Children's Stress and Their Health:
Cortisol and the Common Cold

by

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Abstract

Many studies document associations between high levels of stress in early childhood and poor adult health, but understanding of mechanisms underlying these links is limited. Recent theoretical models focus on physiological mechanisms of stress, particularly dysregulation or modification of the hypothalamic-pituitary-adrenocortical (HPA) axis. However, little empirical research examines the proposed links. I examined associations between family risk factors, cortisol, and health in a sample of preschool-aged children from a midsize, South-Eastern city. I hypothesized that children's abnormally high and abnormally low cortisol levels, along with flat patterns of cortisol change over the day, would mediate associations between risk factors (low job status (i.e., prestige), minority status, and education) and poor health. Risk factors and cortisol predicted between 11% and 16% of the variance in child health. However, there was no evidence of mediation. Rather, risk factors and higher morning cortisol levels made independent contributions to the prediction of children's health. Future studies should examine mechanisms linking early risk factors, HPA-axis factors, and health longitudinally, beginning in early childhood.

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Table of Contents

List of Tables

List of Figures

I. INTRODUCTION

Children who experience high levels of stress early in life suffer the consequences in the form of poor health in their adult years. Even after a stressor has come and gone, the effects are still evident; stress leaves its mark.

Miller, Chen, and Parker (2011) define stress as exposure to a stimulus that a person judges as too threatening to manage. A stressor is simply the stimulus itself (Miller et al., 2011). Moments after animals experience a stressor, the hypothalamic-pituitary-adrenal axis, or HPA-axis, releases cortisol. Cortisol is considered the most important hormonal actor in the stress response, and this, in addition to the ease with which cortisol can be measured, has led to its use as a marker of HPA-axis activity in many studies, including ours. Cortisol is a hormone that exists in the body at all times, but it is released in large amounts at the onset of stress. When cortisol enters the bloodstream, it goes to work prepping the body for the strenuous, exhausting activities that stress brings on; heart rate, respiration, and blood pressure increase while functions of the reproductive system, digestive system, immune system, and excretory system are suppressed (Sapolsky, 2004).

In normal circumstances, cortisol follows a circadian rhythm. Highest values occur in early morning, usually just after waking, followed by a steep decline across the morning, and then gradually declining levels through the afternoon and nighttime hours.

Cortisol normally reaches its nadir around midnight, and then begins to increase again to prepare the body for rising and the day's activities.

Without cortisol, the human body cannot function properly. However, when the body is exposed to too much cortisol, such as when an individual experiences repeated stress or when cortisol is taken as a treatment for a disease, the effects can be destructive. Among these negative effects are heightened risk for a variety of diseases, poor sleep quality, low levels of testosterone in men and estrogen in women, memory loss, and decreases in bone formation (Sapolsky, 2004). Chronically high cortisol can even lead to Cushing Syndrome, a disease that includes symptoms such as skin necrosis, excessive sweating and weight gain, depression, and anxiety (Orth, 1995). Conversely, abnormally low cortisol is associated with adverse symptoms like weakness, headaches, fatigue, and even autoimmune disorders (Sapolsky, 2004). Most dramatically, extremely low cortisol is known as Addison disease, a disorder that causes weakness, irritability, nausea, low blood pressure, weight loss, acute adrenal failure, and even death in some cases (Ten, New, & Maclaren, 2001).

Although we generally think of the stress response system as engaging only in extreme situations like animal attacks or other life-threatening circumstances, in the modern world, most disruptions in normal cortisol patterns are caused by recurring daily stressors like low socioeconomic status, conflict, and abuse in the family. A large body of research now shows that such recurring daily stressors in childhood are associated with higher rates of disease and dysfunction in adulthood. In a 2009 study by Anda et al., researchers found that adults who experienced stressors like family violence, abuse, and neglect as children were one-and-a-half to two times more likely than individuals who

did not report such early life stressors to be diagnosed with cardiovascular disease, autoimmune disorders, and even to experience premature death. Similarly, Springer, Sheridan, Kuo, and Carnes (2003) found that abuse in childhood was linked with increased diagnoses of fibromyalgia, chronic fatigue syndrome, and irritable bowel syndrome in adulthood. Fallitti et al. (1998) found that adults who reported stress in the home during childhood in the form of child abuse, mother abuse, mental illness of family members, drug use by family members, or incarceration of family members were at a significantly higher risk for diagnoses of ischemic heart disease, chronic lung disease, skeletal fractures, liver disease, and even cancer. These studies are just the tip of the iceberg; it is now clear that young children who experience recurring negative circumstances in their homes grow up to face life-long health consequences.

There is limited understanding, however, of why early stressors have such corrosive effects on long-term health. Attention has now shifted from documenting links between early stressful life circumstances and later health to understanding the mechanisms through which early stress can negatively affect health across the lifespan. Historically in the field of social and behavioral sciences, we have focused on social and cognitive mechanisms, such as reinforcement and modeling, but more recent foci have been on physiological mechanisms of stress, including epigenetics, DNA, and the dysregulation or modification of the HPA-axis.

Perhaps the most promising current approach identifies disruption in HPA-axis activity as the primary mechanism in the link between early stress and health. Thus, recent studies show that stress in early childhood is linked to deviations from typical patterns of cortisol secretion in adulthood (Ashman et al., 2002; Nicholson, 2003).

Disruptions can take the form of lower than typical daily cortisol production (Flinn & England, 1997; Hiem et al., 2000; Yehuda, Southwick, Nussbaum, & Wahby, 1990); higher than typical daily cortisol production (Carlson & Earls, 1997; Cicchetti & Rogosch, 2001; Hart, Gunnar, & Cicchetti, 1996; Hessl et al., 1998; Li, Power, Kelly, Kirshbaum, & Hertman, 2007); or deviations from the typical circadian patterning of cortisol production, with slightly lower morning values and slightly higher evening values, resulting in flattened circadian patterns (Ball, Anderson, Minto, & Halonen, 2006; Gunnar & Vasquez, 2001). For a conceptual model illustrating links between early stress, cortisol, and adult health in existing literature, see Figure 1.

If HPA-axis activity mediates links between early childhood stress and later health, then it is reasonable to suggest that similar links between cortisol and concurrent health may be apparent in early life. There is limited evidence existing with which to evaluate this hypothesis, however. A possible reason for the surprisingly small body of research on this topic is the complexity involved in identifying HPA-axis disruptions. Because higher than normal, lower than normal, and flattened daily cortisol patterns are all associated with negative health outcomes, criteria for identifying children at risk for negative health outcomes should entail a Goldilocks approach: children at risk could have cortisol levels that are too high, too low, or too flat. In other words, any cortisol pattern other than "just right" could be a marker for disease risk.

Stressful living conditions and poor care have been associated with the three deviations from normal cortisol patterning just described. For example, Flinn and England found in 1997 that children who lived in households with unstable care had abnormal cortisol levels, but paradoxically, some children's levels were abnormally high

whereas other children's were abnormally low, and still others had flattened patterns. Similarly, in 2008, Bevans, Cerbone, and Overstreet found that some types of stressors were associated with high afternoon cortisol levels in children whereas others were related to low cortisol levels in the morning and high cortisol levels in the afternoon. Needless to say, it seems clear that deviations from typical cortisol patterning that mark risk are not unidirectional; instead, timing of stressors, type of stressors, and specific traits of individuals affect cortisol patterns and ultimately, HPA-axis functioning.

To our knowledge, only one article exists today that specifically studies the links between stress in early childhood, HPA-functioning, and health concurrently. This 1997 work was completed by Flinn and England with a sample of 264 children ages two-months to eighteen-years old from a rural Caribbean township. They found that socioeconomic conditions (SES), family composition, and traumatic experiences were related to health. For example, children who lived with distant relatives or with single mothers who did not have support from other members of the family were sick more often than children who lived with both their father and mother, close relatives, or with mothers who had support from kin. In households containing one child who was the genetic offspring of parents and one child who was the step-offspring of parents, stepchildren were sick more often than genetic children. Traumatic experiences like conflict and abuse in the family or residence changes, more than any other stressors studied by Flinn and England, were associated with heightened cortisol. However, Flinn and England also found that children who were exposed to chronic traumatic experiences had flattened cortisol responses, again suggesting that chronic stress can cause a variety of forms of abnormal HPA-axis activity.

The purpose of this study is to further explore links between HPA-axis activity and concurrent health in young children. Uncovering additional information about whether and how cortisol patterning is associated with concurrent health would shed light on the mechanisms affecting long-term health and could inform practice in a variety of fields such as pediatric medicine, social work, early education, parent education, and counseling. In particular, I suggest that greater dysregulation of HPA-axis activity in early childhood will be associated concurrently with poorer health. I examined this issue in a sample of preschool children whose families' SES range from quite low to quite high. I looked at associations between children's HPA-axis activity as measured through salivary cortisol and children's health as reported by parents. A number of studies show that stress can be associated with high cortisol, low cortisol, high total cortisol output over a day, low total cortisol output over a day, and flattened daily cortisol patterns. Based on these studies, I examind morning cortisol level, afternoon cortisol level, total daily cortisol output, and cortisol change from morning to afternoon. Specifically, I defined HPA-axis dysregulation as deviations from typical morning cortisol values, afternoon cortisol values, total daily cortisol output, and cortisol change across the day. In determining typical cortisol values, I was guided by both previous research establishing ranges of expected cortisol values (Knutson et al., 1997) and exploratory analyses of our data set.

Data for the study were collected as part of a larger research project, called the Childcare Quality Enhancement Project, which investigated the cognitive, social, and physiological experiences of four-year-old children in childcare, exploring how each of these was related to children's competence and adjustment both concurrently and a year

later in the kindergarten environment. Cortisol was measured in saliva samples collected while children were at their child care centers. Parents provided information about children's health and demographic characteristics of the family. Analyses focused on associations between children's health and their morning cortisol levels, afternoon cortisol levels, total daily cortisol output, and change in cortisol from morning to afternoon. Also, I will examine the possible mediation of cortisol in the relationship between risk and health. Specific hypotheses investigated include:

H1: Lower morning cortisol levels will be correlated with poorer health.

H2: Higher afternoon cortisol levels will be correlated with poorer health.

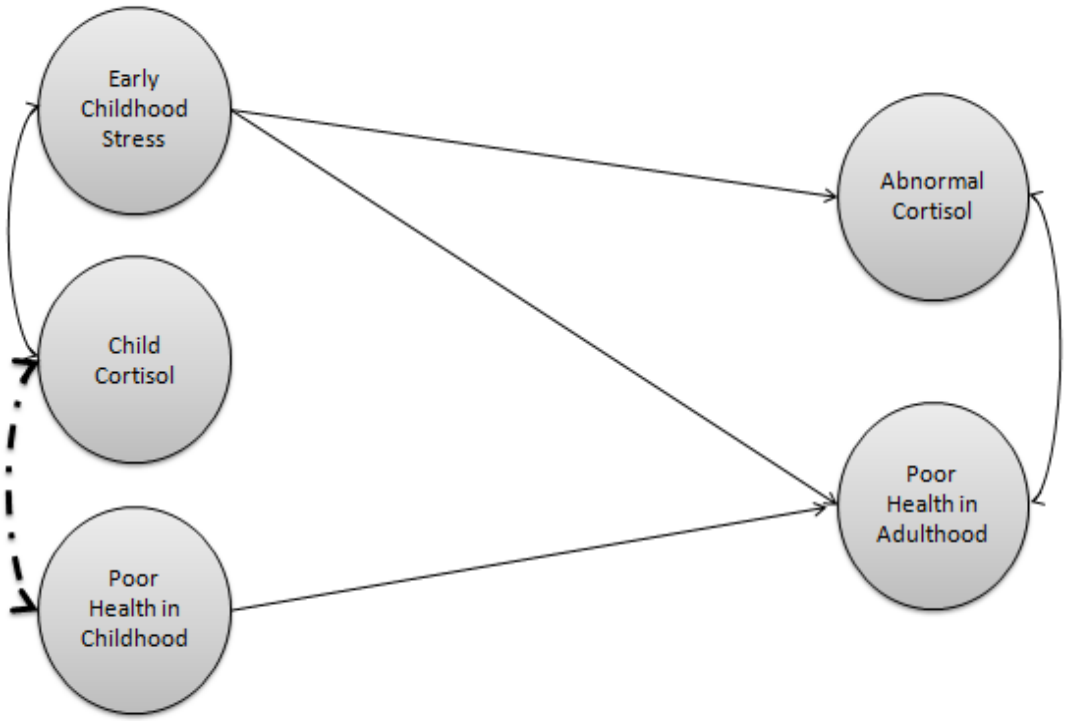
H3: Lesser declines, or increases, in morning to afternoon cortisol values (i.e., flattened or increasing morning to afternoon slopes) will be correlated with poorer health.

H4: Higher total daily output will be associated with poorer health.

H5: Cortisol will mediate associations between demographic and risk factors and children's health.

Figure 1.

Conceptual model of existing studies linking early stress, cortisol, and adult health.



Notes. Solid lines represent associations for which a body of research exists. Dotted line represents the author's research question

II. REVIEW OF LITERATURE

The purpose of this chapter is to explore the existing research on the topics of cortisol and its role in the body, stress and adverse childhood experiences, and how stress can affect health both concurrently and throughout the lifespan. Specifically, I will first explore the idea of stress, defining the words "stress" and "stressor" as used in this thesis, and looking at the workings of the HPA-axis. Second, I will examine the role cortisol plays in the human body. Next, I will review the possible influences that children's circumstances and individual characteristics have on their cortisol. Then, I will review highlights from the extensive body of existing studies on children's stress and the long term health effects that are associated with that early stress. Fifth, I will explore the many relationships represented in the theoretical model in Figure 1. Finally, I will delve into the only existing study Iknow of that specifically looks at cortisol levels and health in children concurrently (Flinn & England, 1997).

Stress and the HPA-axis

Stress and stressors. In the Miller et al. (2011) review of articles on HPA-axis functioning, stress is defined as exposure to an event or condition that a person does not believe he or she can handle, whereas a stressor is simply the event or condition itself. This all-encompassing definition accounts for the vast number of circumstances that people consider stressful, from trouble at work and test anxiety to the loss of a loved one and physical threats to bodily safety like rape, abuse, and murder. For example, the ocean

may be a stressor to a person who cannot swim; they see it as an obstacle that is too threatening to manage. Conversely, to an avid swimmer, vessel captain, or oceanographer, the ocean is not a stressor; in fact it can be a therapeutic entity to some. Stressors can come in countless forms varying from person to person, but it is clear that stressors in all their many forms prompt the body to respond.

HPA-axis functioning. Robert Sapolsky notes the evolutionary importance of the body's reaction to stressors in his book, *Why zebras don't get ulcers* (2004). He cites the example of a zebra running from a hungry lion on the savannah; in this case, there is little need for digestion, urination, reproduction, or immune response when life is at stake. What is important to the zebra in this scenario is increased circulation to the legs to increase running speed. In our world, stressors are usually much less novel than this, but our bodies react the same way to anxiety over our academic performance or conflict between friends as they do to threats for our safety. This biological function may serve well on the savannah; however, in the life of a human, long-term exposure to stress can be devastatingly detrimental in terms of health. Without the normative functions of the digestive, reproductive, nervous, circulatory, endocrine, immune, muscular, and lymphatic systems, not only do humans fail to grow and develop normatively, but also humans fall prey to a myriad of illnesses.

The body's response to stress. To understand more about what happens in the body during stress, basic concepts about the nervous system must be grasped. When animals experience any type of stress, their bodies respond with changes in two major systems: the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS is composed of the brain and the spinal cord, whereas the PNS is composed of the

nerves within the rest of the body. Two parts of the PNS are the sympathetic nervous system (SNS), which stimulates fight and flight responses, and the parasympathetic nervous system (PSNS), which stimulates rest and digest functions (Nelson, 2000).

The stress response involves the production and release of several chemicals, some of which take immediate effect and others of which are more long-acting. At the onset of a stressor, the SNS releases norepinephrine, and the CNS secretes epinephrine immediately. These two chemicals together are called catecholamines. Catecholamines increase blood glucose levels so that muscles have access to the energy they need in order to fight or flee. Epinephrine also stimulates the respiratory and cardiovascular systems, again to aide in the fight or flight response. Catecholamines are cleared from the body very quickly. Immediately, Corticotropin Releasing Hormone (CRH) is released by the hypothalamus. CRH works to stimulate production of Adrenocorticotropic Hormone (ACTH) from the pituitary gland. All these actions only take a few seconds. Over the course of several minutes, ACTH stimulates the production and release of inflammation-reducing corticosteroids (or cortisol as it is called in humans) into the blood stream (Nelson, 2000). Cortisol is more long-lasting than catecholamines, working in terms of minutes and hours instead of seconds.

Cortisol

Cortisol is necessary for survival, and it exists in the body at all times. When a person suffers from too high or too low cortisol, the effects can be painful, debilitating, and even deadly. As mentioned earlier, too much cortisol can have effects that are detrimental; chronically high cortisol is called Cushing Syndrome, a disease that includes symptoms such as skin necrosis, excessive sweating and weight gain, depression, and

anxiety (Orth, 1995). Similarly, chronically low cortisol is called Addison Disease, a painful disease that causes weakness, irritability, nausea, low blood pressure, weight loss, acute adrenal failure, and even death in some cases (Ten, New, & Maclaren, 2001). Interestingly, President John F. Kennedy suffered from Addison Disease, although he kept it a secret through most of his life. After experiencing two public fainting spells and suffering on a daily basis for most of his life, many would assume that the debilitating pain would weaken this man's political drive and limit his power. However, Robert Gilbert, an American author who studied Kennedy's life thoroughly, says, "rather than adversely affecting him politically, Kennedy's physical ailments vitally contributed to the development of his character and the formation of his political personality" (2010).

Cortisol's daily patterns. During normal daily tasks, cortisol levels fluctuate through a pattern of daily values. This normal pattern is called a circadian rhythm. According to Nelson in his 2000 work, *Introduction to behavioral endocrinology*, circadian rhythms include all of the cortisol released in a 24 hour period. Typically, cortisol levels are highest after waking, and levels plummet throughout the day, reaching lowest levels around midnight. Over the course of the day, life-sustaining amounts of cortisol are constantly flowing through these circadian cycles.

At the onset of a stressor, cortisol is released in large amounts, interrupting the normal circadian rhythm. This surge of cortisol increases blood sugar longer-term than catecholamines do and supplies a source of energy for fight and flight responses. It also suppresses the immune system and decreases bone formation so that the body's energy sources are not being used on long-term improvements and can instead be used for immediate action in the face of a stressor (Sapolsky, 2004). All of these responses are

life-saving in the face of danger, but are superfluous and even harmful in the midst of typical daily social stressors.

Cortisol as a measure of stress. Traditionally, cortisol has been measured through blood and urine samples (Kirschbaum & Hellhammer, 1994). However, recent developments in the field have allowed for less invasive means of collecting cortisol for testing; saliva is now an approved vehicle in which to measure cortisol levels (Kirschbaum & Hellhammer, 1994). Previously, obtaining accurate cortisol measures from taking a child's blood was arguably impossible, because the trauma of anticipating multiple finger pricks was increasing children's stress, and thus affecting cortisol readings. Because of this exciting innovation, studies concentrating on children's stress are now more practical for researchers to take on.

Salivary cortisol is highly correlated with serum cortisol taken from blood or urine (r = .71 to r = .91; Kirschbaum & Hellhammer, 1994). Saliva samples can be taken in one of three ways: one, a child can spit into a cup (also known as passive drooling); two, a child can moisten cotton in their mouths; or three, researchers can use Sorbettes, which are small devices with absorbent tips that can be held under the child's tongue. All three of these collection strategies are considered valid.

Although salivary cortisol collection is arguably easier and simpler than traditional collection methods, it is still vital to the validity of the study that cortisol is measured correctly. A number of factors can result in measurement errors if they are not controlled. Among these is acidity of saliva, time of day collection is taken, and the presence of blood or particulates in saliva. In reputable, well-designed studies, these factors are controlled for methodologically (by taking samples from all children at the

same time of day, and avoiding taking samples immediately after the child has eaten, drank, or suffered oral trauma) or statistically (by controlling for time of collection.) Another factor at play is hydration; researchers must insure that each participant stays adequately hydrated to ensure that he or she can produce enough saliva for analysis (Salimetrics, 2000).

Recently, researchers have debated the use of candy, sweetened drink mixes, or gum to increase saliva production. Some argue that the use of these saliva stimulants can change the pH of saliva, thus biasing cortisol assays (Schwartz et al., 1998). Because of this, researchers chose to use only un-stimulated saliva samples for this study.

Influences on cortisol in children

In the past few years, researchers studying cortisol in children have discovered that most of the variation in cortisol levels is attributable to ever-changing, state-like factors instead of unique differences in individuals. For example, Shirtcliff, Granger, Booth, and Johnson report in a 2005 article that 70% of the variation in cortisol levels in children is attributable to state-like factors, and only 30% is attributable to trait-like factors. Thus, stressful events have more effect on cortisol production than inherent characteristics of individual children. In agreement with these findings is the work by French, Smith, Gleason, Birnie, Mustoe, and Korgan (2012), which found that in sets of identical twin marmoset monkeys, stress responses were not similar between the two twins. Because the study focuses on twins who have identical DNA, we know that inherited trait-like influences had little bearing on HPA- axis activity, and that state-like influences were the factor affecting cortisol secretion. Between person differences can be better identified when more than one sample is used, because relatively little of the

differences in cortisol levels is due to stable, within-person factors. Thus, in the current study, Iwill analyze four saliva samples instead of just one.

Trait-like influences on cortisol. As noted in the previous paragraph, trait-like influences account for only 30% of the variation in cortisol values. However, it is important to note one common trait-like variable, as it is in play in nearly every study involving human cortisol. This factor is SES. A 2010 article by Chen, Cohen, and Miller (2010) found that children from low-income homes show higher daily cortisol levels over a two-year period than children from high-income homes. However, it seems reasonable to believe that other factors like poor nutrition or parental stress that are probably present in low-SES homes could be the causal factor at work here. To eliminate these questions of causality, Fernald and Gunnar (2009) supplemented incomes for low-income families, and found that the high cortisol values of children in these families disappeared, unlike the cortisol values for children from non-supplemented families. Thus, we can assume that in our study, SES will be associated with cortisol.

At best, research exploring how long childhood SES continues to affect cortisol remains unclear. Some researchers find that the influence of SES on HPA- axis reactivity disappears before children make the transition into high school (Lupien, King, Meaney, & McEwan, 2001). However, Miller and colleagues find that children who experience low SES early in life have higher daily cortisol outputs well into adulthood than do children who never experienced low SES (2012). Regardless of whether these links are transient or long-lasting, it is important to examine the potential relationship between SES and HPA-axis activity when assessing cortisol in children. In fact, HPA axis activity may mediate the relationship between demographic factors and health. Another trait-like

influence is temperament; however, as this construct will not be included in the study, it is not reviewed.

State-like influences on cortisol. State-like influences on cortisol are transient and usually affect individuals only periodically. Often, the social context preceding saliva sampling can be mildly stressful, and thus this environment contributes to changes in cortisol. Two examples of state-like influences on cortisol that are of particular interest in the present study are the presence of comforting adults and the quality of the childcare environment.

While it is clear from the massive existing literature on cortisol and the preceding review that stress, fear, and anxiety increase cortisol (Gunnar, Talge, & Herrara, 2008), it may be less obvious that comfort and relief from fear can decrease cortisol levels. For instance, social interactions with comforting adults can lower cortisol in young children (Gunnar, Brodersen, Nachimias, Buss, & Rigatuso, 1996; Gunner & Cheatham, 2003; Gunnar & Donzella, 2002). In fact, if an adult with whom a child has a secure attachment is present, that person can serve as a protective factor from the effects that stress would otherwise have produced by either lowering already high cortisol or prohibiting cortisol from rising (Gunnar, et al., 1996; Lisonbee, Mize, Payne, & Granger, 2009). In the present study, levels of stress that are chronic at home may be masked by the presence of a comforting teacher or other adult in the childcare environment; or conversely, chronic stress in the childcare environment may be due to lack of adult supports in that environment, and thus chronic stress may be limited to the childcare environment and nonexistent at home.

Similarly, the quality of the childcare environment within which saliva samples were collected may serve as a state-like influence on cortisol. Because of this, literature on cortisol in the childcare environment will be considered in some detail in this section. Many studies in the past several years have investigated the relationship between children's cortisol and childcare quality, and conclude that this association is probably causal, not due to selection factors (Geoffroy, Cote, Parent, & Seguin, 2006; Ouellet-Morin, Tremblay, Boivin, Meaney, Kramer, & Sylvana, 2010). For example, in 2003, Watamura, Donzella, Alwin, and Gunnar compared children in their homes and in their preschools, finding that many children showed morning to afternoon increases in cortisol while at preschool, which is at odds with normal, healthy circadian rhythms. However, these same children did not show these increases while at home. In fact, most of these children experienced healthy circadian rhythms with cortisol falling throughout the day. Dettling, Parker, Lane, Sebanc, and Gunnar also found these patterns among preschool children. Fifty-five percent of children showed increases in cortisol levels throughout the day while at preschool, whereas 68% of children showed healthy, falling cortisol circadian rhythms at home (2000).

These findings seem to suggest that children are particularly vulnerable to dysfunctional changes in cortisol levels in the school environment. Particularly, research shows that preschoolers are at most risk for experiencing this phenomenon (Geoffroy et al., 2006). Findings from Dettling, Gunnar, and Donzella's 1999 work are in agreement with Geoffroy and colleagues, finding that 80% of three-year-olds, 60% of four-year-olds, and 50% of five- and six-year-olds experience increases in cortisol from morning to afternoon, instead of normal decreases expected in the circadian rhythm.

However, the reasons why preschoolers experience more dysregulated cortisol rhythms are unclear. Perhaps this dysregulation is due to the social stress that comes with negotiating peer relationships (Gunnar & Donzella, 2002). Infants have fewer interactions with peers, and so they may not experience this stressor. Similarly, by the time a child reaches formal schooling, they may feel more comfortable with peers, and thus have less social stress.

Research suggests that quality of the childcare environment also plays a role. Geoffroy and colleagues discovered that children who attend high-quality daycares experience decreasing levels of cortisol throughout the day on average, whereas children who attend low-quality daycares tend to experience increasing levels of cortisol throughout the day. Building on this research, many scholars are investigating specific traits that comprise the quality of a childcare center. Legendre (2003) explored the relationship between numbers of children in the classroom and classroom space, finding that children displayed increases in daily cortisol when more than 15 children were in the room or when there was not enough space available for each child.

The following section will further explore other issues related to cortisol.

Dysregulated cortisol

When cortisol is repeatedly elevated from its normal levels, cells become less sensitive to cortisol. One way this happens is through the reduction in the number of receptors for cortisol on a cell's surface, referred to as down regulation. Down regulation of cortisol results in higher circulating levels of cortisol, because cell receptors are able to take in less of the circulating cortisol (Nelson, 2000). Because cortisol is a powerful anti-inflammatory, having less cortisol within the cell increases inflammatory response

(Miller et al., 2011). Usually, cortisol works as an anti-inflammatory, but as stress becomes chronic, down regulation of cortisol occurs, and inflammation is likely to spiral out of control. This is a primary mechanism thought to link stress in childhood with later health problems (Miller et al., 2011). In fact, long-term inflammation has been linked to a myriad of negative health outcomes in adulthood including cardiovascular disease, autoimmune disorders, and even premature death (Anda et al., 2009). Even experiencing stress as a child, therefore, can affect health in adulthood (Miller, et al., 2011). Long-term health consequences of dysregulated HPA-axis activity will be discussed below.

Early Stress and Cortisol Outcomes

Children's stress and adulthood cortisol. Problems with cortisol look even grimmer when we consider the stability of cortisol over time. Researchers investigating the link between stress in childhood and cortisol in adulthood are finding that stressors that occur in childhood elevate cortisol over the life-span; even after a stressor has come and gone, research is showing that elevated cortisol does not decline. Intriguingly, van der Hal-Van Raalte, Bakermans-Kranenburg, & van Ijzendoorn (2008) discovered that male survivors of the Holocaust have elevated cortisol levels in adulthood, even after controlling for depression, loss of parents, and physical health! Similarly, Nicholson's 2004 study finds that men who lost a parent during childhood had higher basal cortisol in adulthood, even after controlling for health. It is possible that the body never fully returns to pre-stress functioning after traumatic stressors plague it.

Children's stress and concurrent cortisol. Because we understand the workings of the HPA- axis and cortisol, we know that cortisol takes effect within minutes of a stressor. We know that stressors vary from person to person, and studies exploring a

variety of stressors and their effects on cortisol exist. Findings from these studies paint a rather confusing portrait; stress can result in abnormally high levels of cortisol, abnormally low levels of cortisol, or abnormally flattened cortisol rhythms. For example, one study explored the typical, daily types of stress that children undergo, finding that life-stress, whether positive or negative (like making a sports team, parents getting a new job, or having trouble with a new teacher) during the past year is related to high levels of afternoon cortisol in children (Bevans, Cerbone, & Overstreet, 2008). Seemingly contradictory is their finding that trauma stress like abuse or violence in the home was actually correlated with both low morning cortisol and high afternoon cortisol. Contradictorily, another study by Saridjan, Huisink, Koetsier, Jaddoe, Mackenbach, Hofman, Kirschbaum, Verhulst, and Tiemeier (2010) find that children from low SES have higher morning cortisol and lower afternoon cortisol than their higher SES peers. However, this finding is in agreement with Cicchetti and Rogosch who found in 2001 that school-aged children who were the victims of abuse during their lives experienced higher morning cortisol levels. Evans and Kim (2007) also found links to higher cortisol when they discovered that New York teenagers who live in poverty had chronically high cortisol compared to those not living in poverty, with greater number of years living in poverty related to higher cortisol levels. As is clear from the above segments, the body's reaction to stress is fast-occurring, but can be long-lasting.

Early Childhood Stress and Health Outcomes

Children's stress and adulthood health. In recent years, the field of stress literature has been infiltrated with research on children's stress and adult health; scientists, developmental specialists, psychologists, doctors, and laymen alike are

fascinated with the idea that our early stressors, some of which we may not remember, affect our health well into adulthood. The research on the topic is mind-bogglingly large, with everything from diabetes to cancer connected with stress in childhood. Because this body of literature is so large, only a few particularly interesting, representative studies will be mentioned here.

In 2009, Anda et al. found that most of America's leading causes of death are related to the negative experiences of childhood, with adults who faced family violence, abuse, and neglect during childhood nearly doubling their likelihood of being diagnosed with cardiovascular disease, autoimmune disorders, and even experiencing premature death! Falitti et al. found in a 1998 work that adverse experiences in childhood like abuse, drug use by family members, and mental illness of family members are significantly related to chronic lung disease, skeletal fractures, liver disease, and even cancer. Galobardes, Lynch, and Smith (2004, 2008), discovered that adults who were from low SES families in childhood were more likely to experience death from chronic heart disease and several types of cancer, even controlling for current SES. Sparen and colleagues found that children who experience starvation, especially around the onset of puberty, are at increased risk of cardiovascular disease in adulthood (2003). Finally, Springer, Sheridan, Kuo, and Carnes note in their 2003 article that abuse in childhood is linked to increased diagnoses of fibromyalgia, chronic fatigue syndrome, and irritable bowel syndrome in adulthood.

The biological embedding of childhood adversity model. Miller and colleagues explored the myriad of articles on the topic and came up with a hypothesized model of the ways in which childhood stress could affect health in adult years (2011). This model

is called the biological embedding of childhood adversity model. It uses ideas from a myriad of scientific theories including fetal-origins literature, life-course epidemiology, socioeconomic development, stress physiology, and behavioral immunity. Specifically, Miller and colleagues posit that stress embeds itself into tissues in the body through biological programming.

The following paragraphs will outline the main ideas of this theory. First, children are in a vulnerable period of development during which immune function is more plastic than it will be later in life. Normally, when body tissues are exposed to pathogens or experience a trauma, inflammatory responses are vital players in the healing process. However, when the trauma or infection is over, this process must stop, so inflammation-inhibitor cells are released. Cortisol is a very strong inflammatory-inhibitor, or anti-inflammatory, so when the process must stop, cortisol binds to receptors and limits inflammation. According to Bilbo and Schwartz (2009) and others (Coe & Lubach, 2007), psychosocial environments also produce these same inflammatory responses (Finch & Crimmins, 2004; McDade, 2005). Thus, when children experience stressors like low SES, violence in the family, cold or distant parents, or maltreatment, their bodies react with inflammatory responses. Miller and colleagues hypothesize that through epigenetic pathways, post-translation modifications, and tissue remodeling, stress becomes embedded into cells, setting them up for a lifetime of excessive inflammatory responses. These concepts are outside of the realm of this study, and thus will not be mentioned further.

Second, children's cells become immune to cortisol, and are less able to limit inflammation because of down regulation of cortisol receptors. Because inflammatory

responses are chronically engaged, and cortisol is unable to stop this reaction, the inflammatory response is sustained throughout the lifetime, causing a myriad of negative health consequences.

Third, not only are physiological responses altered by stress in childhood, but also psychological damage results. Miller and colleagues suggest that when children are exposed to threats and adversity in childhood, the amygdala, which is a part of the brain that shapes the way ambiguous information is processed, is altered (2012). When this occurs, children who grow up with adversity become adults who have "altered corticolimbic responsivity to emotional stimuli" (Miller et al., 2012). This means that children who experience stress are more likely than children who did not experience stress to interpret ambiguous situations as negative or threatening throughout their lifetimes and respond with activation of emotional centers of the brain and the HPA-axis.

Fourth, the reshaping of these brain parts gives rise to a phenotype that is highly discountive of the future, and thus individuals behave impulsively. These individuals have a propensity to engage in health compromising behaviors like smoking, drinking, engaging in unsafe sex, and more. These risky behaviors are related to even more chronic inflammation; for example, studies show that smokers have chronic low-grade inflammation even 10 years after quitting (Yanbeava, Dentener, Creutzberg, Wesseling, & Wouters, 2007).

The combination of these occurrences, which all spring from adversity in childhood, create the perfect storm for negative health outcomes to transpire. Perhaps this explains why research consistently finds that childhood stress is related to poor health in adulthood.

The current section documents that a myriad of frightening and life-threatening illnesses in adulthood are all linked to childhood stress, but is it possible that the effects of stress could be observable earlier in life? Because HPA-axis functioning supposedly mediates the link between stress and health, it seems reasonable that effects of stress may appear in early life.

Children's stress and concurrent health. From casual observation alone we can see that children who are stressed are sick. It seems that students always suffer from stomach viruses or respiratory illnesses at the end of the semester during final exams. We know from experience that our own health plummets in the face of stress. Thus, it seems obvious that poor health could result from early childhood stress. In fact, research is confirming our assumptions. The following studies comprise some of the most interesting studies in the small, yet growing field of research. In 2010, Quinn, Kaufman, Siddiqi, and Yeatts noted that housing stressors, like too many people living in one space, inability to pay for services like power, water, and rent, and experiencing landlord troubles were related to higher rates of asthma in Chicagoan children. Authors note that this finding supports the idea from Wright, Cohen, Cohen (2005) and Wright (2005) that "psychological stress [is] a 'social pollutant' that may be 'breathed' into the body." This statement agrees well will Miller and colleagues' Biological Embedding of Childhood Adversity model.

On a related note, Flaherty and colleagues explored the link between children's exposure to adversity, such as caregiver depression, violence in the home, and criminal behavior and health. They found that one exposure doubles the risk of general poor health in children and that four or more adverse exposures triple the risk of illness requiring

medical treatment in children (2006). It seems reasonable to believe that children who are experiencing chronic stress have high numbers of poor health outcomes.

We know that a link exists between stress and concurrent health, but we do not know why. Classically, the link between early childhood stress and poor health has been attributed to environmental factors that are correlated with stressors, factors such as poor diet, exposure to mold or pests, and poor sleep quality. There is some evidence that these items contribute to poor health. However, only some of the variation in health is accounted for. Thus, researchers are now searching for clues within the realm of stress. As noted earlier, some of the chemicals of the stress response system take effect in the body immediately, while others are more long-lasting. Also, we know that in the face of a stressor, the body halts non-essential activities (like immune response) and uses energy on life-saving activities only. Because of this, it seems logical to assume that some negative health outcomes of living in adverse circumstances could be attributed to the body's stress response. Perhaps quick-acting hormones impact health immediately, whereas long-acting hormones affect health for years to come. Another factor at play could be the suppression of immune responses, which exposes the body to illnesses that would have otherwise been nonthreatening. At the root of these complex interactions is cortisol, the main actor in the body's stress response. Recently, scholars have identified cortisol as a possible player in the link between stressors and health, and Iplan to investigate this idea further.

Children's Cortisol and Concurrent Health

Existing research. To date, only one research study exists that explores the relationship between cortisol and concurrent physical health in children. This study was

published in two parts, one in 1995, and another in 1997. Flinn and England examined a group of 264 children ages 2 months to 18 years from a rural Caribbean township, finding impressive support for the theory. For example, Flinn and England (1997) identified household composition as a major factor—children living with single mothers or nonrelatives had significantly higher cortisol on average than children living with close family. Also, step-children experience higher daily cortisol on average than genetic off-spring. More than any other variable observed, trauma in the family was associated with elevations in cortisol across every age group. Moreover, cortisol and illness were positively correlated. Illnesses like diarrhea, the common cold, and asthma were more commonly observed in children who experienced heightened cortisol levels in the previous days (Flinn & England, 1995). Thus, children who experience higher average cortisol also experienced more sickness.

Children living in households with consistent, stable caretaking had moderate cortisol levels, whereas children living in households with unstable caretaking had abnormally high or abnormally low cortisol. As may be recalled, both higher-than-normal and lower-than-normal cortisol levels are dysfunctional and unhealthy. Interestingly, households that were considered stable and consistent in terms of caretaking may have experienced similar numbers of stressful and traumatic events to households with unstable caretaking, but positive affectionate events, security of attachment between caregiver and child, and development of coping techniques in children may have shielded these children in affectionate homes from the negative effects that were expected.

The present study. The current study examines links between measures of cortisol collected in childcare centers when children were four years old and their health, as

measured by mother's responses to the Rand Health questionnaire and respiratory health questions. This study will help flesh out the meager body of research on cortisol and health in early childhood.

III. METHOD

The National Science Foundation funded the Child Care Quality Enhancement Project (CQEP), from which the data for this study were taken (NSF #0126584 to J. Mize). This short-term longitudinal study sought to investigate the cognitive, social, and physiological experiences of children in childcare, exploring how each of these is related to children's competence and adjustment concurrently and a year later in the kindergarten environment. The data were collected in three cohorts of children enrolled in childcare programs, and follow-up data were collected when children entered formal education (i.e., one year later in kindergarten). The Institutional Review Board and the Office of Human Subjects Research approved all procedures for the CQEP (IRB #00 – 141 MR 0006), as well as the secondary analyses reported here (IRB #12 – 328 EX 1210 to A. Newberry).

This report focuses on cortisol, which was measured in saliva samples collected on several occasions from children in the childcare setting. In particular, in order to estimate HPA-axis patterns, this study considers cortisol measured in saliva samples collected in the mornings and afternoons. Procedures for collecting saliva samples and assaying cortisol will be discussed later in this chapter. Estimates of children's health were derived from parent report. Parents also supplied demographic information regarding their and their partners' employment and level of education.

Participants

Participants were recruited from four-year-old classrooms in 12 different childcare centers in two medium-size communities in the southeastern U.S. Over three years, data were collected in 47 different groups, or classrooms, of children. Over this time, 507 children received permission to participate and provided some data. For every child that participated, the director of their center, their teacher, and their parent signed informed consent papers. No differences as a function of race or child sex existed between children who were given consent to participate and children who were not.

Small monetary gifts ($5) were offered to each childcare center for each family and child that participated. For each classroom with over 75% of children participating, additional monetary incentives were offered. In addition, each teacher who completed questionnaires regarding their background, education, experience, and teaching philosophy received $20. The average rate of participation across all cohorts and groups was 81%.

Focus Sample. Because of monetary constraints, saliva samples were not obtained from all 507 participants. Instead, a subset of classrooms was selected in each year of the study to complete the more time-consuming and expensive procedures; children in these classrooms are referred to as the focus sample. Sixteen classrooms participated as focus classrooms over the three years of data collection in preschool. In the focus classrooms, 209 children received parental permission to participate over the course of the study. There was one difference between children in the focus and non-focus classrooms.: Children from the non-focus group were slightly younger (51.8 months vs. 52.33 months) There were no other differences between focus and non-focus children.

Focus Sample Demographics. As a result of family moves in the middle of the year, children's inability to produce enough saliva for tests, and children's varied daily schedules, only 189 of the 209 children enrolled in focus-sample classrooms had saliva samples. Of these 189 children, 127 were Caucasian ("majority"); 62, or about 33% of the sample, were from other ethnic or racial backgrounds ("minority"). One-hundred-and-two boys were in the sample. Average age of children was 52.33 months, with a range of 36 months to 67 months. Based on occupational prestige scores (Entwisle & Astone, 1994; see Measures) and parent education, most families were middle class, but there was a wide range of occupations (unemployed, unskilled, managers, professionals) and educational levels represented.

Measures

Socioeconomic indicators. Parents were asked to report job title and place of employment for themselves and for their partners. Parents' responses were later coded into U.S. Census Bureau occupational codes and assigned occupational prestige scores based on recommendations of Entwisle and Astone (1994) and Nakao and Treas (1994). Occupational prestige scores can range from 0 to 100; in these rankings, based on 1990 Census data, male civil engineers receive a score of 82.67, whereas male laundry workers have a score of 33.14, and textile sewing machine operators receive a score of 18.18. For families with two employed parents, the higher occupational prestige score was used. Median score was 64 (elementary school teachers, fire lieutenants, medical technicians, and paramedics received occupational prestige scores of 64), with a range of 10 to 93.34.

Parents also reported the highest level of education they completed, with 1 indicating "some high school" and 6 indicating "post-college." The sample was well

educated, with over half having completed at least a college degree (58%). However, a wide range of education levels was represented with almost 10% (9.3%) having a high-school education or less.

Health. Parents completed the short version of the RAND health questionnaire (Eisen, Donald, Ware, & Brooke, 1980; Nakao & Treas, 1994) to which CQEP researchers added two items related to respiratory illnesses. Eisen et al. (1980) note that the original version of this measure has adequate reliability ($\alpha = 0.77$). Several questions focused on the child's general health status (e.g., "In general, would you say this child's health is excellent, good, fair or poor?"), whereas other items focused on specific health problems and the frequency with which the child experienced the problems (e.g., "This child has had an earache or earache with fever," rated as occurring "not at all" to "almost always"). It was necessary to reverse score five items so that higher scores consistently reflected worse health. Because some sections of the questionnaire offered 4-point response scales, whereas other sections offered 5-point response scales, items were standardized over the full sample prior to analysis. For this study, I constructed a composite, *poor health*, by averaging five items that reflect persistent health problems. The composite had adequate internal consistency (alpha = .75) and was made from the standardized forms of the following items: (a) "In general, would you say this child's health is excellent, good, fair, or poor?"; (b) "This child's health is excellent" (reverse scored); (c) "This child seems to resist illness very well (reverse scored); (d) "This child has had allergies without asthma"; (e) "A doctor has said this child has asthma." Information about individual health items can be found in Table 1. An examination of individual items that made up the composite revealed that most parents generally rated

their children as in excellent or good health and as resisting illness very well or well. However, mothers report that 17% of children were described as having been diagnosed with asthma and 28% of the children were said to have allergies.

Table 1

Descriptions of individual items composing poor health composite

	N	*M*	*SD*	Range
General health [a]	152	1.30	.54	1 – 4
Not excellent health [b]	152	1.39	.69	1 – 5
Poor illness resistance [c]	152	2.03	.95	1 – 5
Asthma [d]	152	.61	1.07	0 – 4
Allergies [e]	152	.49	1.18	0 – 4

Note. Higher scores reflect worse health in all cases.
[a] "In general, would you say this child's health is excellent, good, fair, or poor?" excellent = 1, poor = 4. [b] "This child's health is excellent." Definitely false = 5, Definitely true = 1. [c] "This child seems to resist illness very well." Definitely false = 5, Definitely true = 1 [d] "This child has had allergies without asthma." not at all = 0, almost always = 4. [e] "A doctor has said this child has asthma." not at all = 0, almost always =

Saliva collection, storage, and assay procedures. To examine children's HPA-axis activity, nine saliva samples were collected from children at their child care centers. However, only values from samples collected on mornings and afternoons of two days are of interest in this study. The other samples were collected before and after a stressful task and after a teacher-child interaction, and thus do not necessarily reflect daily cortisol patterning.

Over the course of the fall semester of each year, children from the focus group were taught to spit or drool into a cup and were given the opportunity to practice the task twice (morning and afternoon of one day). By allowing this practice session, children were introduced to the procedures of the study, thus reducing potential cortisol elevations that may have occurred simply because of the novelty of the task (see Flinn, 1999). The samples that resulted from practice sessions were not analyzed, with the exception of six cases in which children could not produce enough saliva for analysis during one of the later sample collections. In these six cases the practice sample was substituted for the missing sample.

On saliva collection days, children were gathered in small groups at a table to "play the spitting game" at mid-morning during free-play time and again in mid-afternoon during free play (at approximately 10:00 a.m. and 2:00 p.m., respectively). Children seemed to enjoy these sessions and did not seem to experience stress during saliva collection. Researchers developed a number of strategies to help children produce sufficient saliva without the use of stimulants. For instance, children were shown pictures of flavorful foods (e.g., pizza, ice cream) and asked to imagine they were eating the foods. The precise time at which each child contributed each saliva sample was recorded.

Care was taken to ensure that saliva samples were not contaminated with food or blood. The presence of food in saliva can change its pH and thereby affect assay results. The presence of blood in saliva dramatically increases the amount of cortisol in the sample because concentration of cortisol in blood is much higher than it is in saliva. The lab that conducted assays screened for and excluded samples with blood contamination. In addition, children who appeared to be ill were excluded from saliva collection

(samples were obtained on another day when the child was well) because illness dramatically increases cortisol. For detailed descriptions of saliva collection procedures, see Lisonbee, 2004.

After collection, saliva samples were immediately placed on ice in a cooler at the center. These were then transported to a freezer in the research offices. Frozen samples were sent to Salimetrics Laboratory on the Pennsylvania State University campus for assaying. Each of these samples was assayed in duplicate in order to validate the concentrations of cortisol. Correlations between duplicate assays of the same sample exceeded .99. Thus, duplicate values were averaged to provide more reliable estimates of true values at each collection time. As is common practice in the field of cortisol research, extremely high cortisol values that likely reflected blood contamination or fever, rather than valid values, were deleted from the sample.

For the current study, cortisol values from the two morning collections were significantly correlated ($r = .30$, $p < .001$) and values from the two afternoon saliva collections were marginally correlated ($r = .13$, $p < .11$). Itherefore averaged the two morning and two afternoon values to create average *morning cortisol* and average *afternoon cortisol,* respectively. Because cortisol values are highly positively skewed, they were transformed using a log 10 transformation prior to analysis. However, descriptive statistics are reported in the original untransformed values of micrograms of cortisol per deciliter of saliva (µg/dl).

Because both the total amount of cortisol produced over the day and the diurnal pattern (i.e., change over the day-light hours) have been implicated in studies of health,Ialso created measures from the average morning cortisol and average afternoon

cortisol values to provide an approximate reflection of these constructs. *Total cortisol* was computed as the sum of the average morning and average afternoon cortisol values. *Daily change cortisol* was computed as the average afternoon value minus the average morning value of cortisol. Steeper declines in cortisol across the day are reflected in more extreme negative daily change values. Children whose cortisol increased over the day have positive daily change scores. For easy reference, descriptions of the cortisol variables can be found in Table 2.

Table 2

Cortisol variables definitions

Variable Name	Definition
Morning cortisol	Average of 2 morning salivary cortisol values (one winter and one spring), taken in classroom
Afternoon cortisol	Average of 2 afternoon salivary cortisol values (one winter and one spring), taken in classroom
Total	Morning cortisol + Afternoon cortisol
Change	Afternoon cortisol – Morning cortisol

Note. All non-transformed cortisol values are measured in micrograms of cortisol per deciliter of saliva (μg / dl).

The cortisol variables were, for the most part, correlated in expected ways. Children with higher morning cortisol had marginally higher afternoon cortisol (r = .12, p < .10). Of course, both morning and afternoon cortisol values were significantly associated with composites made from these variables (absolute values of r = .64 –.76).

Time since wake up. Because cortisol levels normally peak in the morning hours after waking up and then decline throughout the day, Icontrolled for children's wake time for analyses involving cortisol. Parents reported the time their child had awakened when they dropped children off for school. Time between waking and morning and afternoon saliva collections were computed to form *time since waking (morning)* and *time since waking* (*afternoon)*, respectively. The morning and afternoon time measures were significantly correlated ($r = .57$, $p < .001$), morning times were moderately correlated with morning cortisol values ($r = -.38$, $p < .001$), and afternoon times were modestly correlated with afternoon cortisol values ($r = .14$, $p < .05$). However, controlling for morning time, the correlation between afternoon time and afternoon cortisol was no longer significant ($r = .15$, $p > .05$). Therefore, in order to preserve power, I will use a single average time in minutes from waking to morning saliva collection (*time since waking)* in analyses. Time since waking ranged from less than 30 minutes to more than 4 hours ($M = 2.41$ hours).

IV. RESULTS

First, I examined stem-and-leaf plots, scatter plots, and box plots for all study variables. Only cortisol values deviated substantially from normality. In the sections to follow, I will present descriptive statistics of study variables, analyses examining differences in study variables as a function of child sex and minority status, and bivariate correlations among study variables. Then I will present results of partial correlations examining hypotheses one through four. Finally, to examine hypothesis five, I will present results of mediational analyses using path analysis.

Preliminary and descriptive analyses

Demographic. Descriptive statistics for main study variables can be found in Table 3. As would be expected based on individual items that made up the poor health composite, the composite was positively skewed, indicating that, for the most part, children in the sample were described as healthy. Although the means of morning and afternoon cortisol do not appear to be different in Table 3, repeated measures analysis of variance using transformed cortisol scores indicated that afternoon cortisol was significantly lower than morning cortisol (*M*s = -.80 and -.82, respectively, $F = 15.82$ (1, 160), $p < .001$).

Table 3

Descriptive statistics for main study variables

Statistic	*N*	*Mean*	*SD*	Range
Morning cortisol	184	.20	.24	.06 – 2.85
Afternoon cortisol	183	.20	.24	.05 – 3.07
Total cortisol [a]	179	.40	.45	.11 – 5.92
Daily change [b]	179	-.01	.15	-.61 – .55
Poor Health	152	-.06	.68	-.68 – 3.08
Occupational Prestige	144	67.49	18.61	10 – 93.34
Education	150	4.83	.95	1 – 6
Age in months	189	53.22	4.20	36 – 67

[a] *morning cortisol + afternoon cortisol.* [b] *afternoon cortisol – morning cortisol.*

Associations among individual demographic and health variables were largely as expected based on existing literature (see Tables 4 and 5). No differences in study variables existed as a function of sex. Parents with higher levels of occupational prestige had higher levels of education ($r = .65$, $p < .0001$). A number of differences as a function of minority status emerged, as can be seen in Table 4. The parents of minority status children had completed less schooling and had lower occupational prestige scores. Minority children had higher morning cortisol and higher total cortisol than did majority (white) children. Minority status children also had considerably poorer health. Child age was associated with several of the main study variables (see Tables 5 and 6). The parents

of older children had lower occupational prestige scores and less education. Controlling for time since waking, older children had lower morning cortisol.

Table 4

Study variables as a function of minority status

Variables	Mean		*f* (significance)	*Df*
	Non-minority (white)	Minority		
Cortisol (raw)				
Morning	.17	.22	5.56*	1, 182
Afternoon	.18	.23	1.76	1, 182
Daily change	.01	-.04	4.62*	1, 177
Total	.36	.41	3.48^{+}	1, 177
Health				
Poor health [a]	-.22	.27	19.32****	1, 150
Demographics				
Occupational Prestige	67.49	49.92	25.09****	1, 142
Parent Education [b]	4.83	3.96	19.16****	1, 148

Note. Untransformed cortisol values are reported.
[a] standardized composite. [b] highest level of education completed by caregiver who responded to the demographic questionnaire. 0 = *some high school*, 6 = *post college.*
$^{+}p < .10$; $*p < .05$; $****p < .001$.

Table 5

Bivariate and partial correlations of cortisol with health and demographic variables.

	Cortisol			
Variables	Morning	Afternoon	Daily change	Total
Poor Health	.20****	-.05	-.20****	.09
	.22**	**-.05**	**-.21****	**.11**
Occupational Prestige	-.07	-.07	.03	-.09
	-.16*	**-.07**	**.10**	**-.14***
Education	.05	.00	-.03	.04
	.00	**.00**	**.02**	**.01**
Race	.20	.07	-.12	.17*
	-.21**	**.07**	**-.12**	**.17***
Age	-.07	-.03	.02	-.08
	-.14*	**-.03**	**.06**	**-.12**

Note. Partial correlations (controlling for time since waking) are shown in bold

*$p < .05$; **$p < .01$. One tailed.

Examination of Study Hypotheses

To examine the first four hypotheses concerning associations between cortisol and poor health, a series of partial correlations between cortisol and poor health were computed controlling for time between waking and morning saliva collection. To examine meditational relationships, a series of path analyses were fitted.

The first hypothesis stated that children with poorer health would have lower morning cortisol. There was no support for this hypothesis. Rather, as can be seen in Table 5, children with poorer health had higher morning cortisol.

The second hypothesis stated that children with poorer health would have lower afternoon cortisol. There was no support for this hypothesis. As can be seen in Table 5, there was no association between afternoon cortisol and poor health.

The third hypothesis stated that flatter, less steep declines in cortisol from morning to afternoon, or increasing cortisol from morning to afternoon, would be associated with poorer health. There was no support for this hypothesis. In fact the opposite pattern to that predicted was obtained: the correlation between daily change and poor health was negative. One may recall that steeper declines are reflected in more extreme negative change scores. No change would be reflected by a score of 0, and increases over the day would be reflected by positive daily change scores. Children with flatter, less steep declines in cortisol over the day had poorer health (See Table 5). To clarify the nature of these differences further, the sample was split into thirds based on daily changes in cortisol: the declining group had the poorest health, the flat group had somewhat better health, and the increasing group had the best health (*M*s for poor health = .18, -.10, and -.20, respectively).

The fourth hypothesis stated that children with higher total cortisol would have poorer health. Although the correlation was in the predicted direction, it was not significant (See Table 5). Thus, there was no support for the fourth hypothesis.

The fifth hypothesis was that cortisol would mediate observed associations between measures of family risk and child health. According to Baron and Kenny

(1986), in order to establish a mediational relationship, it is necessary to first show that (a) the predictor is significantly associated with the proposed mediator; (b) the predictor is significantly associated with the outcome of interest; and (c) that the proposed mediator is significantly associated with the outcome. If these three conditions are met, it is then permissible to test for mediation. Table 6 presents bivariate correlations between demographic and health variables and Table 7 presents a summary of our examination of these criteria. Partial correlations were used to evaluate these criteria.

Table 6

Bivariate correlations between demographic and health variables

Variables	Poor health	Occupational prestige	Education	Race	Age
Poor Health	1				
Occupational prestige	-.26**	1			
Education	-.34**	.65**	1		
Race	.34**	-.39**	-.34**	1	
Age	.08	-.22**	-.21**	.12	1

**$p < .01$

Table 7

Table of Mediational Criteria

Risk Factor	Cortisol measure	Results of Correlational Analyses: Risk Factor – Cortisol	Cortisol - Poor Health	Risk Factor – Poor Health	Were all three criteria stipulated by Baron and Kenny satisfied?
Minority Status					
	Morning	+	+	+	Yes
	Afternoon	0	0	0	No
	Change	^	+	+	No
	Total	+	^	+	No
Occupational Status					
	Morning	+	+	+	Yes
	Afternoon	0	0	+	No
	Change	0	+	+	No
	Total	+	0	+	No
Parent Education					
	Morning	0	0	+	No
	Afternoon	0	0	+	No
	Change	0	0	+	No
	Total	0	0	+	No

+ A significant correlation or significant partial correlation (controlling for time since waking in correlations with cortisol variables) was obtained; ^ A marginally significant correlation or marginally significant partial correlation (controlling for time since waking in correlations with cortisol) variables was obtained; 0 Correlation between the relevant measures was not significant.

Two paths met these conditions. First, controlling for time since waking, occupational prestige was significantly negatively correlated with morning cortisol (r = -.16, $p < .05$) and with poor health (r = -.26, $p < .01$), and health was significantly positively correlated with morning cortisol (children with poorer health had higher morning cortisol) (r = .22, $p < .01$). Second, minority status was significantly correlated with morning cortisol (r = - .21, $p < .01$) and poor health (r = .34, $p < .01$), and morning cortisol was significantly correlated with poor health (r = .22, $p < .01$). MPlus was used to examine the two potential mediated paths. Full Information Maximum Likelihood Estimation was used to estimate missing values, and therefore to preserve sample Ns. The proportion of data present for variables used in path analysis ranged from 73% to 97% with a median of 78%. Visual depiction of these paths can be found in Figures 2 and 3, respectively.

Test of first potential mediational path: Occupational prestige through morning cortisol to child health. I fit a path model using MPlus to determine whether data were consistent with the hypothesis that morning cortisol would mediate the association between parents' occupational prestige and poor health in children. I estimated effects of time since waking on morning cortisol and allowed morning cortisol to covary with occupational prestige. I also estimated the direct effects of occupational prestige and morning cortisol on poor health, and the indirect effects of occupational prestige on poor health.

This model fit the data very well. As can be seen in Figure 2, the chi-square test of model fit was not significant. The Tucker Lewis Index (TLI) and the Comparative Fit Index (CFI) demonstrate how much better the model fits compared to a baseline model

(in which there are no paths). A value between .90 and 1 indicates the model is a good fit. For the model with occupational prestige, the TLI was 1.06 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). 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 .01, which again suggests the model was a good fit.

As can be seen in Figure 2, both parents' occupational prestige and morning cortisol significantly predicted child health. Additionally, children whose parents had lower occupational prestige had been awake longer when morning saliva samples were collected. However, the indirect path from parent occupational prestige through morning cortisol to poor health was not significant (unstandardized indirect effect = -.02, standardized indirect effect = .00, p = .20). Children with higher morning cortisol and whose parents had lower occupational prestige had poorer health. That is, both children's morning cortisol and parents' occupational prestige made unique, independent contributions to children's poor health. The model predicted 11% of the variance in child health.

Test of second potential mediational path: Minority status through morning cortisol to child health. To examine the hypothesis that minority status would be associated with children's poor health through its effects on morning cortisol, I fit a path model similar to the one described in the previous paragraph, except that minority status was used in place of occupational prestige (see Figure 3).

The model was a good fit. The chi-square test of model fit was not significant (χ^2 = 1.21, *df* = 1, *p* = .27). The TLI was .98 and the CFI was .99, indicating good fit. The RMSEA for this model also indicated that the model was a good fit (RMSEA = .03, *p* = .40). The value for the SRMR was .02, which again suggests the model was a good fit. As can be seen in Figure 3, minority status children had higher morning cortisol and poorer health. Again, however the indirect path from minority status through morning cortisol to poor health was not significant (unstandardized indirect effect = .04, standardized indirect effect = -.01, *p* = .62). Minority status, but not cortisol, predicted poorer health. The model predicted 13% of the variance in child health.

Combined model: Occupational prestige and minority status through morning cortisol to child health. To provide a more comprehensive and stringent test of contributions to children's health, I fit a third path model in which poor health was regressed on occupational prestige, minority status, and morning cortisol, controlling for the effects of waking time on morning cortisol. This model also fit the data very well, with excellent fit indices. The chi-square test of model fit was not significant (χ^2 = .51, *df* = 1, *p* = .47). The TLI was 1.06 and the CFI was 1.00, indicating good fit. The RMSEA for this model also indicated that the model was a good fit (RMSEA = .00, *p* = .57). The value for the SRMR was .01, which again suggests the model was a good fit. For the most part, results were consistent with the first two models. As can be seen in Figure 4, both lower occupational prestige and minority status significantly predicted poorer child health. Tests of indirect effects of occupational prestige and minority status on poor health were also insignificant (unstandardized = .04 and .00; standardardized = .01 and -.01 for minority and occupational prestige, respectively, all *p*s > .10. With both minority

status and parents' occupational prestige in the model, higher morning cortisol marginally predicted poorer health. This model predicted 16% of the variance in children's health.

Figure 2. Fitted path diagram (unstandardized solution): The regression of poor health on morning cortisol and occupational prestige, controlling for time since waking (with estimated correlations in parentheses).

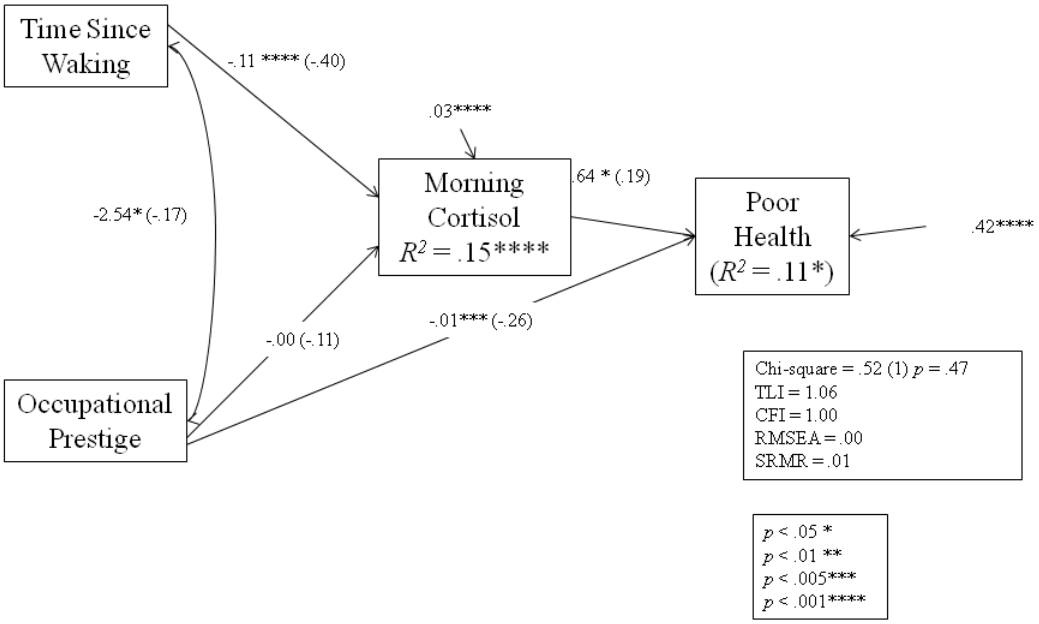


Figure 3. Fitted path diagram (unstandardized solution): The regression of poor health on morning cortisol and minority status, controlling for time since waking (with estimated correlations in parentheses).

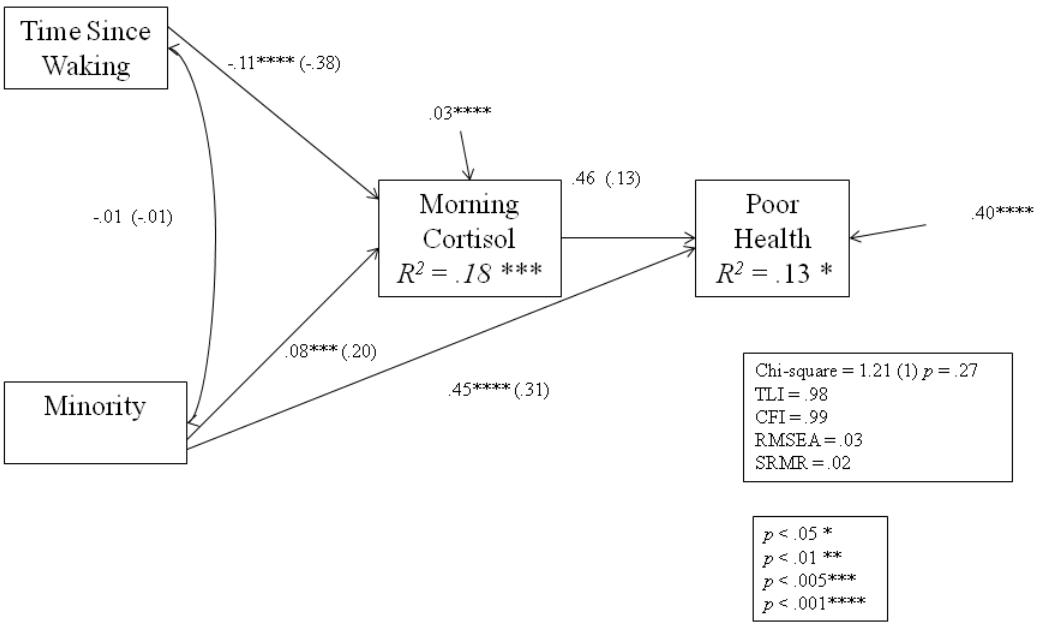
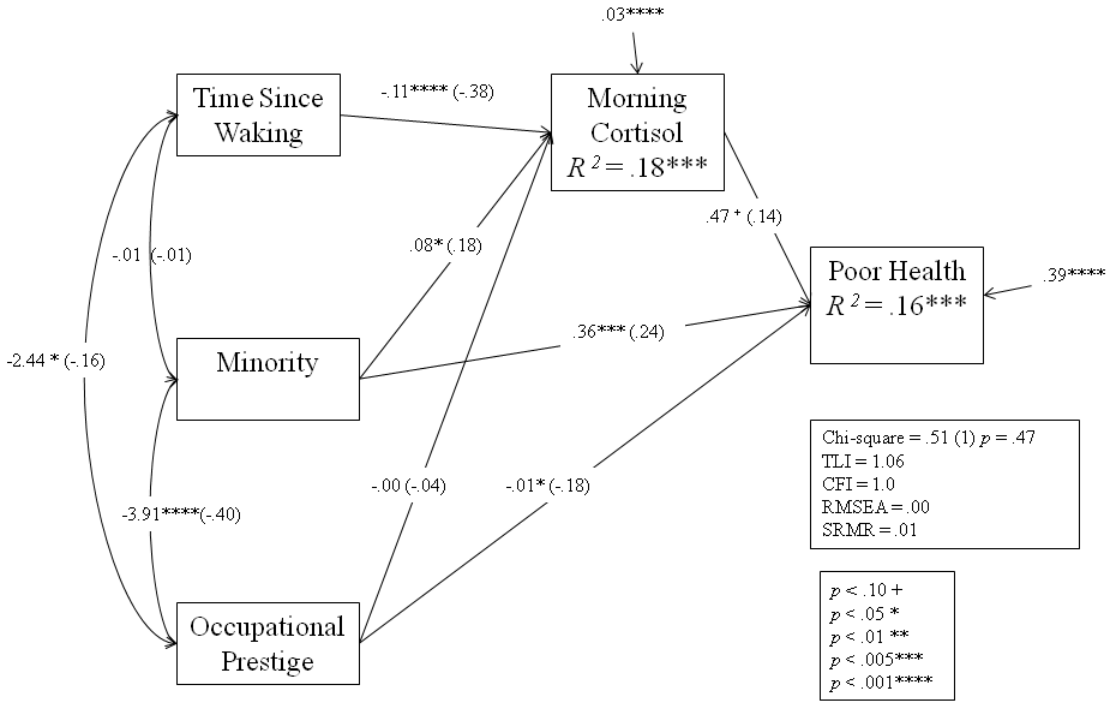


Figure 4. Fitted path diagram (unstandardized solution): the regression of poor health on morning cortisol, occupational prestige, and minority status, controlling for time since waking (with estimated correlations in parentheses).



V. DISCUSSION

The current study finds that family risk factors and children's higher morning cortisol independently predict poorer child health. Although many studies document links between risk factors and adult health, and a few studies link cortisol with adult health, this may be the first study to show independent contributions of both risk and cortisol on concurrent health in young children. These data suggest that the corrosive effects of stress on the human body start early and are manifest in cortisol patterns and poor health.

I had expected that cortisol would mediate the associations between risk factors and poor health. However, rather than mediating links between risk and health, cortisol and risk additively contributed to poor health. This was true for two of the risk factors examined in this study, parent low occupational status and minority status, when each was examined separately with cortisol. The fact that minority status still predicted some of the variance while occpuational prestige was in the model suggests that minorities do not have poor health simply because they have fewer resoureces available to them. Had this been the case, occpuational prestige would have captured all of the effects that race contributed to poor health. Instead, it seems that minority status itself is actually contributing to health independently, suggesting that it descrimintation towards minorities regardless of their occupational prestige is at play.

Associations between risk status and poor health across all stages of the life span are well documented (Anda et al., 2009; Falitti et al., 1998; Galobardez et al., 2004;

2008; Miller et al., 2011). Therefore, in this study I was particularly interested in whether cortisol would be associated with health in young children, and if so, whether cortisol would mediate any associations between risk factors and health. I had predicted that atypical cortisol patterns -- low (rather than high) morning, high (rather than low) afternoon, flattened (rather than steeply falling) diurnal -- would be associated with poorer health. Contrary to these predictions, for all significant cortisol – health associations, it was more extreme "normal" patterns that were linked to poorer health. It was children with higher morning cortisol and steeper morning to afternoon declines in cortisol who had poorer health. Perhaps the atypical cortisol patterns I expected have not yet emerged for the young children in our sample. The only other existing study examining the links between HPA-axis activity and children's health concurrently analyzes a sample with children ages 2 to 18 years, and does not analyze young children separately (Flinn & England, 1995; 1997). Perhaps for children living in adversity, atypical rhythms emerge in later childhood. To my knowledge, however, studies supporting this idea do not yet exist. Future studies should examine these issues longitudinally in order to determine when atypical patterns emerge for children living in adverse circumstances.

Second, even though I controlled for time since waking, it is possible that this was not an adequate method of eliminating the effects of differences in children's schedules. This is particularly true for children who had been up for extremely long periods of time. Cortisol naturally plummets soon after waking, but the rate of decline slows over the morning and afternoon hours. Thus, for instance, cortisol declines for children who had been awake for 4 hours would not be twice the declines of children who had been awake

for two hours. However, as far as I know, there are no algorithms to account for the variation in rate of decline over the day. Future research could aim to manage this issue by having parents take samples from children at a specific number of minutes post-wake up. For example, participants could donate samples within 30 minutes of waking to eliminate the effects of wake time on cortisol.

Finally, collection of saliva samples while children were in childcare settings distorts typical circadian rhythms. For example, previous studies show that children who display normal circadian rhythms at home show atypical or flattened patterns in the childcare setting (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Watamura, Donzella, Alwin, & Gunnar, 2003). The fact that saliva samples were collected at childcare may also explain why there were no associations between afternoon cortisol values and either health or risk factors. It is possible that children's living conditions at home more strongly predict morning cortisol levels, but that conditions at child care predict afternoon values. In fact, previous research indicates that factors such as poor child care quality, more children in a class, and less classroom space are associated with higher afternoon cortisol when children are at child care (Geoffroy et al., 2006; Legendre, 2003; Sims, Guilfoyle, & Parry, 2005)

Of the three risk factors examined in this study, two, parents' occupational prestige and minority status, were associated with children's cortisol. Minority children had higher morning cortisol, higher total daily cortisol output, and steeper declines in cortisol across the day than did white children. Children whose parents had lower job status had higher morning cortisol and higher total daily output of cortisol. These findings are not consistent with what is known about adult cortisol patterns. Normally, stress leads

to lower morning cortisol, higher afternoon cortisol, and flattened patterns over the course of the day. For instance, Cohen, Schwartz, Epel, Kirschbaum, Sidney, and Seeman report, in a 2006 study of young adults, that being African-American and being from a low SES background were associated with higher afternoon cortisol and less steep declines in cortisol over the day.

Why I found patterns opposite to those typically reported for studies of cortisol and health in adulthood is unclear. Again, it is possible that among very young children, HPA-axis activity does not yet reflect risk factors in the same way that it will in late childhood and adulthood. Alternatively, as suggested earlier, cortisol measured in child care settings may not reflect children's typical patterns. Future research should attempt to disentangle contextual and developmental effects on links between HPA-axis activity and risk factors. This could be achieved by embarking on research ventures that explore the links between children's cortisol and adult's cortisol. More information about how stress effects children's cortisol and HPA axis activity in ways that are different from adults would help shed light on the topic.

Patterns of association among risk factors and between individual risk factors and health were consistent with prior research. Parents with lower occupational status had lower levels of education, a finding consistent with a large body of research showing that education is a key to job success (Ng & Feldman, 2009). Our data testify to the robustness of the education – job link because the current sample contained a number of parents who were university students, many of whom may have had low job status but high levels of education. The minority parents in this study had completed less schooling and had lower status jobs, which, again, is consistent with a large body of research (e.g.,

Williams, Mohammed, Leavell, & Collins, 2010). Thus, our data are evidence of the robustness of these associations because a number of the minority parents in this sample were highly educated legal aliens pursuing advanced degrees.

Less studied is the fact that children whose parents had lower job status had been awake longer at the time of morning saliva collection. This probably reflects the requirement of many lower-status workers to report to work early. Many factories, hospitals, and food service operations begin morning shifts at 7:00 a.m. Single-parent families and families in which both parents occupy low-status jobs would necessarily waken children early to take them to child care. The fact that parents of older children had less education and lower status jobs may be a quirk of this sample; to our knowledge, it has not been reported previously.

Numerous studies, including this one, report that lower SES children (including children whose parents have less education and lower job status) and minority children have poorer health (Case, Lubotsky, & Paxson, 2001; Quinn, Kaufman, Seddiqi, Yeatts, 2010). The fact that these findings are in keeping with literature validates our measure of health.Traditional explanations for links between risk factors and health have included social, behavioral, or cognitive propensities. For instance, parents with lower job status, and thus lower income, may provide less nutritious food and seek medical care less often. In this study children of parents with lower job status woke up earlier, at least on days saliva was collected. It is possible that children of parents who have lower status jobs get insufficient sleep and that their health suffers as a result. However, this explanation is not supported by our data in that neither the correlation between children's average wake-up time on saliva collection days and poor health nor the correlation between children's

typical wake up time and poor health (also reported by mothers) were significant (*r*s = -.08 and -.01, respectively).

Limitations. One of the most obvious limitations of this study is that it is cross sectional. In this study, I examined a model of the effects of risk factors in early childhood on poor health as mediated by cortisol. However, all data were collected essentially concurrently. Mediational models necessarily imply that effects occur over time, but when measures of the proposed predictor, outcome, and mediator are collected at the same time, it is impossible to be certain of the causal order among the variables. In this study, I reasoned that the risk factors, for most families, had been stable for months, if not years, prior to the data collection. Although parent questionnaires were distributed early in the year, data are not available on whether or not questionnaires were returned prior to saliva collection. Obviously, to definitively establish temporal associations among risk factors, HPA-axis dysregulation, and child health, longitudinal data are required (MacKinnon, 2008).

A second limitation is that cortisol was assessed only when children were in child care. Given that cortisol, particularly afternoon cortisol, tends to be higher in child care environments than when children are at home (Dettling et al., 1999; Dettling et al., 2000; Geoffroy et al., 2006), cortisol levels probably do not reflect the true basal levels I could expect if saliva samples were taken in the home. Multiple factors that were not controlled in this study, such as quality of the child care program, space, and teacher-child relationship quality (Geoffroy et al., 2006; Legendre, 2003; Lisonbee et al., 2004), surely affected children's cortisol in this study. Ideally, future studies would obtain estimates of children's cortisol in multiple settings, including home and child care.

Another limitation is that estimates of children's cortisol were based on the averages of only two morning and two afternoon saliva samples per child. Because a great deal of the variation in cortisol is the result of transient, situational factors (Hellhammer, Fries, Schweisthal, Scholtz, Stone, Hagemann, 2007; Shirtcliff, Granger, Booth, & Johnson, 2005), greater reliability is afforded by having many samples that can be used to create a composite or latent construct. Clearly, when possible, more than two samples for any given context of interest should be obtained. However, costs of cortisol assays are prohibitively expensive (currently over $17 per sample at Salimetrics lab) without considerable outside funding. Moreover, although only two saliva samples were used to estimate morning cortisol, two risk factors showed significant correlations with morning cortisol, and morning cortisol contributed significantly to the prediction of children's health, even controlling for the risk factors. The fact that the most robust pattern of associations was found with morning cortisol is consistent with claims that morning cortisol is relatively more influenced by trait-like characteristics, whereas cortisol during afternoon and evening hours is more influenced by transient, or state-like, factors (Kertes & van Dulmen, 2012). Thus, despite the fact that only two morning and two afternoon saliva samples were available, the cortisol measures, particularly morning cortisol, appeared to capture meaningful individual differences in cortisol.

Another limitation is that, although minority representation was relatively high (about a third of the sample); members of the minority sample were not a homogenous group. The minority group in this study was made up of highly educated Asians attending graduate school or working for local industry, African Americans, and Latinos. A larger

sample of minority status families would have allowed analyses as a function of specific minority group.

Finally, the family and health measures used in this study are probably not as valid or reliable as would be ideal. The health measure, although typical of those used in studies of children's health, relied on parent report and variability was limited. Most parents indicated that their children were very healthy, and a good bit of the variability came from items about asthma and allergies. Future studies would benefit from methods that incorporate additional ways to measure health, including diary records, medical records, and measures of immune function. Along the same lines, my measures of SES and demographic risk factors were fairly weak. Parents were asked to report how much education they had and to describe their jobs. Although there was considerable variation in these measures, particularly in the occupational prestige scores derived from job descriptions, information about family income, residence, harshness of parenting, and abuse in the home would have created a more detailed picture of risk. Moreover, the sample may not have included families at very high risk because children were recruited through child care programs. Many families at very high risk are isolated and children are not enrolled in child care. Future studies might find more powerful effects of risk on cortisol and health by including a more diverse sample and a broader set of risk factors.

Conclusions. Childhood adversity is associated withlife-long poor health. Traditional models have proved inadequate to explain this link. Recent biosocial models suggest that dysregulation of the HPA axis results from stress brought on by prolonged early adversity, and that HPA-axis dysregulation, in turn, is physiologically damaging to numerous other body systems. However, there are virtually no data with which to

evaluate these proposals (or the implied mediational models), even in adults. I chose to explore predictions based on these models during a time period when the HPA axis and the immune system presumably are still being molded.

Even though the data in this study were not consistent with a model in which cortisol mediates the early risk – poor health association, it is important to note that current theoretical descriptions (e.g., Miller et al., 2011) do not specify the time frame in which the proposed effects should occur. It is possible that evidence for mediational effects would have been found if children were older when health was measured. Nonetheless, these data partially support current theory (e.g., Miller et al., 2011) in that both health and HPA-axis activity, as measured by salivary cortisol, were associated with risk, and cortisol was associated with health. At this young age, it appears that risk factors and cortisol make independent contributions to the variance in children's health.

This study takes the field one step further toward understanding how early adversity affects HPA-axis regulation and life-long health. This raises the hope of finding ways to reduce stress-induced disease and promote greater long-term health.

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