mutually positive affect, the quality of child compliance requests and prohibitions, and maternal control as correlates of early internalization. *Child Development, 66,* 236 – 254.

Kochanska, G., Murray, K. T., & Harlen, E. T. (2000). Effortful control in early childhood: Continuity and change, antecedents, and implications for social development. *Developmental Psychology, 36*, 220 – 232.

Kruk, M.R., Halasz, J., Meelis, W., & Haller, J. (2004). Fast positive feedback between adrenocortical stress response and a brain mechanism involved in aggressive behavior. *Behavioral Neuroscience, 118,* 1062-1070.

Legendre, A. (2003). Environmental features influencing toddlers' bioemotional reaction in day care centers. *Environment and Behavior*, *35*, 523-549.

Lisonbee, J. (2004). Teacher-child relationships and preschool children's cortisol fluctuations (Doctoral dissertation, Auburn University, 2004).

Lisonbee, J.A., Mize, J., Lapp Payne, A., & Granger, D.A. (2008). Children's cortisol and the quality of teacher-child relationships in child care. *Child Development, 79,* 1818-1832.

Lisonbee, J.A., Mize, J., Payne, A., & Reeves, K. (2002). *Saliva Collection Protocol: Childcare Quality Enhancement Project*. Unpublished document, Auburn University.

Loney, B.R., Butler, M.A., Lima, E.N., Counts, C.A., & Eckel, L.A. (2006). The relation between salivary cortisol, callous-unemotional traits, and conduct problems in an adolescent non-referred sample. *Journal of Child Psychology and Psychiatry, 47,* 30-36.

Lopez-Duran, N.L., Hajal, N.J, Olson, S.L., Felt, B.T., Vazquez, D.M. (2009). Individual differences in cortisol responses to fear and frustration during middle childhood. *Journal of Experimental Child Psychology*, *103,* 285-295.

Lorber, M.F. (2004). Psychophysiology of aggression, psychopathy, and conduct problems: A meta-analysis. *Psychological Bulletin, 130,* 531-552.

Lupien, S., King, S., Meaney, M.J., McEwen, B.S. (2000) Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biological Psychiatry, 48,* 976-980.

Lupien, S.J., King, S., Meaney, M.J., & McEwan, B.S. (2001). Can poverty get under your skin? Basal cortisol levels and cognitive function in children from low and high socioeconomic status. *Development and Psychopathology, 13,* 653-676.

Mayer, M., & Mayer, M. (1975). *One frog too many*. New York: Dial Press.

McBurnett, K., Lahey, B.B., Rathouz, P.J., & Loeber, R. (2000). Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Archive of General Psychiatry, 57,* 38-43.

McLoyd, V. (1998). Socioeconomic disadvantage and child development. *American Psychologist, 53,* 185–204.

Mesman, J., Alink, L.R.A., van Zeijl, J., Stolk, M.N., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., Juffer, F., & Koot, H.M. (2008). Observation of early childhood physical aggression: A psychometric study of the system for coding early physical aggression. *Aggressive Behavior, 34,* 539-552.

Miller, G.E., Chen, E., Fok, A.K., Walker, H., Lim, A., Nicholls, E.F., Cole, S., & Kobor, M.S. (2009). Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 106,* 14716-14721.

Mistry, R.S., Vandewater, E.A., Huston, A.C., & McLoyd, V.C. (2002). Economic well-being and children's social adjustment: The role of family process in an ethnically diverse low-income sample. *Child Development, 73,* 935–951.

Moffitt, T.E. (2007). A review of research on the taxonomy of life-course persistent versus adolescence-limited antisocial behavior. In D.J. Flannery, A.T. Vazsonyi, & I.D. Waldman (Eds.), *The Cambridge handbook of violent behavior and aggression* (pp. 49-74). New York: Cambridge University Press.

Nelson, R.J. & Trainor, B.C. (2007). Neural mechanisms of aggression. *Nature Reviews Neuroscience, 8,* 536-546.

Nock, M. & Kurtz, S.M.S. (2005). Direct behavioral observation in school settings: Bringing science to practice. *Cognitive and Behavioral Practice, 12,* 359-370.

Olweus, D. (1979). Stability of aggressive reaction patterns in males: A review. *Psychological Bulletin, 86,* 852-875.Ortiz, J. & Raine, A. (2004). Heart rate level and antisocial behavior in children and adolescents: A meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry, 43,* 154-162.

O'Neal, C.R., Brotman, L.M., Huang, K-Y, Gouley, K.K., Kamboukos, D., Calzada, E.J., & Pine, D.S. (2010). Understanding relations among early family environment, cortisol response, and child aggression via a prevention experiment. *Child Development, 81,* 290-305.

Ouellet-morin, I., Tremblay, R.E., Boivin, M., Meaney, M., Kramer, M., & Cote, S.M. (2010). Diurnal cortisol secretion at home and in child care: A prospective study of 2-year-old toddlers. *Journal of Child Psychology and Psychiatry, 51,* 295-303.

Perez-Edgar, K., Schmidt, L.A., Henderson, H.A., Schulkin, J., & Fox, N.A. (2008). Salivary cortisol levels and infant temperament shape developmental trajectories in boys at risk for behavioral maladjustment. *Psychoneuroendocrinology, 33,* 918-925.

Pajer, K., Gardner, W., Rubin, R.T., Perel, J., & Neal, S. (2001). Decreased cortisol levels in adolescent girls with conduct disorder. *Archives of General Psychiatry, 58,* 297-302.

Poggi-Davis, E., Donzella, B., Krueger, W.K., Gunnar, M.R. (1999). The start of a new school year: Individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology, 35,* 188-196.

Poustka, L. Maras, A., Hohm, E., Fellinger, J., Holtmann, M., Banaschewski, T., Lewicka, S., Schmidt, M.H., Esser, G., Laucht, M. (2010). Negative association between plasma cortisol levels and aggression in a high-risk community sample of adolescents. *Journal of Neural Transmission, 117,* 621-627.

Raine, A. (2002). Annotation: The role of prefrontal deficits, low autonomic arousal, and early health factors in the development of antisocial and aggressive behavior in children. *Journal of Child Psychology and Psychiatry, 43,* 417-434.

Raine, A., Venables, P.H., Dalais, C., Mellingen, K., Reynolds, C., & Mednick, S.A. (2001). Early educational and health enrichment at age 3-5 years is associated with increased autonomic and central nervous system arousal and orienting at age 11 years: Evidence from the Mauritius Child Health Project. *Psychophysiology, 38,* 254-266.

Rothbart, M. K., Ahadi, S. A., Hershey, K. L., & Fisher, P. (2001). Investigation of temperament at three to seven years: The Children's Behavior Questionnaire. *Child Development, 72,* 1394 – 1408.

Salimetrics LLC. (2000). *HS cortisol kit information.* Unpublished manuscript. State College, PA: Pennsylvania State University.

Sapolsky, R.M. (2004). *Why zebras don't get ulcers.* New York, New York: Henry Holt and Company.

Scarpa, A. & Raine, A. (1997). Psychophysiology of anger and violent behavior. *The Psychiatric Clinics of North America, 20,* 375-394.

Scarpa, A. & Raine, A. (2006). The psychophysiology of human antisocial behavior. In RJ Nelson, (Ed.) *The biology of aggression* (pp. 447-461). New York: Oxford.

Schmidt, L.A., Santesso, D.L., Schulkin, J., Segalowitz, S.J. (2007). Shyness is a necessary but not sufficient condition for high salivary cortisol in typically developing 10-year-old children. *Personality and Individual Differences, 43,* 1541-1551.

Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development, 69,* 1503-1513.

Shaw, D.S., Gilliom, M., Ingoldsby, E.M., Nagin, D.S. (2003). Trajectories leading to school-age conduct problems. *Developmental Psychology, 39,* 189-200.

Shirtcliff, E.A., & Essex, M.J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology, 50,* 690-703.

Shirtcliff, E.A., Granger, D.A., Booth, A., & Johnson, D. (2005). Low salivary cortisol levels and externalizing behavior problems in youth. *Development and Psychopathology, 17,* 167-184.

Skinner, C. H., Dittmer, K. I., & Howell, L. A. (2000). Direct observation in school settings: Theoretical issues. In E. S. Shapiro & T. R. Kratochwill (Eds.), *Behavioral assessment in* *schools: Theory, research, and clinical foundations* (2nd ed., pp. 19-45). New York: Guilford Press.

Sims, M., Guilfoyle, A., & Parry, T.S. (2005). Children's cortisol levels and quality of child care provision. *Child: Care, Health, and Development, 32,* 453-466.

Smider, N.A., Essex, M.J., Kalin, N.H., Buss, K.A., Klein, M.H., Davidson, R.J., & Goldsmith, H.H. (2002). Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective study. *Child Development, 73,* 75-92.

Smiley, P. A., & Dweck, C. S. (1994). Individual differences in achievement goals among young children. *Child Development, 65,* 1723 – 1743.

Snoek, H., van Goozen, S.H.M., Matthys, W., Buitelaar, J.K., & van Engeland, H. (2004). Stress responsivity in children with externalizing behavior disorders. *Development and Psychopathology, 16,* 389-406.

Stansbury, K. & Harris, M.L. (2000). Individual differences in stress reactions during a peer entry episode: Effects of age, temperament, approach behavior, and self-perceived peer competence. *Journal of Experimental Child Psychology, 76,* 50-63.

Tarullo, A., & Gunnar, M. (2006). Child maltreatment and the developing HPA axis. *Hormones and Behavior*, *50,* 632-639.

van Bakel, H.J.A. & Riksen-Walraven, J.M. (2004). Stress reactivity in 15-month-old infants: Links with infant temperament, cognitive competence, and attachment security. *Developmental Psychobiology, 44,* 157-167.

van Bokhoven, I., van Goozen, S.H.M., van Engeland, H., Schaal, B., Arseneault, L, Seguin, J.R., Nagin, D.S., Vitaro, F., & Tremblay, R.E. (2005). Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *Journal of Neural Transmission, 112*, 1083-1096.

van Goozen, S.H.M, Matthys, W., Cohen-Kettenis, P.T., Buitelarr, J.K., & van Engeland, H. (2000). Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *Journal of the American Academy of Child and Adolescent Psychiatry, 39,* 1438-1445.

van Goozen, S.H., Matthys, W., Cohen-Kettenis, P.T., Gispen-de-Wied, C., Weigant, V.M., van Engeland, H. (1998). Salivary cortisol and cardiovascular activity during stress in oppositional-defiant-disorder boys and normal controls. *Biological Psychiatry, 43,* 531-539.

Vermeer, H.J. & van IJzendoorn, M.H. (2006). Children’s elevated cortisol levels at daycare: A review and meta-analysis. *Early Childhood Research Quarterly, 21,* 390-401.

Watamura, S.E., Donzella, B., Alwin, J., & Gunnar, M.R., (2003). Morning-to-afternoon increases in cortisol concentrations for infants and toddlers at child care: Age differences and behavioral correlates. *Child Development, 74,* 1006-1020.

Ziegert, D.I., Kistner, J.A., Castro, R., & Robertson, B. (2001). Longitudinal study of young children’s responses to challenging achievement situations. *Child Development, 72*, 609-624.

APPENDICES

Appendix A

IRB Approval Form

AUBURN

UNIVERSITY

Office of Research Compliance
307 Samford Hall
Auburn University, AL 36849

Telephone: 334-844-5966
Fax: 334-844-4391
hsubjec@auburn.edu

June 15, 2010

MEMORANDUM TO: Ms. Diana Reinicke
Department of Human Development and Family Studies

PROTOCOL TITLE: "Preschool Children's Cortisol Levels at Childcare and Aggression One Year Later in Kindergarten"

IRB FILE NO.: 10-158 EX 1006

APPROVAL DATE: June 12, 2010
EXPIRATION DATE: June 11, 2011

The referenced protocol was approved "Exempt" by the IRB under 45 CFR 46.101 (b) (4):

> "Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects."

You should retain this letter in your files, along with a copy of the revised protocol and other pertinent information concerning your study. If you anticipate a change in any of the procedures authorized in this protocol, you must request and receive IRB approval prior to implementation of any revision. Please reference the above IRB file number in any correspondence regarding this project.

If you will be unable to file a Final Report on your project before June 11, 2011, you must submit a request for an extension of approval to the IRB no later than May 20, 2010. If your IRB authorization expires and/or you have not received written notice that a request for an extension has been approved prior to June 11, 2011 you must suspend the project immediately and contact the Office of Research Compliance.

<u>A Final Report will be required to close your IRB project file</u>.

If you have any questions concerning this Board action, please contact the Office of Research Compliance.

Sincerely,

Kathy Jo Ellison

Kathy Jo Ellison, RN, DSN, CIP
Chair of the Institutional Review Board
for the Use of Human Subjects in Research

cc: Dr. Leanne Lamke
Dr. Jacqueline Mize

Appendix B

Teacher's Checklist of Children's Peer Relationships

For each of the following statements, please circle the number that best applies.
Use the following scale to determine the best applying number:

Circle 1 if this statement is <u>never</u> true of this child.	1 = NEVER
Circle 2 if this statement is <u>rarely</u> true of this child.	2 = RARELY, ALMOST NEVER
Circle 3 if this statement is <u>sometimes</u> true of this child.	3 = SOMETIMES
Circle 4 if this statement is <u>usually</u> true of this child.	4 = USUALLY
Circle 5 if this statement is <u>almost always</u> true of this child.	5 = ALMOST ALWAYS

	PEER RELATIONS					
1.	This child gets along well with peers of the same sex.	1	2	3	4	5
2.	This child gets along well with peers of the opposite sex.	1	2	3	4	5
3.	This child isolates him/her self from the peer group.	1	2	3	4	5
4.	This child is accepted by the peer group.	1	2	3	4	5
5.	Other children like this child and seek him or her out for play.	1	2	3	4	5
6.	Other children dislike this child and reject him or her from their play.	1	2	3	4	5
	AGGRESSION AND COERCION					
7.	This child starts fights with peers.	1	2	3	4	5
8.	The child gets into verbal arguments with peers.	1	2	3	4	5
9.	This child says mean things to peers, in teasing or name calling.	1	2	3	4	5
10.	This child refuses to share things with peers.	1	2	3	4	5
11.	This child disrupts the peer group by inappropriate or attention getting behavior.	1	2	3	4	5

12.	Tells another child he or she won't let the child play unless that child does what he or she asks.	1	2	3	4	5
13.	Tells others not to play with or be someone else's friend.	1	2	3	4	5
14.	When mad at another child, tries to keep that child from being in a play group.	1	2	3	4	5
15.	Tells a peer that they won't be invited to their party unless he or she does what the child wants.	1	2	3	4	5
16.	Tries to get others to dislike a peer.	1	2	3	4	5
17.	Verbally threatens to keep a peer out of a playgroup if the peer doesn't do what the child asks.	1	2	3	4	5
18.	Grabs or takes thing from other children	1	2	3	4	5
19.	Pushes or shoves other children	1	2	3	4	5
20.	Hits, pinches, bites or otherwise hurts other children	1	2	3	4	5
21.	Is mean to other children	1	2	3	4	5

How good is the child at each of the following skills?

Circle the appropriate response. Use the following scale in answering:

Circle 1 if this child is very poor at this skill most of the time.	1 = VERY POOR
Circle 2 if this child performs somewhat poorly at this time.	2 = SOMEWHAT POORLY
Circle 3 if this child performs about average.	3 = AVERAGE
Circle 4 if this child performs well at this skill.	4 = WELL
Circle 5 if this child performs very well at this skill.	5 = VERY WELL

22.	Understanding others feelings	1	2	3	4	5
23.	Being socially aware of what is happening in a situation	1	2	3	4	5
24.	Accurately interpreting what a peer is trying to do	1	2	3	4	5
25.	Refraining from over-impulsive responding	1	2	3	4	5
26.	Generating many solution to interpersonal problems	1	2	3	4	5
27.	Generating good quality solutions to interpersonal problems	1	2	3	4	5

28.	Being aware of the effects of his or her behavior on others	1	2	3	4	5
29.	Expressing feelings in socially appropriate ways	1	2	3	4	5
30.	Regulating his/her emotions	1	2	3	4	5

Appendix C

Children's Behavior Questionnaire
Short Form Version l

Subject No. ___________

Today's Date ____________

Sex of Child ____________

Date of Child's Birth:

______ ______ ______
Month Day Year

Age of Child ______ ______
Years months

<u>Instructions</u>: <u>Please read carefully before starting</u>:

On the next pages you will see a set of statements that describe children's reactions to a number of situations. We would like you to tell us what <u>your</u> child's reaction is likely to be in those situations. There are of course no "correct" ways of reacting; children differ widely in their reactions, and it is these differences we are trying to learn about. Please read each statement and decide whether it is a "<u>true</u>" or "<u>untrue</u>" description of your child's reaction <u>within the past six months</u>. Use the following scale to indicate how well a statement describes your child:

Circle #	If the statement is:
l	extremely untrue of your child
2	quite untrue of your child
3	slightly untrue of your child
4	neither true nor false of your child
5	slightly true of your child
6	quite true of your child
7	extremely true of your child

If you cannot answer one of the items because you have never seen the child in that situation, for example, if the statement is about the child's reaction to your singing and you have never sung to your child, then circle NA (not applicable). Please be sure to circle a number or NA for every item.

1. Seems always in a big hurry to get from one place to another.

   l 2 3 4 5 6 7 NA

2. Gets angry when told s/he has to go to bed.

   l 2 3 4 5 6 7 NA

3. Is not very bothered by pain.

   l 2 3 4 5 6 7 NA

4. Likes going down high slides or other adventurous activities.

   l 2 3 4 5 6 7 NA

5. Notices the smoothness or roughness of objects s/he touches.

   l 2 3 4 5 6 7 NA

6. Gets so worked up before an exciting event that s/he has trouble sitting still.

   l 2 3 4 5 6 7 NA

7. Usually rushes into an activity without thinking about it.

   l 2 3 4 5 6 7 NA

8. Cries sadly when a favorite toy gets lost or broken.

   l 2 3 4 5 6 7 NA

9. Becomes quite uncomfortable when cold and/or wet.

   l 2 3 4 5 6 7 NA

10. Likes to play so wild and recklessly that s/he might get hurt.

l 2 3 4 5 6 7 NA

11. Seems to be at ease with almost any person.

l 2 3 4 5 6 7 NA

12. Tends to run rather than walk from room to room.

l 2 3 4 5 6 7 NA

13. Notices it when parents are wearing new clothing.

l 2 3 4 5 6 7 NA

14. Has temper tantrums when s/he doesn't get what s/he wants.

l 2 3 4 5 6 7 NA

15. Gets very enthusiastic about the things s/he does

l 2 3 4 5 6 7 NA

16. When practicing an activity, has a hard time keeping her/his mind on it.

l 2 3 4 5 6 7 NA

17. Is afraid of burglars or the "boogie man."

l 2 3 4 5 6 7 NA

18. When outside, often sits quietly.

l 2 3 4 5 6 7 NA

19. Enjoys funny stories but usually doesn=t laugh at them.

l 2 3 4 5 6 7 NA

20. Tends to become sad if the family's plans don't work out.

l 2 3 4 5 6 7 NA

21. Will move from one task to another without completing any of them.

l 2 3 4 5 6 7 NA

22. Moves about actively (runs, climbs, jumps) when playing in the house.

l 2 3 4 5 6 7 NA

23. Is afraid of loud noises.

l 2 3 4 5 6 7 NA

24. Seems to listen to even quiet sounds.

l 2 3 4 5 6 7 NA

25. Has a hard time settling down after an exciting activity.

l 2 3 4 5 6 7 NA

26. Enjoys taking warm baths.

l 2 3 4 5 6 7 NA

27. Seems to feel depressed when unable to accomplish some task.

l 2 3 4 5 6 7 NA

28. Often rushes into new situations.

l 2 3 4 5 6 7 NA

29. Is quite upset by a little cut or bruise.

l 2 3 4 5 6 7 NA

30. Gets quite frustrated when prevented from doing something s/he wants to do.

l 2 3 4 5 6 7 NA

31. Becomes upset when loved relatives or friends are getting ready to leave following a visit.

l 2 3 4 5 6 7 NA

32. Comments when a parent has changed his/her appearance.

l 2 3 4 5 6 7 NA

33. Enjoys activities such as being chased, spun around by the arms, etc.

l 2 3 4 5 6 7 NA

34. When angry about something, s/he tends to stay upset for ten minutes or longer.

l 2 3 4 5 6 7 NA

35. Is not afraid of the dark.

l 2 3 4 5 6 7 NA

36. Takes a long time in approaching new situations.

l 2 3 4 5 6 7 NA

37. Is sometimes shy even around people s/he has known a long time.

l 2 3 4 5 6 7 NA

38. Can wait before entering into new activities if s/he is asked to.

l 2 3 4 5 6 7 NA

39. Enjoys "snuggling up" next to a parent or babysitter.

l 2 3 4 5 6 7 NA

40. Gets angry when s/he can't find something s/he wants to play with.

l 2 3 4 5 6 7 NA

41. Is afraid of fire.

l 2 3 4 5 6 7 NA

42. Sometimes seems nervous when talking to adults s/he has just met.

l 2 3 4 5 6 7 NA

43. Is slow and unhurried in deciding what to do next.

l 2 3 4 5 6 7 NA

44. Changes from being upset to feeling much better within a few minutes.

l 2 3 4 5 6 7 NA

45. Prepares for trips and outings by planning things s/he will need..

l 2 3 4 5 6 7 NA

46. Becomes very excited while planning for trips.

l 2 3 4 5 6 7 NA

47. Is quickly aware of some new item in the living room.

l 2 3 4 5 6 7 NA

48. Hardly ever laughs out loud during play with other children.

l 2 3 4 5 6 7 NA

49. Is not very upset at minor cuts or bruises.

l 2 3 4 5 6 7 NA

50. Prefers quiet activities to active games.

l 2 3 4 5 6 7 NA

51. Tends to say the first thing that comes to mind, without stopping to think about it.

l 2 3 4 5 6 7 NA

52. Acts shy around new people.

l 2 3 4 5 6 7 NA

53. Has trouble sitting still when s/he is told to (at movies, church, etc.).

l 2 3 4 5 6 7 NA

54. Rarely cries when s/he hears a sad story.

l 2 3 4 5 6 7 NA

55. Sometimes smiles or giggles playing by her/himself.

l 2 3 4 5 6 7 NA

56. Rarely becomes upset when watching a sad event in a TV show.

l 2 3 4 5 6 7 NA

57. Enjoys just being talked to.

l 2 3 4 5 6 7 NA

58. Becomes very excited before an outing (e.g., picnic, party).

l 2 3 4 5 6 7 NA

59. If upset, cheers up quickly when s/he thinks about something else.

l 2 3 4 5 6 7 NA

60. Is comfortable asking other children to play.

l 2 3 4 5 6 7 NA

61. Rarely gets upset when told s/he has to go to bed.

l 2 3 4 5 6 7 NA

62. When drawing or coloring in a book, shows strong concentration.

l 2 3 4 5 6 7 NA

63. Is afraid of the dark.

l 2 3 4 5 6 7 NA

64. Is likely to cry when even a little bit hurt.

l 2 3 4 5 6 7 NA

65. Enjoys looking at picture books.

l 2 3 4 5 6 7 NA

66. Is easy to soothe when s/he is upset.

l 2 3 4 5 6 7 NA

67. Is good at following instructions.

l 2 3 4 5 6 7 NA

68. Is rarely frightened by "monsters" seen on TV or at movies.

l 2 3 4 5 6 7 NA

69. Likes to go high and fast when pushed on a swing.

l 2 3 4 5 6 7 NA

70. Sometimes turns away shyly from new acquaintances.

l 2 3 4 5 6 7 NA

71. When building or putting something together, becomes very involved in what s/he is doing, and works for long periods.

l 2 3 4 5 6 7 NA

72. Likes being sung to.

l 2 3 4 5 6 7 NA

73. Approaches places s/he has been told are dangerous slowly and cautiously.

l 2 3 4 5 6 7 NA

74. Rarely becomes discouraged when s/he has trouble making something work.

l 2 3 4 5 6 7 NA

75. Is very difficult to soothe when s/he has become upset.

l 2 3 4 5 6 7 NA

76. Likes the sound of words, such as nursery rhymes.

l 2 3 4 5 6 7 NA

77. Smiles a lot at people s/he likes.

l 2 3 4 5 6 7 NA

78. Dislikes rough and rowdy games.

l 2 3 4 5 6 7 NA

79. Often laughs out loud in play with other children.

l 2 3 4 5 6 7 NA

80. Rarely laughs aloud while watching TV or movie comedies.

l 2 3 4 5 6 7 NA

81. Can easily stop an activity when s/he is told "no."

l 2 3 4 5 6 7 NA

82. Is among the last children to try out a new activity.

l 2 3 4 5 6 7 NA

83. Doesn't usually notice odors such as perfume, smoke, cooking, etc.

l 2 3 4 5 6 7 NA

84. Is easily distracted when listening to a story.

l 2 3 4 5 6 7 NA

85. Is full of energy, even in the evening.

l 2 3 4 5 6 7 NA

86. Enjoys sitting on parent's lap.

l 2 3 4 5 6 7 NA

87. Gets angry when called in from play before s/he is ready to quit.

l 2 3 4 5 6 7 NA

88. Enjoys riding a tricycle or bicycle fast and recklessly.

l 2 3 4 5 6 7 NA

89. Sometimes becomes absorbed in a picture book and looks at it for a long time.

l 2 3 4 5 6 7 NA

90. Remains pretty calm about upcoming desserts like ice cream.

l 2 3 4 5 6 7 NA

91. Hardly ever complains when ill with a cold.

l 2 3 4 5 6 7 NA

92. Looks forward to family outings, but does not get too excited about them.

l 2 3 4 5 6 7 NA

93. Likes to sit quietly and watch people do things.

l 2 3 4 5 6 7 NA

94. Enjoys gentle rhythmic activities, such as rocking or swaying.

l 2 3 4 5 6 7 NA

Please check back to make sure you have completed all the pages of the questionnaire. Thank you very much for your help!

Appendix D

Saliva Collection Protocol

Childcare Quality Enhancement Project

Jared Lisonbee
Jacquelyn Mize
Amie Lapp
Kaye Reeves

Revised July 18, 2002

Childcare Quality Enhancement Project
Saliva Collection Protocol

Pre-Collection:

Prior to going to the daycare centers to collect saliva samples, check the “Saliva Collection Kit” (large Rubbermaid container labeled “Saliva Samples”) to assure that the necessary materials are present. The kit should contain:

* Small (3 oz.) disposable plastic cups (at least 4 cups for each child: two are used to give the child water 20 to 30 minutes prior to each collection and two are used for the actual saliva collection). Write the study children’s names on the cups that will be used to collect the saliva prior to going to the center.
* A plastic container to hold water for children to swish their mouths out.
* Pre-labeled, 2 ml plastic storage vials for the saliva. Be sure that there are enough vials for each collection as well as a few “back up” vials to be safe. If possible, use vial numbers that are sequential to make cataloguing for shipping easier.
* A box of disposable latex gloves with sufficient gloves for each research team member who will be directly in contact with handling the saliva.
* Cartoon character lab coats for each team member who will be directly involved in collecting saliva.
* Saliva Collection Roster with the names of each child from whom saliva will be collected printed in the left column.
* Permanent marker for writing the child’s “start” and “end” time on the saliva collection cup to be recorded on the Saliva Collection Roster.
* Pens or pencils for recording vial numbers and start and end times for the collection on the Saliva Collection Roster (these pencils should be kept in a container in the Saliva Collection Kit and should be treated as if they are potentially contaminated [i.e., should not be used for anything other than recording saliva collection information]).
* Cardboard tray to hold completed saliva sample cups while transferring saliva from the collection cups into storage vials.

* A roll of reward stickers to thank the children for playing the Spitting Game.

* A gallon-size plastic jug with a screw-on lid for holding the used saliva collection cups and gloves to return to the lab for disinfecting prior to disposal.

* Sealable plastic storage bags to collect all the filled vials from the collection batch to transport in the cooler back to the lab to be stored in the saliva storage freezer.

* A roll of paper towels and a spray bottle of antibacterial cleaner to disinfect the collection area following the collection procedure.

* The manilla envelope containing the laminated pictures of a variety of foods to be used to help the children salivate (especially with the first saliva collection).

* A pack of <u>Trident</u> gum (original flavor) to be used with children who have difficulty producing enough saliva.

* **Immediately before leaving for the center**, remove the small cooler with the frozen, reusable ice-packs from the freezer to take with you to the center to hold the samples for transporting the samples back to the lab.

On the day before the saliva collection, take a “Wake-up Time Recording Sheet” to the daycare center and arrange to attach the sheet to the classrooms regular sign-in sheet for the following day for parents to record the child’s wake-up time when the parent signs the child in on the day of the saliva collection. The center director and classroom teacher should have been contacted already about the saliva collection date to ensure that there are no conflicts with field trips or other special activities, but this visit should be used to confirm the collection time with the director and teacher. Be sure to discuss with the teacher when the best time for the collection is (prior to the morning snack is ideal, but if that is not possible, work around the teacher’s classroom schedule).

Instruction and Initial Saliva Collection:

The first saliva collection serves to introduce the child(ren) to the saliva collection procedures. Give each child a drink of water (use some of the small plastic cups from the Saliva Collection Kit) and tell each child to be sure to swish the water around their mouth well to get rid of any food that they may have in their mouth. Wait for 20 to 30 minutes following giving the children water before beginning the saliva collection procedure. Select a small group of children (usually between 3 and 5 children) and seat them at a small table in the classroom. Explain to the other study children in the classroom who are not selected for the first group that they will get a chance to play the game soon. One or two research assistants sit at the table to assist the children (one RA records the start and end times and the saliva vial numbers when the saliva is transferred from the collection cups to the storage vials). Each child is given a small (3 oz. size) plastic cup that has the child's name written on it with a permanent magic marker (prior to going to the center). The child's start time is recorded on a Saliva Collection Roster as the child is given the cup. Since there is a possibility that the novelty and potential stress of the collection procedure can elevate the child's cortisol level, the goal is to complete the procedure for each child in less than 10 minutes (in our experience, after becoming familiar with the "spitting game," most children provide a sufficient sample in less than 5 minutes, and often in less than 1 minute).

When each child has received his or her own plastic cup, tell the children:

> "We are going to play the spitting game today. Do any of you know what the wet stuff in your mouth is called?" [children respond] "That wet stuff [spit, slobber, etc., depending on what the children respond] is also called saliva and I want to get some saliva from you so I can find out what's in it. Your saliva is important because it helps you to eat food. In fact, did you know that sometimes when you are getting ready to eat or when you see or smell some yummy food, your mouth makes extra saliva. I brought some pictures of some yummy food that I want to show to you today to help you make lots of good saliva. When I show you a picture of food, I want to see how much you like the food by how much saliva you can get in the cup. Now when you put your spit in this cup, you don't have to blow it like you are spitting because that sometimes makes your spit go everywhere and that is yucky. Here's the best way to do it."

[Demonstrate for the children how to drool into the cup by pressing the cup against the middle of your bottom lip and pushing saliva out onto your lips then "scoop" the cup upward to catch the saliva].

> "Okay, now you try it. I will show you some pictures of food and I want to see how much you like the food by how much saliva you make. Here is the first picture, it looks like some __________. MMMM, let's see how much you like______. Go ahead and catch your saliva in your cup."

[Continue showing pictures of food while watching how much saliva the children produce. It may be necessary (especially at first) to help some of the children by using their cup to scoop off the saliva as they push it out onto their lips. Be sure to praise the children for their efforts. If children are having difficulties producing enough saliva, you can try other things with them such having them use their tongues as "brooms" to sweep the saliva out from all around the inside of their mouths and push it forward. Other possible helps are to have the children make a “fish face” by puckering up their mouths like a fish and, with their mouths closed, make chewing motions to stimulate saliva secretion and push the saliva to the front of the mouth. You might also hum a preschool song with the children while they are doing “fish face” to let them build up saliva to spit into the cup. Another option for children who are having difficulty is to have them pretend to be a firefighter and tell them to try to put out a pretend fire on the bottom of the cup with their spit. Remember, some children may need a lot of individual attention and assistance with spitting, especially during the first saliva collection procedure(s).]

When a child has provided enough saliva (at least 1 ml--usually when the saliva nearly covers the bottom of the 3 oz. disposable cup), praise the child for the great job that she or he did, take the cup from the child, and write the end time on the plastic cup with a permanent marker. Place the cup in the cardboard tray so that you can help any remaining children with producing their saliva sample. If there is a research team member with you who is not directly involved with saliva collection (someone who is not wearing latex gloves) have that person give the child a sticker and let the child return to the regular classroom activities (otherwise, let the child go rejoin the classroom activities and distribute the reward stickers all at once when all the children have completed the saliva collection procedure). If there are two researchers involved in the saliva collection, have one of the researchers transfer the saliva from the collection cups into the storage vials and record the vial number and end time on the Saliva Collection Roster while the other researcher is continuing to work with any remaining children who are still spitting. If there is only one researcher collecting saliva, just write the end time on the completed cup with the permanent marker and place the cup in the tray while helping the remaining child(ren) to finish providing the sample(s) (as long as it does not take more than five minutes to complete the remaining child(ren)s collection). Place the filled vials into a plastic, sealable storage bag, write the date, location, and collection information (e.g., “P.M. Baseline”) on the storage bag and place the filled storage bag into the cooler for transport back to the lab for storage in the storage freezer. Repeat the saliva collection procedure with the rest of the children in the classroom until saliva has been collected from all the participants. Place all used saliva collection cups and latex gloves into the plastic waste storage jug to transport back to the lab for disinfecting prior to disposal.

When all the children have finished providing samples, clean the table well with the antibacterial cleaner (let the cleaner stay on the table for 30 seconds prior to wiping down the table with paper towels to kill germs). Thank the teacher for allowing you to come in to the classroom and remind him or her that you will be returning in the afternoon for the afternoon collection. Verify with the teacher about what time to return for the afternoon collection. Take the cooler with the saliva to the lab to store the samples in the freezer as soon as possible.

Subsequent Saliva Collections:

Subsequent saliva collections do not need the instruction and demonstration required in the initial saliva collection. Most children seem to catch on quite quickly and may not even need to use the pictures of food or some of the other “games” used to help the children spit.

Give the children a drink of water (using the disposable plastic cups) and instruct each child to swish the water around in her or his mouth to be sure to clear out any food particles. Wait for 20 to 30 minutes following the mouth swishing before beginning the saliva collection procedure.

Have a small group of children (3 to 5 children) sit at a small table in the classroom and give each child a disposable plastic cup with their name written on it in permanent marker to collect their saliva. Record the start time as you give the cups to the children. Remind the children about how to put their spit into the cups (demonstrate again as needed), and let the children begin. Many children will not need any additional help or instruction but will proceed with drooling into their cup on their own. Other children may require some of the procedures and games described previously such as showing pictures of food to see which foods they like the most by how much spit they produce, doing “fishyface,” spitting out the fire on the bottom of the cup, etc.. Monitor the children to see how they are progressing and collect their saliva collection cup as soon as they are finished. Be sure to record the end time on the cup with a permanent marker and transfer the saliva into a vial as soon as possible. Place the filled vials in a plastic sealable storage bag. Write the date, location, and collection information (e.g., “P.M. Baseline”) on the storage bag and place the filled storage bag into the cooler for transport back to the lab for storage. Place all used saliva collection cups and latex gloves into the plastic waste storage jug to transport back to the lab for disinfecting prior to disposal.

With some children who have difficulty providing saliva, it may be necessary to work with them individually (not in a small group setting). It may be helpful for the researcher to hold the cup to scoop the saliva off the child’s lip and, for some children, it may be easier to just use the plastic vial to scoop the saliva off the child’s lip instead of using the cup. This may be especially helpful with children who are having difficulty producing saliva because some saliva will naturally adhere to the sides of the plastic cup, requiring the collection of slightly more saliva when using a cup instead of just using the vial. If the child is having a lot of difficulty providing enough saliva and it looks like the collection procedure may drag on, use a small piece of Trident gum (usually just half a piece is needed) for the child to chew to stimulate saliva secretion. After chewing for a moment, have the child hold the piece of gum while he or she drools into the cup or vial. Repeat as needed. Be sure to make a note on the Saliva Collection Roster that Trident was used for this sample.

When all the study children have finished providing saliva samples, clean the area with antibacterial cleaner (allowing the cleaner to remain on the table for at least 30 seconds before wiping with paper towels).

Notes, Precautions, and Considerations:

Be sure that you have spent time in the classroom interacting with the children prior to the day of the saliva collection so that the children know you and are comfortable interacting with you. This is to try to avoid influencing cortisol levels due to novelty or discomfort with the situation for the child.

If faced with a choice between getting more saliva or keeping the procedure short, go with trying to keep the procedure short

DO NOT encourage the children to spit by setting up competition between the children (such as “let’s see which of you can finish spitting first.”) because creating a competitive situation might influence hormonal levels.

Be sure to record any information that may be potentially important for understanding the results. Note any discoloration in the saliva (find out what the child ate recently or if the child bumped his or her mouth etc. to try to determine if discoloration is from colorful food [such as punch, chips, or candy with artificial colors] or if there is a possibility of blood contamination. Also note if you notice that the child seems to have a cold or seems ill in any way. Note if the child seems to have any canker sores or dental problems that may cause blood or other contaminants to be present in the saliva.