EFFECTS OF PHYSICAL ACTIVITY ON CORTISOL LEVELS IN AFRICAN

AMERICAN TODDLERS ATTENDING FULL-TIME DAYCARE

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# EFFECTS OF PHYSICAL ACTIVITY ON CORTISOL LEVELS IN AFRICAN AMERICAN TODDLERS ATTENDING FULL-TIME DAYCARE

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DISSERTATION ABSTRACT

EFFECTS OF PHYSICAL ACTIVITY ON CORTISOL LEVELS IN AFRICAN AMERICAN TODDLERS ATTENDING FULL-TIME DAYCARE

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Cortisol is a glucocorticoid hormone secreted by the adrenal cortex in response to both physical and psychological stressors. A baseline, diurnal pattern of activity, which peaks soon after waking and declines throughout the day to a low point in the evening, begins to establish itself during infancy. However, research now suggests that cortisol levels are atypical (i.e., elevated during the afternoon) in young toddlers on days when they attend full-time daycare.

The implications of long-term elevated cortisol levels on the functioning of the immune system, as well as on cognitive and brain development, appear to be detrimental, so research is warranted to identify interventions that result in positive changes towards more typical cortisol patterning. Exercise has been shown to be related to increased positive affect and lower cortisol levels, and these findings suggest that physical activity may be an option for regulating cortisol response in toddlers attending full-time, center-based daycare.

In this study, participants were 22 African American toddlers who attended low-income full-time daycare in Alabama, all of whom were sampled under control

and physical play conditions. For both conditions saliva samples were collected at 9:45 a.m., 10:35 a.m., 11:30 a.m., and 3:30 p.m., and the outdoor physical play treatment session was conducted at 10:00 a.m. Actiheart™ heart rate monitors and video analysis were used to monitor the children's engagement in the physical play. The saliva sample was collected using a Sorbette, without stimulant.

Results showed a significant lowering of mean cortisol levels at mid-afternoon on days with physical play in the morning when compared to the control days. No change in cortisol levels was seen pre- to post-physical activity as had been expected, however a significant increase was observed between the 10:35 a.m. and 11:30 a.m. samples under both conditions. Heart rates were significantly higher during the play condition in comparison to the control condition, but only a weak to moderate relationship was found between higher heart rate during the physical play and lower cortisol mid-afternoon.

Future research is needed to better understand the influence that physical activity may have on the hypothalamic-pituitary-adrenocortical stress cascade in young children.

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# CHAPTER I

# INTRODUCTION

It was estimated in 2000 that 49% of 3– and 4–year-old children attended some form of childcare in the US (Annie E. Casey Foundation, 2000). Of these, an increasingly large number are enrolled in full-time daycare (National Research Council & Institute of Medicine [NRC], 2000). There are many positive outcomes for children attending daycare, particularly one of high quality. For example, children's social skills have been shown to significantly improve so that they are able to more competently deal with peer and adult interactions. A review by the NRC indicates that most research findings show significant improvements in many domains (e.g., language and cognition) for at-risk children. Some studies argue that all children benefit regardless of whether or not they are at-risk (NICHD Early Child Care Research Network, 2000).

Recently, though, it was discovered that young children, in full-time daycare are exhibiting elevations in the glucocorticoid hormone cortisol in the afternoon (Tout, de Haan, Kipp Campbell, & Gunnar, 1998), which may put them in jeopardy for developmental problems. These elevations are context-sensitive; that is, the same children do not show these increases on days at home (Dettling, Gunnar, & Donzella, 1999). It seems that older toddlers and preschoolers, ages 21 – 70 months, are at particular risk of exhibiting this atypical pattern (Gunnar & Donzella, 2002). Currently, it

is not understood how much threat increased cortisol poses to the developing child, but it does have an influence on the hippocampus and thus memory, both of which are still evolving in this age group. In large enough doses, cortisol also plays a role in suppressing the immune system. Therefore, these chronic elevations may have implications for brain and cognitive development as well as the health of children attending full-time daycare (Dickerson & Kemeny, 2004; Nelson & Carver, 1998). Regular activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, when cortisol is elevated over and above basal, nonstressed levels, has been shown to alter the number of brain receptors for cortisol and its precursors in rat populations (Sutanto, Rosenfeld, de Kloet, & Levine, 1996). Whether a similar adaptation occurs in humans is currently under study, but it is known that certain psychological disorders, for example clinical depression, are characterized by disturbances in HPA axis regulation (Gunnar & Cheatham, 2003). It seems warranted, under the circumstances, to attempt to find ways of controlling cortisol secretion over the childcare day so that it more closely mimics typical secretion levels.

One possibility might be introducing regular stress-relief to the curriculum in the form of physical activity. Exercising once a week has been shown to increase positive, and decrease negative, affect for adults (Steinberg at al., 1998), and there appears to be a relationship between positive affect and lower cortisol (Rudolph & McAuley, 1998). Changes in mood states following exercise have also been found in 9- and 10-year-old children. Williamson, Dewey, and Steinberg (2001) reported increases in positive mood and decreases in negative mood after exercise, with the reverse being seen in a control group who watched a video. Analyses on 30 minutes of exercise at 60% of maximal oxygen consumption ($VO_2$ max) revealed a trend towards lower recovery cortisol levels

in adults who were trained runners (Rudolph & McAuley, 1998). Acute increases in serum cortisol levels during and immediately after exercise in a study with 10-year-old boys (del Corral, Mahon, Duncan, Howe, & Craig, 1994) were similar to those expected in adults. These findings suggest that moderate to high intensity exercise may be an option for full-time daycares to implement for reducing atypical levels of cortisol in toddlers.

## Statement of Research Objectives

The purpose of this study was to determine the effects of an acute bout of physical play on atypical cortisol levels observed in toddlers attending full-time daycare, and to examine their cortisol response to and recovery from this form of acute physical activity.

The child development literature presents causal factors for the observed rise in cortisol, but none indicate any prescription for trying to lower it. From a scholarly viewpoint little is known about toddlers' response to exercise and how it influences the regulation of cortisol levels in young children. This investigation not only allowed the opportunity to find out whether moderate to high intensity physical activity can be used effectively as a means of stress relief in young children, but it also explored the physiological stress recovery process from an acute stress response (never before observed in this age group).

Demographics for previous studies have been heavily weighted in favor of middle income, predominantly Caucasian families whose children were attending daycare. There is no literature on low income African American children of this age attending daycare. Consequently, the need for such investigation has been highlighted by other researchers

(Keenan, Gunthorpe, & Young, 2002; Watamura, Donzella, Alwin, & Gunnar, 2003). There is also evidence that adult African American males have significantly different epinephrine and norepinephrine responses to high intensity exercise than their Caucasian counterparts (Walker et al., 1992). At rest these differences were not apparent. This was supported recently when no differences were noted in overnight epinephrine or cortisol production for ethnically diverse participants (Masi, Rickett, Hawkley, & Cacioppo, 2004). Information is lacking on all young children with regard to their physiological response to exercise.

Furthermore, there are educational implications. If the hypotheses are supported, the study would highlight the necessity for planned physical activity play to be included regularly within the daycare curriculum. To date, toddlers experiencing atypical elevated cortisol levels during the day while attending full-time daycare appear to be at future risk of developmental problems including possible impairment of self-regulation and attentional capacities (Gunnar, 1998), and more immediately, suppression of the immune system and physical development (NRC, 2000). Since numerous children in the U.S. attend full-time daycare, the long-term outlook for health and educational problems is of concern.

## Hypotheses

Several hypotheses were postulated for this study.

1. Physical play in the morning will result in lower cortisol levels mid-afternoon (3:30 p.m.) than at mid-afternoon on days without physical play.

2. Toddlers will show elevated cortisol levels immediately after physical activity (10:35 a.m.) as compared to levels prior to physical activity (9:45 a.m.), and in a similar manner to older children and adults.

3. Post-activity cortisol levels (10:35 a.m.) will return to pre-activity levels within an hour (11:30 a.m.).

4. Heart rate during physical play will inversely correlate to mid-afternoon cortisol levels (3:30 p.m.).

## Operational Definitions

**Cortisol:** when measured in saliva, cortisol levels represent the 'free' or active portion of the circulating hormone (i.e., the element not bound to corticotropin binding globulin, or CBG). At baseline the acting portion is approximately 10% of circulating cortisol (Gunnar & Cheatham, 2003).

**Cortisol response:** for this study, the peak increase seen in cortisol levels following a stressor, is implied. In the literature this may also be termed reactivity.

**Cortisol recovery:** for this study, the return, from peak response, to baseline or pre-stressor cortisol levels, is implied. In the literature this may also be termed regulation or response dampening.

**µg/dl:** (micrograms per deciliter) unit of measurement for cortisol. 1 µg/dl is equivalent to 27.6 nmols/L.

**Toddlers:** young children from age 21 months to age 45 months will be classified, in this study, as toddlers.

**Daycare:** center-based childcare (i.e., not home-based) will be implied when using the term daycare.

**Full-time daycare:** attendance at childcare for all or most of the day, for all or most of the working week (i.e., more than 25 hours per week).

**Investigator contact:** period of time during study conditions (typically 10:00 – 10:30 a.m.) that was spent by the investigator engaging with the participants.

**Physical play:** child-initiated movement play. In this study physical play is facilitated by the use of an outdoor mastery motivational climate motor program, promoting moderate to high intensity physical activity. The climate included two researchers modeling physical play.

**Physical activity:** moderate to high intensity activity (or exercise) in the form of outdoor physical play will be implied.

**$VO_2$:** maximal oxygen uptake per minute. Per minute can be notated using a dot above V, but is implied in this study.

**Resting heart rate (RHR):** calculated as the mean of the lowest consecutive 20 minutes of heart rate during nap time (Logan, Reilly, Grant, & Paton, 2000).

**Physical activity heart rate index (PAHR-50):** an index of high intensity activity, calculated by multiplying RHR by 1.5 (Logan, Reilly, Grant, & Paton, 2000).

**Activity count:** unit of measure for the accelerometer data output. Equivalent to the average activity level within a given epoch, calculated by summing the areas under the activity curves sampled per second (Gomy & Allen, 1999).

## Delimitations

Delimitations setting the scope for this study were as follows.

- Participants were 22 African American toddlers.
- Participants attended a subsidized daycare setting in Auburn, Alabama.
- The play condition utilized an already established motor program within the daycare, which involved a mastery motivational motor skills/physical play climate.
- Saliva was collected four times a day during both control and play conditions, and each condition was conducted twice (i.e., a maximum of 16 samples were collected from each toddler).
- The dependant measures were cortisol level and heart rate.

## Limitations

This study was conducted in a naturalistic setting (i.e., daycare). Attempts were made to control for confounding factors such as diet, and sleep, because the possibility exists that factors like these can influence the participants' cortisol levels. For example, sampling times were carefully chosen to allow for cortisol levels to recover after eating and napping before saliva collection. Pilot data resulted in no changes in pH, indicating that food confounds had been successfully controlled for when collecting saliva at the proposed times throughout the day. However, daily timetabling at the daycare is subject to fluctuation and disturbances, and as such, it was not always possible to adhere strictly to the sampling times chosen without disruption to the classroom. In these instances sampling took place within the allotted 15 minutes from the designated time.

The Actiheart™ monitors that were used to measure heart rate, have been validated for use with adults (Brage, Brage, Franks, Ekelund, & Wareham, 2005), but there is no current published literature on their use with such a young population. However, colleagues have shown high correlations between physical activity and heart rate in toddlers using the Actiheart™ monitors (Parish, St. Onge, Rudisill, Weimar, & Wall, 2005). The experimenter believed the use of video as a manipulation check would support data provided by the monitors.

The mastery motivational climate employed for the play condition increases heart rate and involvement in vigorous physical activity (Parish et al., 2005), however, other motor skill climates may also result in these benefits and/or may affect levels of cortisol.

The control and treatment conditions occurred indoors and outside respectively. It is possible that this difference in environment may contribute to the results found.

# CHAPTER II

# REVIEW OF LITERATURE

The purpose of this study was to determine the effects of an acute bout of physical play on atypical cortisol levels observed in toddlers attending full-time daycare, and to examine their cortisol response to and recovery from this form of acute physical activity. This chapter presents a review of literature for the study and consists of the following sections: (a) cortisol and its function, (b) cortisol effects on the developing brain, (c) cortisol patterns in toddlers, (d) exercise and cortisol, (e) exercise, mood enhancement, and stress management, and (f) summary.

## Cortisol and its Function

Cortisol is a glucocorticoid hormone which typically, in adults, exhibits a diurnal secretory pattern peaking soon after waking, declining rapidly, followed by a slower continuous decline throughout the day, and reaching a low point in the evening. Interindividual cortisol levels are highly variable; however intraindividual levels are extremely stable day to day (Knutsson et al., 1997). Basal levels fluctuate in response to daily living activities such as sleeping and eating, and can be influenced by various factors (e.g., personality traits). Cortisol is also released in response to stress, and is the end product of a cascade of processes that occurs within the hypothalamic-pituitary-

adrenocortical (HPA) system. Stress can be defined as any disruption of or challenge to homeostasis (Miller & O'Callaghan, 2002; Sapolsky, 2000), and the cortisol response can be stimulated by either physical or psychological stressors. Examples of the metabolic function of cortisol are mobilizing energy stores (e.g., free fatty acids) in a more chronic response than, say, epinephrine, to ensure that fuel is available for longer bouts of activity, and the catabolism of tissue protein which provides amino acids for repair in cases of injury (Dickerson & Kemeny, 2004; Powers & Howley, 2001).

Activation of the hypothalamus releases corticotropin-releasing hormone or factor (CRH or CRF) that, in turn, stimulates the anterior pituitary to produce adrenocorticotropin hormone (ACTH). This triggers the adrenal cortex to secrete cortisol into the bloodstream 15 – 20 minutes from stressor onset, and begins a negative feedback loop that restores homeostasis. Suppression of the chain reaction within the HPA axis is mediated by glucocorticoid receptors located in both the anterior pituitary and the hypothalamus (de Kloet, Rosenfeld, Van Eekelen, Sutanto, & Levine, 1988; Dickerson & Kemeny, 2004). Refer to Figure 1 for a representation of cortisol release.

Because of its role in regulating other physiological systems that promote survival, the production of cortisol can be regarded as a beneficial adjustment to stress within the environment so long as it can be efficiently turned off (de Kloet et al., 1988; McEwen, 2000; Sapolsky, 2000). In the short run, that is, in response to an acute stressor, cortisol is responsible for positive effects on the physiological and immune systems, and on brain function. Examples of positive responses to an acute stressor are providing for longer term energy release, anti-inflammatory effects, increasing the body's response to

pathogens, and enhancing the formation of threatening or emotional memories (Dickerson & Kemeny, 2004; McEwen, 2000, 2002; Sapolsky, 2000). However, prolonged cortisol release, which may be caused by several mechanisms (e.g., in response to ongoing chronic stress, or failure to terminate response due to malfunction of the feedback loop), is believed to be detrimental to development and health (Dickerson & Kemeny, 2004; NRC, 2000). Thus, negative effects to protracted increases in cortisol above baseline include depletion of energy stores, immune suppression, and in extreme cases atrophy of the hippocampus (McEwen, 2002; Sapolsky, 2000).

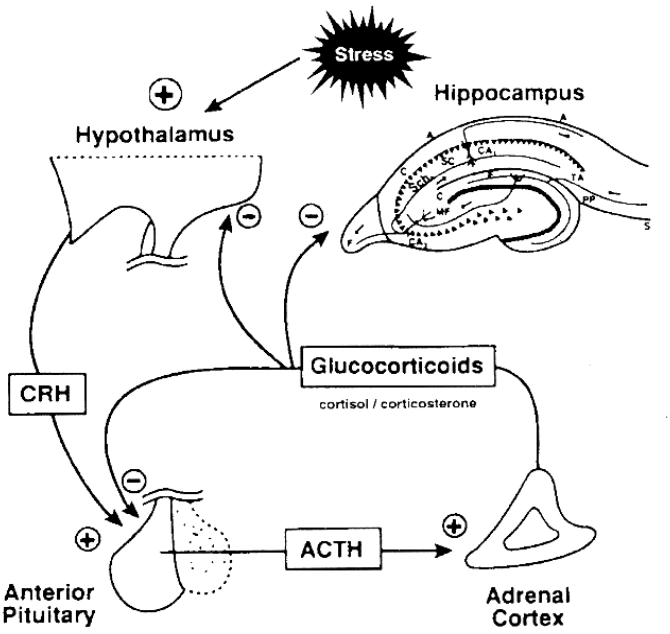


*Figure 1.* Schematic of the stress cascade (Miller & O'Callaghan, 2002).

As mentioned earlier, cortisol typically exhibits a pronounced diurnal rhythm in adults, with an early morning peak that may have several responsibilities. De Kloet reasoned its purpose may be in mobilizing energy stores required after waking (as cited in Gunnar, Bruce, & Hickman, 2001). In addition, cortisol also plays a role in organizing

and regulating other circadian activities such as sleep-related events (de Kloet et al., 1988; deRijk, Schaaf, & de Kloet, 2002). It can be empirically measured in urine, plasma, or saliva samples, and is most often reported in the units µg/dl or nmols/L. Therefore, when comparing results across studies it is important to ensure that cortisol was acquired in the same manner, and apply the conversion correction when necessary (see definitions in Chapter I).

The temporal pattern for stress response in infants is similar to that seen in cortisol studies with adults. Ramsey and Lewis (2003) investigated cortisol responses in 6-month-old infants, and suggested that in order to examine the regulation (or response dampening) of cortisol levels, multiple poststressor samples needed to be collected after the initial reaction to the stressor. Infants in their study showed on average a peak response 20 minutes from stressor onset, and at 30 minutes were still high but beginning to decline. Ramsey and Lewis suggested that a return to baseline would have been seen by 40 – 45 minutes poststressor. It is speculated that toddlers, preschoolers, and older children all manifest a temporal pattern of cortisol response similar to that of infants and adults (del Corral, Mahon, Duncan, Howe, & Craig, 1994).

## Cortisol Effects on the Developing Brain

Two receptors for cortisol are located in various areas of the brain – mineralocorticoid receptors (MRs), and glucocorticoid receptors (GRs) (de Kloet et al., 1988; Gunnar et al., 2001). The former have a high affinity for cortisol, and the latter low affinity. As cortisol secretion increases a continuum can be seen with regards to occupancy of the receptors. During baseline secretion MRs are heavily occupied with

cortisol, while GRs have only low occupancy. Under mild stress MRs become saturated and GRs moderately occupied, and in contrast, during stressful conditions GRs become heavily occupied (Sapolsky, 2003). The hippocampus and amygdala (limbic structures involved in explicit and implicit memory), and the anterior cingulate (involved in effortful control) have ample quantities of both receptors. These and other areas containing cortisol receptors develop largely postnatally (Gunnar et al., 2001). One of the ways these receptors are thought to mediate the effects of cortisol is that MRs facilitate electrical impulses while GRs lower excitability in neuronal pathways (de Kloet, 2004; Sapolsky, 2003).

While the body's stress reaction to acute stressors is indicative of positive adaptation, and can have beneficial effects for brain functioning such as focusing attention and even enhanced cognition, the longer timeframe for reaction to trauma and/or chronic stress may have more deleterious consequences. For both practical and ethical reasons, there are little data on early human brain development from either a functional or mechanical (neurobiological) perspective, or the relationship between the two. There is information available, however, from studies on animals, older children, and adults which warrants discussion when looking at the possible implications for regular elevations in cortisol on the developing brain. In rats, administering high doses of glucocorticoids inhibits cell proliferation, and retards myelination and synaptogenesis (de Kloet et al., 1988). These effects may be permanent depending on the window of exposure, that is whether exposure occurs early or late in the development period. Alternatively, strength of effect may be linked to length of exposure (Gunnar, 1998). Also, Krieger (1972) found that development of the HPA axis appears to be affected

when rats are administered glucocorticoids neonatally causing delay to the stress response. In children, high levels of corticosteroids may disrupt HPA function and suppress the cortisol response to stress (Nelson & Carver, 1998). Chronic dysregulation of the HPA axis may contribute to allostatic load and its incumbent health risks (McEwen, 2003). The term allostatic load is used to describe the long-term wear and tear on the body caused by repeated activation of the stress response systems.

The plasticity of the developing brain during the first 2 - 3 years means that experiences may provide either positive adaptation or result in negative effects on brain growth and functioning. Positive experiences can be seen as opportunities that result in learning. Negative experiences, on the other hand, represent vulnerability. Experiments with rats have shown that under extreme conditions stress and elevated corticosteroids can result in retraction of dendritic processes in the hippocampus causing cognitive deficit, and this may be implicated in the smaller hippocampi seen in certain human conditions. The process, however, does appear to be reversible (Sapolsky, 2003). In adults, stress and/or cortisol increases have been shown to disrupt retrieval of declarative or explicit memory. This conscious memory function (e.g., the ability to recall a specific object), which is reliant upon the hippocampus and surrounding structures, continues to be refined during early childhood as synaptic connections between these neural structures are formed and mature (Nelson & Carver, 1998). Because of the involvement of the hippocampus with the products of the HPA axis, the hypothesis that high levels of cortisol during this time may impair hippocampal development and/or memory function appears worth investigating (Nelson & Carver, 1998). Recent data reported by Quas, Bauer, and Boyce (2004) seemed to corroborate an effect on memory, but results

supported the notion that under some circumstances elevations in cortisol can be related to positive outcomes. In this instance, children whose cortisol levels increased due to anticipation of a laboratory visit scored better on memory tests, while those whose cortisol response increased during the session did not score as well perhaps because of issues with self-regulation and attentional problems.

Although no human data directly link cortisol levels with development, results from a study by Gunnar and Nelson (1994) show that cortisol might have a 'dampening effect' on hippocampal activity in infants, such that higher cortisol is related to lower activity in the hippocampus during a visual memory task. This is supported by reports on clinical populations of children. Children using steroid-based inhalants for asthma have been shown to exhibit memory, attention, and self-regulation problems following use (Gunnar, 1998; Nelson & Carver, 1998).

Teachers and parents report that preschoolers whose typical day-to-day cortisol pattern is at the higher end of sample norms have poor effortful control (Gunnar, Tout, de Haan, Pierce, & Stansbury, 1997). Effortful control is the ability to inhibit a response, which can be physical or emotional, that is ready to be performed. For example, during movement games such as 'Red Light, Green Light' a child is required to suddenly stop an activity when dictated by the rules (NRC, 2002). Thus, if a child is running, expecting to hear "green light" and suddenly hears "red light" called instead, a degree of effortful control is required to suppress the running behavior. The more excited or faster the child was moving the more effortful control is needed to interrupt the response. How cortisol affects behaviors controlled by the frontal cortex, however, is not fully understood (Gunnar et al., 2001).

*Typical Daily Cortisol Secretion*

While the typical pattern of activity for cortisol (i.e., high early in the morning, decreasing over the day, and reaching a low at night) establishes itself within infancy, cortisol changes during the day remain subject to development over early childhood (Gunnar & Donzella, 2002). Discussion in earlier papers regarding the typical decline expected mid-morning to mid-afternoon may have been confounded by the age ranges included in their analyses (Gunnar et al., 1997; Tout, de Haan, Kipp Campbell, & Gunnar, 1998). Gunnar and Donzella compiled data from a number of studies in their review which show that changes in cortisol between mid-morning and mid-afternoon do not show a significant decline until 4 years of age, indicating that the morning drop observed in children younger than this occurs between waking up and mid-morning. Similarly, the drop to the evening low appears to occur after mid-afternoon. They suggested that developmental changes in baseline cortisol and sleep patterns may be related.

A recent study investigated baseline cortisol production in typically developing young children on days at home. The specific aim of the study was to identify the point at which the transition to more adult-like cortisol patterning occurs (Watamura, Donzella, Kertes, & Gunnar, 2004). Watamura and colleagues believed that the transition may be connected to factors such as giving up daytime naps, and the continued brain development (e.g., myelination in the prefrontal cortex) that supports increased capacity for effortful control. The children participating in the study were between 12- and 36-months of age. Both levels of production and variability in cortisol went down with age,

and all ages exhibited a clear daytime pattern – highest measures were taken at wake-up and lowest at bedtime (refer to Figure 2). However, no significant difference was shown mid-morning to mid-afternoon, even by the 36-month-olds. This suggests that a more mature basal circadian rhythm, showing a continuous drop throughout the day, does not begin to appear until after 3-years of age. The older children who napped less demonstrated a decline in cortisol mid-morning to mid-afternoon which supported the view that the transition may be linked to developing adult-like sleep behaviors. Of interest was the finding that children who were described as higher in effortful control had lower cortisol levels regardless of age.

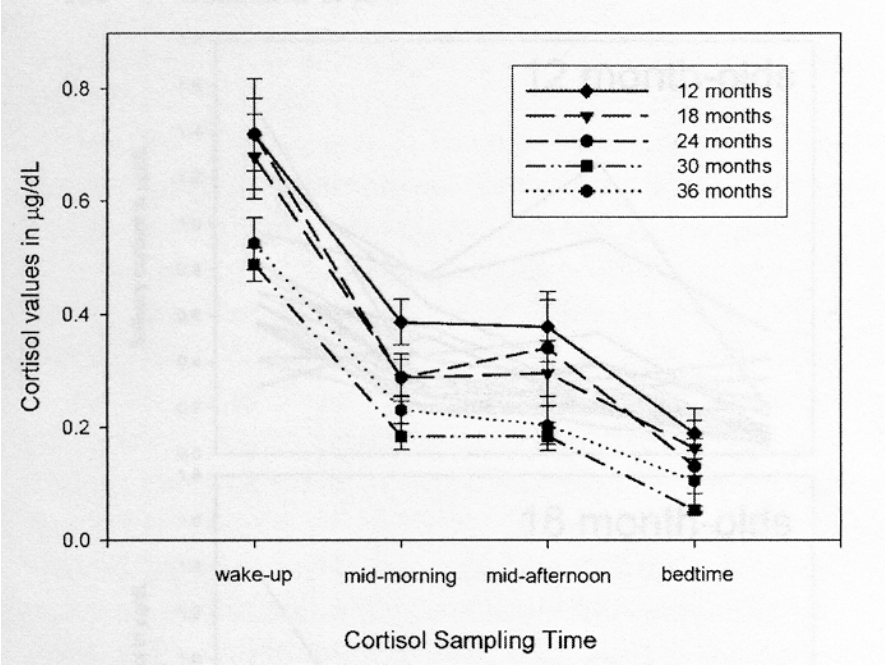


*Figure 2.* Average cortisol patterning across the day for 12-, 18-, 24-, 30-, and 36-month-olds (Watamura et al., 2004).

*Atypical Cortisol Pattern seen at Full-Time Daycare*

In 1998, Tout et al. reported surprising findings from their study of cortisol activity in children attending full-time, center-based daycare. For this study, saliva

samples were collected twice a day (mid-morning and mid-afternoon) for 30 days from children attending two urban daycares. Ten saliva samples for each time of day was the minimum requirement for inclusion in the data analyses, and 75 children (age range 2 years 8 months – 5 years 10 months) met the criteria and completed the study. Morning and afternoon samples were collected, for the most part, after a period of quiet activity, and before snack time. The children sat quietly at their tables and were given $1/16^{th}$ – $1/8^{th}$ teaspoon of sugar-sweetened drink crystals to stimulate saliva flow. They then put a cotton roll in their mouths for approximately 1 minute until saturated. The cotton rolls were syringed, the saliva extracted into vials, and the vials frozen at –20 ºC until the samples could be assayed. Instead of the typical diurnal curve, Tout et al. found that 81% of the children showed a significant rise in cortisol levels from morning (.17 μg/dl) to afternoon (.22 μg/dl). This pattern was not seen in previous research with children attending part-time childcare who appeared to show the typical rhythm (Gunnar et al., 1997). The authors were unable to state conclusively that factors inherent in full-time daycare were responsible for this rise in cortisol activity because no comparison home levels had been taken as part of the study design.

Dettling, Gunnar, and Donzella (1999) were able to replicate the rise in cortisol observed in the study by Tout et al. (1998), but also measured cortisol activity in the sample group on days at home. In this broader study, participants attended an urban preschool or school-age childcare center ($n$ = 36 and $n$ = 34 respectively). Saliva samples were collected twice a day (mid-morning and mid-afternoon) for 2 days both at childcare and at home, although in some cases sufficient saliva was available at only one sampling time. The children were given $1/8^{th}$ teaspoon of sugar-sweetened drink crystals, and then

mouthed cotton rolls. Saliva was syringed into vials, which were frozen and sent for assay. On average, across the total sample, home levels for 80% of children with complete data exhibited the typical pattern expected where mid-afternoon cortisol levels were lower than those taken at mid-morning. Daycare levels for the same children showed the reverse pattern with mid-afternoon cortisol elevated over mid-morning cortisol, and mid-afternoon levels that were significantly higher at childcare from those at home. Further analysis of the data revealed that this significant effect in the cortisol change was seen in the 3- and 4-year-olds. The afternoon cortisol levels at childcare demonstrated by the 5 – 8-year olds were not significantly different from those at home. Thus, Dettling et al. concluded that the rise in cortisol was related to the length of the childcare day, and also showed that younger children (3 – 4 years) were more at risk of the atypical patterning than older children (5 – 8 years). The researchers discussed the likelihood that 3 – 4 year olds are less able to cope with the complexity of group care settings. Napping variables were also suggested as a possible explanation for the results obtained.

Further investigation eliminated napping and/or resting as a rationale for the exaggerated afternoon cortisol levels. The issue of whether sleep patterns during rest time contribute to atypical cortisol levels was investigated with a sample of preschoolers ($N$ = 35) attending a full-day urban childcare center (Watamura, Sebanc, & Gunnar, 2002). Saliva samples were collected daily for 1 week at mid-morning, just before lunch, immediately following rest period, and mid-afternoon. The children had to provide sufficient saliva on a minimum of 2 days to be included in the analyses. If more samples were provided the additional days were also included in the mean calculation. A small

subgroup ($n$ = 8) of children also provided saliva samples at home, collected mid-morning and mid-afternoon. As in previous studies, saliva was stimulated by giving the children sugar-sweetened drink crystals (1/16$^{th}$ teaspoon). Cotton rolls were mouthed and then syringed into plastic vials and frozen. To examine whether napping/resting or not napping/resting played a role in the cortisol elevations, variables such as length of nap, length of rest, and quality of rest were assessed. Mid-morning cortisol levels were slightly lower at daycare than at home, increased by pre-rest, decreased over the rest period, and then increased again post-rest to be significantly higher mid-afternoon at daycare than levels sampled at home (refer to Figure 3).

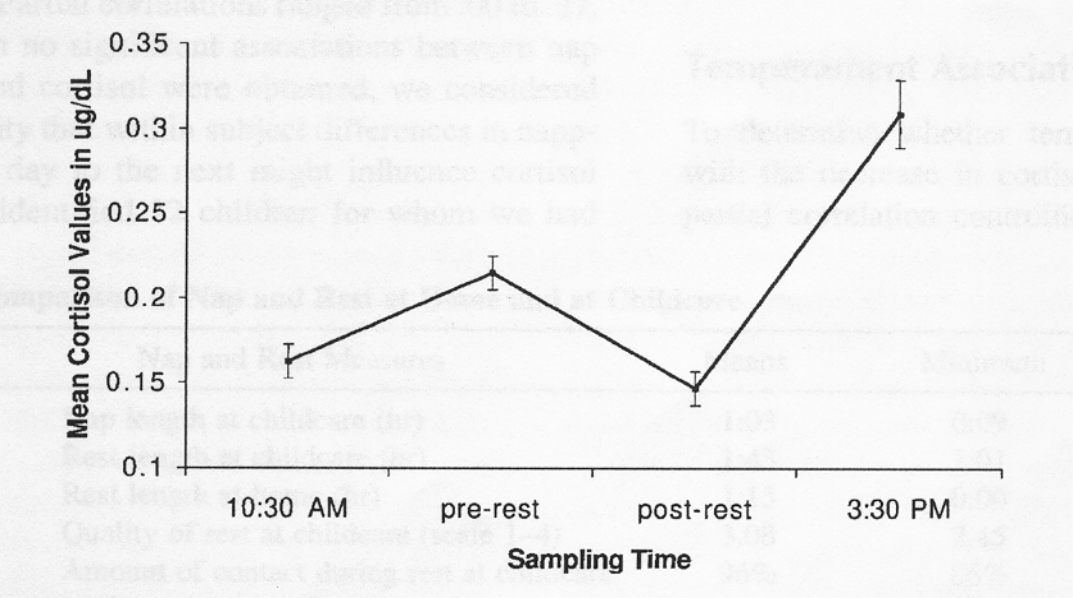


*Figure 3.* Mean cortisol values in µg/dl at childcare across the day. (Watamura et al., 2002).

Analyses showed that the magnitude of the increase seen across the day for 91% of the participants was not related to the decrease in cortisol over the rest period. That is, the change in cortisol seen morning to afternoon was not significantly different whether

the child's cortisol level did, or did not, decrease over the rest period. No relationship was seen between nap variables (e.g., length of rest) and cortisol for the group. Watamura et al. (2002) highlight the fact that the atypical cortisol pattern was already apparent by the pre-rest sample, and that the younger children within the sample showed greater increases over the day than the older ones. They concluded that it seemed likely something other than rest/nap quality or quantity was responsible for the observed increases in cortisol levels in young children attending full-time daycare.

Recently, Gunnar and Donzella (2002) published a comprehensive review of the social regulation of cortisol levels in early childhood. It highlights the data showing increasing levels of cortisol for children attending full-time daycare. The review includes compilation figures that indicate while no increases are seen in many infants and younger toddlers, increases in older toddlers (21 – 40 months) are greater than those observed in preschoolers (41 – 70 months).

Watamura, Donzella, Alwin, and Gunnar (2003) investigated cortisol levels in infants (3 – 16 months) and toddlers (16 – 38 months) attending full-time, center-based urban daycare to examine two competing hypotheses. The first suggested the largest rise in cortisol would be seen in toddler classrooms, but not infant rooms, because of developmental changes occurring for toddlers within play settings. These changes necessitate the negotiation of peer play involving a large element of social skill not ordinarily developed by this age. The second hypothesis revolved around the development of secure attachments, and suggested that older infants would show the largest rise in cortisol because of the long period of separation from parents. Saliva samples were collected at childcare approximately mid-morning and mid-afternoon for

55 children who fulfilled all criteria, and for 36 of these children at home. Similar collection methods were used as in earlier studies. Amount and complexity of peer play amongst the children was coded during free play or gross motor play by using measures of time spent in either parallel, associative, or cooperative play. The results showed that while across the total sample the change in cortisol (mid-afternoon – mid-morning) at home was not significant, at daycare 35% of infants and 71% of toddlers exhibited a rise in cortisol from mid-morning to mid-afternoon. The peak increase in cortisol occurred in toddlers who were between 24 and 36 months old. Thus, the pattern of cortisol rise appears to be age-related and context sensitive (i.e., does not occur in the same children during days at home). Peer play was negatively correlated with cortisol measures in the morning and afternoon, and the relationship decreased with age. Watamura and her colleagues suggest that the toddlers in this study also showed a larger increase in cortisol than preschoolers in previous studies have shown, and argue that separation distress, which would be expected to be greater in infants, does not explain the observed elevations. With regard to the first hypothesis, it was suggested that peer play was not predictive of cortisol rise because no correlation with increases in cortisol production over the childcare day was seen.

A study conducted in France and Hungary (Legendre, 2003) to examine environmental factors that possibly act as stressors to toddlers, showed higher cortisol levels at daycare than at home, but found significant differences in cortisol changes over sampling times across the eight centers included in the study. A total sample of 113 toddlers (18 – 40 months) was used in the data analysis, and environmental data, such as group size and child-to-caregiver ratio, were collected over the 8-month period spanning

saliva sampling in the children. Saliva was collected at 7:30 a.m. (at home, by parents), and at 9:30 a.m. and 10:30 a.m. at the daycare for 3 days to investigate morning cortisol secretion patterns. A subset of toddlers ($n$ = 13) attending one of the observed daycare centers supplied additional saliva data. Saliva samples were collected by parents at 9:30 a.m. and 10:30 a.m. on a home day for comparison with their center-based samples. The procedure for collecting the sample was not as controlled as in the preceding studies; children were allowed to dip their finger twice into a sugar powder. The author states that this was equivalent to less than $1/8^{th}$ teaspoon. As before, cotton rolls were mouthed, syringed into test tubes, and frozen until assayed. It is worth noting that allowing the children to taste the stimulant in this manner appears to have resulted in better compliance with the frequently repeated procedure than achieved in previous studies. Results indicated a dramatic drop in cortisol between wake-up (7:30 a.m.) and first daycare measure (9:30 a.m.), followed by stabilization to the mid-morning measure (10:30 a.m.). The comparison, albeit limited by number, with home data suggests that this stable pattern is atypical as a significant decrease in cortisol level was exhibited between 9:30 a.m. and 10:30 a.m. at home. Intraindividual consistency across samples was also higher at home than at daycare indicating less interruption to the secretory pattern on home days. Analyzing the change in cortisol at each daycare separately indicated that of the eight centers, four showed tendencies for decreasing, one tended to be stable, and three centers showed increasing cortisol levels. The increases seen in two of the latter were significant. These results supported the notion of environmental influence, and findings on the environmental data showed that both actual group size and mean age difference across the group were positively correlated with change in cortisol. The child-

to-caregiver ratio was unexpectedly negatively correlated with cortisol change, and available indoor play space was also negatively correlated independent of the size of the group. Factor analyses allowed Legendre to suggest optimal thresholds for minimizing the impact of these variables on children's stress responses as follows (in order of importance):

- a maximum of 4 caregivers per room (≤ 4 caregivers)
- a minimum of 5 $m^2$ indoor play space per child (≥ 5 $m^2$)
- a maximum of 15 toddlers per room, regardless of available space (≤ 15 toddlers)
- a maximum of 6 months mean age difference between the children per room (≤ 6 months mean age difference).

## Exercise and Cortisol

Studies on the influence of physical activity on cortisol levels are widespread, however, few have examined this relationship in children. It is understood that moderate to high intensity exercise/physical activity acts as an immediate stressor in adults and can lead to a rise in cortisol (Bunt, 1986; Hackney & Viru, 1999; Viru, Karelson, & Smirnova, 1992). This rise appears to be followed by inhibition of adrenocortical activity via the feedback loop and glucocorticoid receptors as discussed earlier in this review (de Kloet et al., 1988). However, contradictory results were reported by Harte, Eifert, and Smith (1995) when adult elite runners evinced no significant change in cortisol levels pre- to post-exercise test. Participants were 31-year-old males selected as elite distance runners ($n$ = 11) or regularly practicing, highly trained meditators ($n$ = 12). Each of these

groups was sampled under two conditions – treatment (running or meditating for 1 hour) and control (quiet inactivity for 1.5 hours). Blood samples were collected pre-condition, immediately post-condition, and 30 minutes post-condition. Not only did Harte and colleagues report no interactions for cortisol samples in either group under either condition, but also there were no significant differences in cortisol levels between runners and meditators at any sampling point during the treatment condition. The only significant difference was seen in pre-test cortisol levels for runners between conditions where they showed higher levels before the control condition than the running treatment. Although not discussed, it may be possible that the anticipation of sitting still for an hour and a half was stressful enough for the runners to initiate a cortisol response. High variance among the individual measures (possibly due to time-of-day effects, or the repeated blood sampling) may explain these unusual results, which the authors warn to interpret with caution.

The effect of exercise intensity on cortisol secretion in adults would seem to underline the importance of this variable. Research on 10 male triathletes (age range 20 – 26 years) examined whether cortisol secretion during the night responded to daytime exercise as a stressor (Kern, Perras, Wodick, Fehm, & Born, 1995). The participants were exposed to 3 conditions – control (i.e., no exercise), long duration exercise (LDE) of low intensity (i.e., biking 40km), and LDE of moderate intensity (i.e., biking 120 – 150km) which elicited significantly higher heart rates. Blood samples were collected pre-condition, 15 minutes into the exercise, immediately post-exercise, and then every 15 minutes during the nighttime sleep (11:00 p.m. – 7:00 a.m.). Post-condition cortisol levels increased significantly after moderate intensity LDE but not after low intensity

LDE. Average cortisol levels during the night were not affected although temporal changes to the secretory pattern were seen. Moderate intensity LDE significantly increased cortisol levels over both other conditions during the first half of nocturnal sleep, and significantly decreased secretion over other treatments in the second half. In the case of the latter result, the interpretation was that typical elevation in cortisol early in the morning was lowered. Refer to Figure 4 for a graphic representation of the results found in the study by Kern and colleagues.

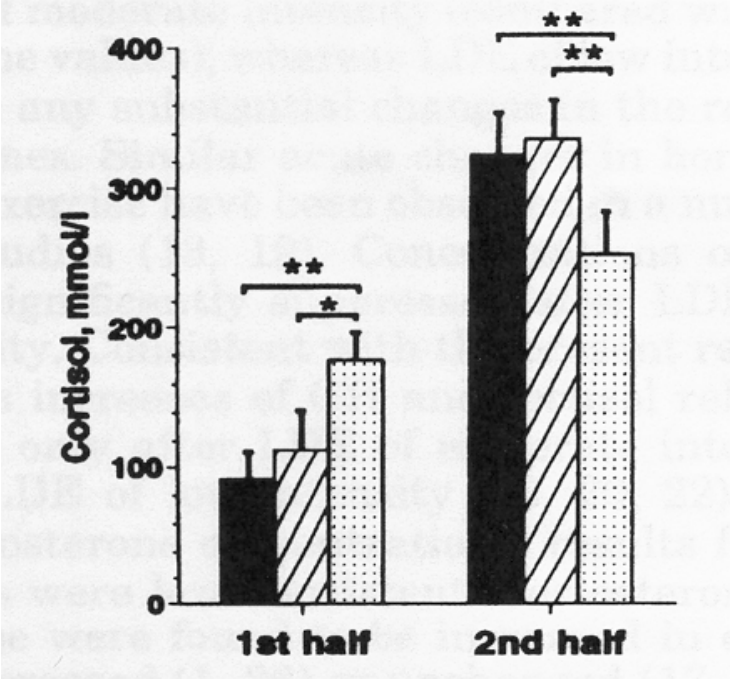


*Figure 4.* Concentration of cortisol averaged separately for first and second half of sleep time after no daytime exercise (depicted in solid bars), LDE of low intensity (depicted in hatched bars), and LDE of moderate intensity (depicted in dotted bars) (Kern et al., 1995). Significant differences are represented: *$p < .05$; **$p < .01$.

Kern et al. (1995) were unable to resolve the neurobiological situation, and stated that the physiological mechanism behind the suppression of cortisol levels during the

night following exercise was unclear. Discussing the cortisol levels seen in connection with moderate intensity LDE, Kern and colleagues suggested that the rise recorded in the first half of sleep was due to ongoing recovery from elevations following post-exercise, which then induced delayed inhibition of cortisol release due to the feedback loop. The results indicated a long-lasting inhibitory effect for moderate intensity LDE on nocturnal cortisol temporal patterning, but whether the authors view this as a positive or negative effect is difficult to discern. For the current study, however, the results support the possibility of a decrease in afternoon cortisol levels following physical activity and naptime.

Hackney and Viru (1999) investigated cortisol responses in adults, ages 18 – 36 years, to multiple exercise bouts of moderate and high intensity. Healthy, physically active males ($N = 17$) participated in three treatment conditions. The control day involved no exercise, while the high-intensity and moderate-intensity exercise days involved an early morning (7:00 a.m.) and late afternoon (5:15 p.m.) session for each condition. Exercise was either treadmill running or cycle ergometry depending on regular training preference. Blood samples were collected pre-session 1, post-session 1 (8:00 a.m.), hourly between sessions, post-session 2 (6:00 p.m.), and then every other hour throughout the night until the last sample was collected at 7:00 a.m. Hackney and Viru found that daytime exercise suppressed nighttime cortisol levels, and that the strength of the effect was moderated by the intensity of the exercise. Both intensities resulted in significantly greater cortisol measures post-condition and greater peak responses after the morning session than evening session. However, post-condition recovery took longer for the high intensity condition. The morning peak response at 9:00 a.m., as well as responses at 8:00

a.m. and 10:00 a.m., was significantly greater for the high intensity condition than the moderate intensity condition. Interestingly, afternoon responses to exercise (6:00 p.m.) were not significantly different between exercise conditions, but both conditions were greater for this sample than on the control day. During the night, moderate and high intensity exercise led to lower levels of cortisol than the control at 10:00 p.m., midnight, and 2:00 a.m. Levels for the high condition continued to be significantly lower than the moderate and control conditions at 4:00 a.m., 6:00 a.m., and 7:00 a.m. Thus, nighttime samples showed that both aerobic (moderate intensity) and anaerobic (high intensity) exercise led to suppression of cortisol at night, again providing support for future study.

Del Corral et al. (1994) looked at both serum and salivary cortisol responses to afternoon physical activity (between 3:00 p.m. and 5:00 p.m.) in a sample of boys ($N$ = 10, $M$ = 10.6 years). Having reached 70% of maximal oxygen consumption ($VO_2$ max) on a cycle ergometer, the participants exercised at this intensity for 30 minutes. Blood and saliva samples were collected pre-trial, at 15 minutes and 30 minutes of exercise, and then 15 minutes post-trial. Serum cortisol levels significantly increased, when compared to rest, at all three measures. As demonstrated in Figure 5, salivary cortisol tended to increase over time and approached significance. There was a significant correlation between serum and salivary cortisol response during and after exercise, and the increases in serum cortisol levels during and after exercise were similar to that expected in adults. It was unclear from the findings whether the increased levels of cortisol were due to increases in secretion or decreases in removal, however based on adult data the authors suggested the former is more likely. The mechanism activating the HPA axis during exercise was not completely understood as discussed by del Corral and colleagues,

although they hypothesized that factors such as increasing lactate levels may have been responsible.

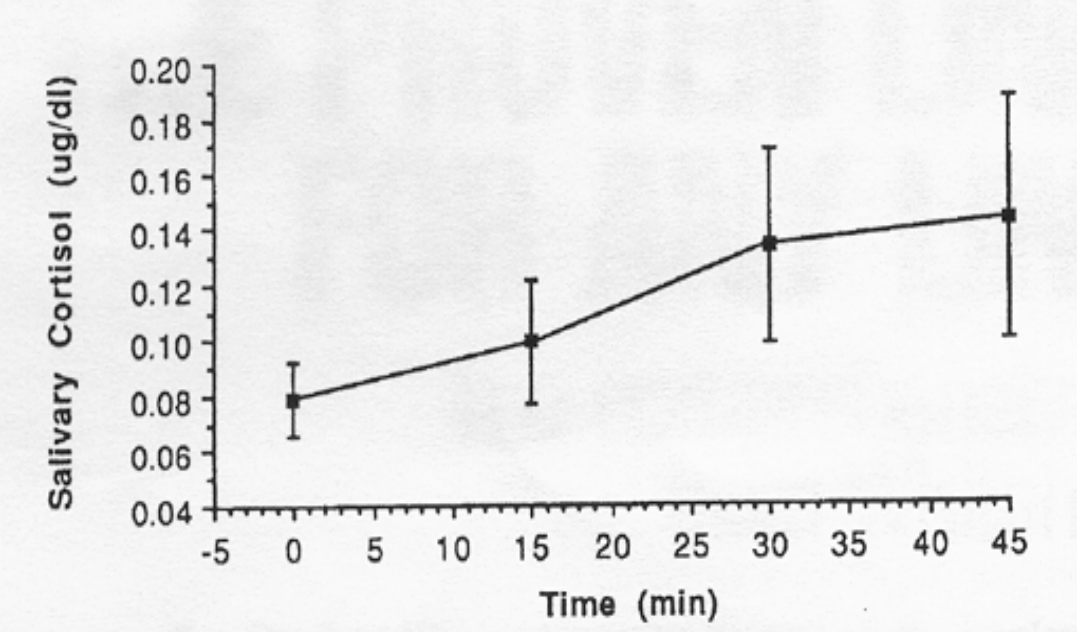


*Figure 5.* Mean salivary cortisol before (0 min), during (15 and 30 min), and after (45 min) exercise (del Corral et al., 1994).

Jansen and colleagues (1999) measured cortisol responses to psychological and physiological tests in children admitted to a child psychiatric in-patient clinic, reiterating that an adequate stress response involves rapid cortisol secretion followed by inhibition via the feedback loop. They compared results to a control group of 15 healthy children ($n$ = 13 boys, $n$ = 2 girls, $M$ = 10 years) attending regular primary school. The physical test for children in both patient and control groups involved 10 minutes cycling on a cycle ergometer at "maximum effort." Saliva samples were collected twice pre-trial (for baseline), immediately post-trial, and then at 20 minutes, 40 minutes, and 60 minutes post-trial. Sampling took place on the test day and a control day (no exercise test) in the afternoon (1:00 p.m. – 3:00 p.m.). The control group produced the highest number of

responders (73%) to the physical challenge, and showed a significant increase in cortisol levels, thus supporting the expected rise in cortisol following exercise. However, neither del Corral and colleagues (1994) nor Jansen et al. looked at whether, for children, cortisol levels during the remainder of the day were affected by the exercise protocol.

Evening physical activity has been shown, however, to correlate with heightened bedtime cortisol for boys although not for girls (Kertes & Gunnar, 2004). This study investigated 72 children ($n$ = 39 girls, $n$ = 33 boys), between 7 and 10 years old. Saliva samples were collected between 8:00 p.m. and 9:00 p.m. on two weekday evenings with activity and two without. Activities were defined as sport or individual lessons/clubs where the child was out of the home after 5:00 p.m. Boys' cortisol levels significantly increased on evenings with activities. Also, boys' cortisol levels were significantly higher than girls' after evenings of sport activity. After analyzing for sport type, boys playing team sports exhibited higher levels than girls in either team or training style sports. Following moderate to intense exercise, the results showed that cortisol levels would typically increase during the recovery phase and since the girls were not showing elevated levels on sports evenings the authors concluded that physical exercise is unlikely the sole explanation for their findings. They suggested that sex may explain the way children respond to exercise because of differences in competitive behavior and parental pressure to perform. There was no discussion within the procedural description on whether the participants were allowed to eat a meal from the time they returned home after the activity session to the time the samples were collected. Lack of control for this factor might have led to increases in cortisol secretion due to food consumption if in fact the boys snacked and girls did not. Another possible confounding variable is that exercise

does not increase cortisol when the exercise is of low intensity. It may be that girls were working at a lower intensity than boys even within a structured sport activity. This would support a documented trend of lower physical activity levels in girls of preschool through adolescent ages (Jackson et al., 2003).

If physical activity leads to lower levels of cortisol several hours later in adults, whatever the neurobiological mechanism involved (de Kloet et al., 1988; Hackney & Viru, 1999; Kern et al., 1995), then perhaps this may also be found for children exhibiting atypical elevations at full-time daycare. Following exercise, collection of multiple samples for examination may indicate whether this is the case.

## Exercise, Mood Enhancement, and Stress Management

CRH stimulates the release of both ACTH and β-endorphin (an opioid) from the anterior pituitary (de Kloet et al., 1988; Harte et al., 1995). The former acts in an excitatory manner, while β-endorphin activity is generally inhibitory. Endorphins are one of three identified endogenous opioids, and it is believed that all three may act in regulating (i.e., attenuating or terminating) stress responses as a defense mechanism. Physical activity results in increased levels of β-endorphin in the bloodstream and the brain (Steinberg, Sykes, & LeBoutillier, 1995), but how exercise influences affect is still unclear (Szabo, Billett, & Turner, 2001). Drolet et al. (2001) believe that endogenous opioids could represent a major modulator in adaptation to chronic stress. Indeed, opioids can act as a homeostatic control on the release of CRH (de Kloet et al., 1988; Drolet et al., 2001).

It is generally accepted by the public, and even the medical profession, that exercise makes you feel better (Edwards, 1984; McEwen & Lasley, 2003; Szabo et al., 2001). Moderate exercise improves cardiovascular function, strength and flexibility, and also appears to improve immune functioning. This may be due to initial prevention of the immune suppression caused by stress (Fleshner, 2000). Which neuroendocrine mechanism is involved is questionable at this point. Fleshner argues for catecholamines, although cortisol is known to suppress the immune system, but does conclude that regular physical activity may prevent harmful consequences of stress. The World Health Organization ([WHO], 2003) stated on their website that physical activity promotes psychological well-being, and reduces stress and anxiety. Empirical data supporting a link between psychological well-being and changes in physiological response are few, however ample literature exists on the positive effects of exercise on psychological well-being. Exercise has been shown to significantly increase positive affect and decrease negative affect (Steinberg et al., 1998), and the change seen in positive affect was greater than that seen for negative affect, supporting the view that exercise positively enhances mood. Meta-analyses by North, McCullagh, and Tran (1990), and Craft and Landers (1998) also show an improvement in non-clinical and clinical depression, respectively, as a result of physical activity.

Discussions on stress management increasingly center around the concept of prevention (Duhault, 2002; Scully, Kremer, Meade, Graham, & Dudgeon, 1998). Physical activity often plays a role in prescriptions for a healthy lifestyle, and increasing physical activity has been suggested as a necessary factor for any health interventions (Duhault, 2002). Improved fitness may aid the capacity to deal with stress (Scully et al.,

1998), however, to date, there is inconclusive evidence to support a relationship between level of fitness and degree of mood enhancement (Daley & Welch, 2004). Although their results were not statistically significant, Rudolph and McAuley (1998) did show a trend towards lower post-exercise cortisol levels in their sample of trained male runners. Greater perception of effort and negative affect, among the non-runners, during exercise suggested higher cortisol post-exercise. Interestingly, there was also a low, but significant, correlation between increased positive affect and lower cortisol post-trial. Rudolph and McAuley highlighted the fact that the mechanism underlying the relationship between physical activity and changes in psychological well-being is not fully understood. It may be possible that cortisol response is attenuated in physically active individuals.

A recent study indicated that the exercise session does not necessarily have to be long for positive affect to improve. Daley and Welch (2004) investigated the effects of both a 15 minute and 30 minute exercise session on university volunteers who regularly participated in physical activity. They found that both durations resulted in increases in positive well-being scores, and decreases in psychological distress and fatigue scores measured by the Subjective Exercise Experience Scale. In addition, these effects lasted for up to 2 hours after finishing the exercise.

Changes in mood states following exercise are also apparent in children. Williamson, Dewey, and Steinberg (2001) reported increases in positive mood and decreases in negative mood after two differing exercise conditions, with the opposite being seen in the control group that watched a video. No significant differences were found between the two exercise conditions – fun run and physical play – although mean

scores provided for both conditions indicated a tendency towards higher positive affect and lower negative affect following the physical play. These results are encouraging, but difficult to fully interpret. Williamson and colleagues classify both exercise conditions as aerobic, however no measure of intensity was taken during the play session. In light of adult literature that shows enhanced positive affect after either aerobic exercise or meditation (Harte et al., 1995), caution is warranted in generalizing the effects found to aerobic exercise only.

## Summary

The preceding review discussed the glucocorticoid hormone cortisol, its metabolic function, response to stress, and mechanism of production. Release of cortisol during the basal circadian pattern and in reaction to acute stress can be seen as typical and positive. For example, it plays a role in energy provision and increases the body's immune system response (McEwen, 2002). Under chronic situations, however, cortisol may be detrimental to both development and health (Dickerson & Kemeny, 2004).

The research reviewed in this chapter indicates that young children attending full-time daycare show atypical cortisol patterning compared to days at home (Dettling et al., 1999; Tout et al., 1998). Higher afternoon cortisol levels at daycare could infer greater risk of immune system suppression, and due to the involvement of the hippocampus with the HPA axis, it is possible that high levels of cortisol during this time may impair hippocampal development and/or memory function. The literature suggests that toddlers show higher increases in afternoon cortisol than preschoolers (Gunnar & Donzella, 2002;

Watamura et al., 2003), supporting the decision to investigate the toddler age group in the current study.

Exercise has been shown to be related to increased positive affect and lower recovery cortisol levels in adults (Rudolph & McAuley, 1998). Changes in mood states following exercise have also been found in 9- and 10-year-old children. Williamson et al. (2001) reported increases in positive mood and decreases in negative mood after exercise, with the reverse being seen in a control group who watched a video. These findings suggest that exercise may be a viable option for full-time daycares to implement for reducing atypical levels of cortisol among toddlers.

The purpose of this study was to determine the effects of an acute bout of physical play on atypical cortisol levels observed in toddlers attending full-time daycare, and to examine their cortisol response to and recovery from this form of acute physical activity. It was hypothesized that toddlers would show similar responses to physical activity, and cortisol regulation, as do adults. Cortisol levels would initially increase in response to moderate to high intensity physical activity with recovery occurring over the following hour. It was expected that an acute bout of physical activity play would elicit a lowering of atypical mid-afternoon cortisol levels seen in toddlers attending full-time daycare.

This study will enhance understanding of cortisol response and recovery in toddlers. Previous researchers have highlighted the need to consider how cortisol levels can be regulated among populations of young children (Watamura et al., 2003).

# CHAPTER III

# METHOD

The purpose of this study was to determine the effects of an acute bout of physical play on atypical cortisol levels observed in toddlers attending full-time daycare, and to examine their cortisol response to and recovery from this form of acute physical activity. Chapter III presents methodology for the study and consists of the following sections: (a) participants, (b) materials and equipment, (c) procedures and design, and (d) data treatment and analysis. The protocol used was approved by the Auburn University Institutional Review Board for Research Involving Human Subjects.

## Participants

The participant sample was 22 toddlers with ages ranging from 26 – 45½ months ($M$ = 34, $SD$ = 6) who attended full-time daycare in Auburn, Alabama. The sample was composed of boys ($n$ = 10) and girls ($n$ = 12), and all participants were African American, classified as low SES. Informed consent to participate in the study was given by parents or guardians for all children. See Appendix A for a sample of the informed consent forms for the saliva sampling. Participants who took steroid medication, or indicated symptoms of bronchial infection and/or fever, were not eligible to be included in the data analysis. In this study, no children were excluded for this reason.

The daycare participating in this study has subsidized programs for infants, toddlers, and preschoolers. The facility that houses the toddlers has two or three classrooms divided by age group. During data collection there were enough children enrolled for three rooms to be open. Room one (age 26 to 30 months) had a ratio of 11 children to 2 caregivers, and is approximately $17m^2$. Room two (age 31 to 38 months) had a ratio of 9 children to 2 caregivers, and is approximately $21m^2$. Room three (age 40 to 45½ months) had a ratio of 7 children to 1 caregiver, and is also approximately $21m^2$.

## Materials and Equipment

### *Saliva Sample Collection*

Salivary cortisol has been shown to validly represent serum cortisol levels in children (Woodside, Winter, & Fisman, 1991), and allows a non-invasive measure, thus simplifying sample collection in this age group. Current established methodology for collecting saliva samples, reviewed in Chapter II, begins with giving the children a variable amount of sugar-based drink crystals which stimulates saliva flow. The amount used in reviewed literature ranges from less than $1/8^{th}$ teaspoon (Legendre, 2003) to less than $1/16^{th}$ teaspoon (Watamura, Donzella, Alwin, & Gunnar, 2003). The children are then given a cotton roll or rope to mouth and the saliva extracted by syringing into a vial. The samples are then frozen and sent for assay at a laboratory. All samples from the same child are assayed in the same batch.

Contrary to earlier child development literature, it was decided to use collection materials that did not include drink mix crystals. Although research has shown that, under controlled circumstances, ingesting known amounts of certain flavor crystals should not

interfere with results from established radioimunnoassay (RIA) (Schwartz, Granger, Susman, Gunnar, & Laird, 1998), it is understood that citric-acid based stimulants used to increase saliva flow can lower pH and interfere with assay results. It was intended to pilot several collection procedures both with and without this stimulant, although compliance issues with this age group have previously necessitated its use, as only one study (Keenan, Gunthorpe, & Young, 2002) was reviewed that used the Salimetrics' enzyme immunoassay (EIA). Keenan et al. investigated African American neonates and it was unnecessary to use a stimulant with this population. Thus using drink mix crystals with the EIA would have been an unknown combination. Unpublished data had also recently been collected on toddlers without the use of oral stimulant (B. Donzella, personal communication, November 29, 2004) which encouraged the decision to exclude stimulant as a potential problem.

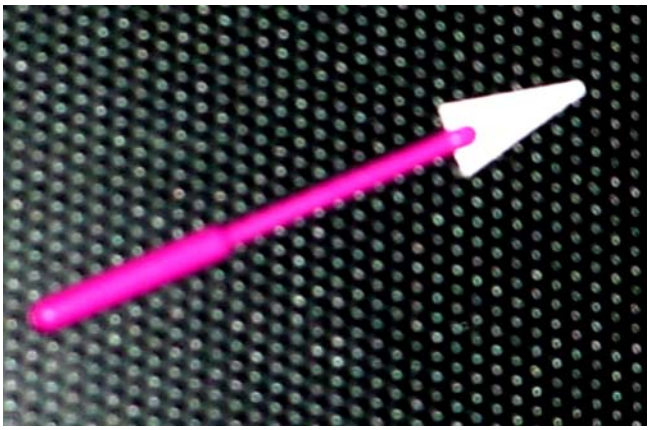

*Figure 6.* Photograph of a Sorbette

Saliva was collected using a small eye spear called a Sorbette (see Figure 6), which consists of a two inch plastic shaft with an absorbent arrowhead. The Sorbette is manufactured by Salimetrics, LLC (item #5029). After trimming the length of the rod, the Sorbette was dropped into a 1.5 microliter microcentrifuge tube (Fisher Scientific, item

#05-406-22), spear end up so that the absorbent material did not interfere with the sample once centrifuged.

*Saliva Assay*

The assay used has been specifically designed for use with human saliva by Salimetrics, LLC. Until very recently RIA developed for use with serum have been adapted for analyzing cortisol in saliva samples. The Salimetrics EIA (catalog number 1-0102/1-0112 96) has a lower limit of sensitivity determined at less than 0.007 µg/dl, and saliva cortisol measured by this method significantly correlates with serum cortisol ($r$ = 0.956, $p < 0.0001$) measured by a comparable RIA. The average intra-assay coefficients of variation (CV) are 3.88% and 7.12% for high and low controls respectively, while the average inter-assay CV are 6.69% and 6.88% (Salimetrics, LLC, 2004). The EIA thus allows for more accurate results, and also has a built-in pH indicator as a warning. Changes in saliva pH (e.g., by using too much stimulant) can compromise the effectiveness of the assay (Schwartz et al., 1998), and samples resulting in pH values less than 3.5 or greater than 9.0 should not be included for analyses (Salimetrics, LLC, 2004). Samples were analyzed by Auburn University College of Veterinary Medicine Endocrine Diagnostic Service. The mean intra-assay CV for participant samples run in this study was 4.43%. No sample in the study violated the pH range.

*Heart Rate Monitors*

Four Actiheart™ heart rate and gross motor activity monitors, (Mini Mitter, a Respironics Company, stock number 510-0001-01) were used to track toddlers' engagement in the physical activity play session. Parish et al. (2005) found the Actiheart™ placement to be well tolerated by young children. The logger itself is small

and was attached to the chest by clipping to pediatric electrodes. The logger and electrodes were then covered to prevent tampering by wrapping elastic tape around the child's chest several times. This type of tape avoids skin irritation and allows for easy removal. The Actiheart™ device is relatively new on the market, and validity and reliability have recently been established for the monitor's use with adults (Brage et al., 2005). Cronbach's alpha for the accelerometer was shown to be 0.9995 ($p < 0.001$) above 1 $m/s^2$, and output for this element was significantly and linearly related to acceleration. Cronbach's alpha for the heart rate monitor was 0.993 ($p < 0.001$) between 30 and 250 beats per minute.

## Procedures and Design

Participants within one classroom were randomly chosen for saliva sampling and assigned the four Actiheart™ monitors. Classrooms were alternated to prevent any training effect occurring from the physical play program, and the order of play and control conditions was randomized at every change of classroom.

The saliva collection procedure was developed as a game (i.e., pretend dentist) and established during the pilot study to ensure that the children were comfortable with the scenario.

Saliva samples were collected within a range of 15 minutes at 9:45 a.m., 10:35 a.m., 11:30 a.m., and 3:30 p.m. under both conditions, outdoor physical play and no physical play. These sampling times were chosen to minimize the influences of food and sleep, both of which cause a short term increase in cortisol. The pilot study indicated no pH influence, suggesting that the data collection times prevented interference from lunch

and snack. Once the sample was collected, it was put into color coded microcentrifuge tubes which had been labeled with the subject identification code and date using a permanent marker. All daily samples for each child were placed inside a zipped freezer bag in a cool box until delivery to the laboratory freezer. Each sample collection was logged on a daily record sheet indicating participant-monitor allocation, time of sample collection, and notes that may help with interpretation of data (e.g., dry mouth). Refer to Appendix B for an example of the day-to-day record of sampling. After the last sample was collected at 3:30 p.m., all microcentrifuge tubes were frozen at -20 $^{o}$C which causes particulate matter, or mucins, to precipitate. Samples were then defrosted and centrifuged to extract the saliva from the absorbent material, and separate mucins from clear sample before the assay was run. The investigator defrosted the required samples by moving them to the refrigerator overnight, and centrifuged the tubes at 10,000 g for 10 minutes. Samples were transported in a cool box to the endocrine diagnostic laboratory where they were refrozen. They were then defrosted and centrifuged again before being assayed. Procedures for the assay can be found in Appendix C.

The outdoor physical play treatment session was conducted between the collection of the first and second cortisol samples. The treatment session consisted of an already established 30-min daycare physical activity program which incorporates a mastery motivational climate for toddlers. This type of motivational climate has been shown to be effective in promoting physical activity (based on heart rate), skill development, and motivation toward physical play (Parish et al., 2005; Rudisill, Wall, Parish, St. Onge, & Goodway, 2003; Valentini & Rudisill, 2004a, 2004b; Wall, Rudisill, Parish, & Goodway, 2004). Parish and colleagues found that heart rate, physical activity levels, and the amount

of time spent in vigorous activity were all significantly higher when toddlers were engaged in the mastery motivational climate in comparison to free play. On the control day, participants did not receive the physical activity program and free play occurred indoors. The investigator was present at the daycare and interacted with the children during the indoor free play to control for any "experimenter effect". See Appendix D for an example of the daily schedule for the toddler daycare facility, and Appendix E for the data collection schedule.

Actiheart™ heart rate/activity monitors were used to monitor the intensity level of the children's physical activity play sessions as well as their heart rates throughout each testing day. The heart rate monitors were placed on the children at the daycare after breakfast (approximately 8:45 a.m.), on treatment and non-treatment testing days, and were removed after the fourth saliva sample was collected (approximately 3:45 p.m.). Heart rate data were collected to ensure that the participants were engaged in moderate to high physical activity and to monitor heart rate activity throughout the day. Four heart rate monitors were randomly assigned and worn by the participants each testing week. Attachment of these monitors involved wiping the chest area with alcohol, putting two electrode pads in place to connect the logger, and wrapping the logger and the pads with elastic tape to secure them. The sensors were placed, as per manufacturer's instructions, to the immediate left of the sternum at the fourth intercostal space, and at the fifth intercostal space in the mid-clavicular line. The participants' ribs were counted from top to bottom by touch to identify the appropriate location for sensor placement. The monitors display a visual signal indicating that the heartbeat is being detected. Each participant's pulse was taken manually at the wrist to ensure the monitor was recording

correctly. Heart rate and activity data were retrieved from the monitor by downloading to a personal computer. Refer to Figure 7 for an example of the data printout using the Actiheart™ software.

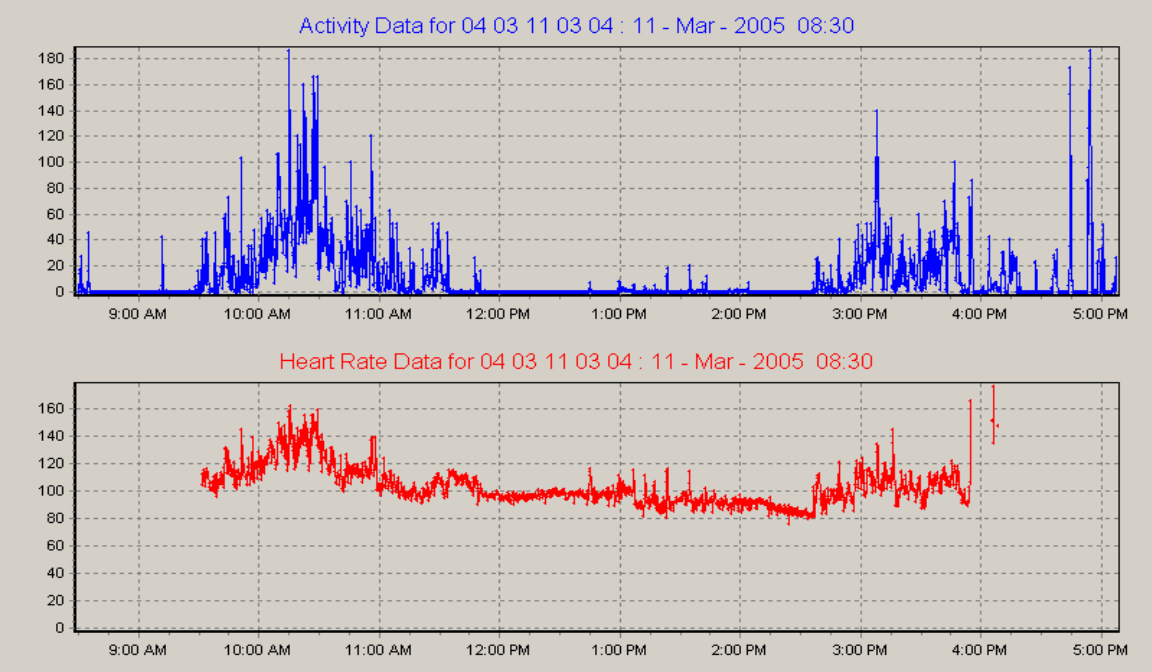


*Figure 7.* Heart rate (bpm) and activity data (counts per min) charts for one participant on a physical play day. (Taken from pilot data collection.)

*Mastery Motivational Climate Physical Activity Program*

The mastery motivational climate physical activity program for toddlers is based on a systematic instructional approach that uses student-centered instruction to target both the motivational level of the student and the processes of learning. See Wall and Rudisill (2004) for a more detailed explanation on how to implement a mastery motivational physical play climate with toddlers. It is a type of climate where the primary emphasis is on the autonomy of the child. The teacher facilitates an instructional environment in which students are given the opportunity to navigate their own learning as

they deem appropriate for their level of development. The focus of a mastery climate is directed toward the process rather than on the product or outcome of learning. Crucial to the mastery climate perspective is the understanding that an effort-mastery relationship helps students build patterns of achievement behavior that have positive long-term implications for learning across the life-span. Other tenets of mastery climate are that students are intrinsically driven to be physically and/or cognitively in control of environmental events and that every child demonstrates a preference for at least some degree of novelty within the learning environment. See Ames (1992), Valentini, Rudisill, and Goodway (1999a, 1999b), and Valentini and Rudisill, (2004a, 2004b) for more information on the theory supporting mastery motivational climates.

*Figure 8.* Photographs representing camera angles which illustrate the playground layout.

Many activities were available to the participants during the physical play session. For example, there was a large throwing target with different sizes and weights of balls or beanbags, and combinations of hockey sticks with various balls, paddles with long and short handles, scarves, a running road, obstacles for leaping or jumping over, pushing and pulling toys, and balancing toys. The playground was set up with a variety of equipment before the play session began (see Figure 8). The toddlers were allowed free choice of activity and level of challenge. The mastery motivational climate was child-directed, with

the researchers engaging in and modeling appropriate physical play. Children were encouraged to take the initiative and develop their own play, and so adaptation of the activities was supported when it advanced fine or gross motor skills and promoted physical activity. Refer to Appendix F for a sample lesson plan for the mastery motivational climate physical play session used in the present study.

*Manipulation Check*

Video analysis was used to monitor the children's physical activities during the outdoor play session, ensuring that the participants were engaged in moderate to high physical activity, and confirming data output from the heart rate monitors. For example, it was possible to clarify behavior and randomly check spikes that were identified at a particular sample time for the heart rate and activity data. Video data were thus used as a means to corroborate intensity levels indicated by the heart rate and activity data. Two cameras were unobtrusively placed so that the entire play area was included. Camera placement was previously established by Parish and colleagues (2005) to alleviate behavioral change in the children and to monitor all physical activity on the playground during the physical play session.

## Data Analysis

Because there is limited research with this population, a preliminary analysis was conducted to explore sex differences in cortisol responses as well as the heart rate data. The daycare cortisol literature reports no differences between boys and girls; however there is speculation that sex may be a factor in research investigating cortisol response to physical activity (Jansen et al., 1999). If significant differences were detected sex was

included in the analyses, otherwise all data were pooled.

A 2 (Condition) x 4 (Sample Time) analysis of variance (ANOVA) with repeated measures on both factors was conducted to examine whether interactions or main effects existed, and a series of one-way repeated measures ANOVA were conducted as follow-ups to investigate stated relationships between variables for each hypothesis. Alpha was set a priori at .05 to control for the probability of Type I error.

To test the first hypothesis that states physical play would result in lower cortisol levels at mid-afternoon than at mid-afternoon on days without physical play, a repeated measures ANOVA was conducted comparing the 3:30 p.m. samples between the play and control conditions.

To test the second hypothesis stating that toddlers would show elevated cortisol levels immediately after physical activity as compared to levels prior to physical activity and in a similar manner to older children and adults, a repeated measures ANOVA was conducted comparing the 9:45 a.m. sample to the 10:35 a.m. sample under the play condition. The response pattern was further elaborated by comparing the pre- and post-physical activity cortisol levels to values reported for older boys (del Corral et al., 1994) and adults (Jacks, Sowash, Anning, McGloughlin, & Andres, 2002) using one sample *t* tests.

To test the third hypothesis suggesting that post-activity cortisol levels would recover and return to pre-activity levels within an hour, a repeated measures ANOVA was conducted comparing the 11:30 a.m. sample with the 9:45 a.m. sample.

Heart rate monitors and video analysis were employed to validate engagement (intensity) in the physical activity play and the latter, incorporated solely as a

manipulation check, was available for corroboration if necessary. These data were used to better understand the cortisol responses to physical activity. A repeated measures ANOVA was run to better understand heart rate changes during the play condition when compared to the control condition.

In order to test the hypothesis that heart rate during physical activity play would inversely correlate to mid-afternoon cortisol levels, a regression was conducted to investigate the relationship between mean heart rate during the physical play session, calculated from downloaded data recorded by the Actiheart™ monitors, and cortisol response at 3:30 p.m. as indicated by the saliva samples. In this instance, alpha was increased to .10.

# CHAPTER IV

# RESULTS

The results of this study are presented in the following sections: (a) preliminary analyses, (b) data treatment, (c) cortisol data, (d) heart rate data, and (e) hypotheses testing. To make recognition of the sampling times for the study less cumbersome when discussing the statistical tests used in this chapter, labels were allocated as explained in Table 1.

| | Control | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Time | 9:45 a.m. | 10:35 a.m. | 11:30 a.m. | 3:30 p.m. | 9:45 a.m. | 10:35 a.m. | 11:30 a.m. | 3:30 p.m. |
| Label | C9 | C10 | C11 | C3 | P9 | P10 | P11 | P3 |

*Table 1.* Labels for saliva sample times.

## Preliminary Analyses

Previous literature has indicated that the cortisol response to exercise differs for adult males and females (Putnam, Chrousos, Nieman, & Rubinow, 2005), although the daycare literature suggests that no differences are seen in daily cortisol levels between young boys and girls (Watamura et al., 2004). In light of this discrepancy, preliminary analyses were performed to ascertain whether sex should be included as a variable.

Two separate repeated measures analyses of variance (ANOVA), with sex as a between subjects variable were conducted. The first analysis, Sex (2) x Cortisol Sample (8), and the second, Sex (2) x HR (2), revealed no significant main effects for sex, $F$ (1, 17) = .16, $p$ = .69, and $F$ (1, 20) = .01, $p$ = .91, respectively. Main effects existed for cortisol sample, $F$ (1, 17) = 7.04, $p < .05$, and HR, $F$ (1, 20) = 165.87, $p < .01$, however, neither analysis revealed a significant interaction, $F$ (1, 17) = .41, $p$ = .53, and $F$ (1, 20) = 1.12, $p$ = .30, respectively. All further analyses, therefore, were conducted on available samples, disregarding sex.

## Data Treatment

A within subjects design was implemented and all participants were sampled under two conditions – physical play and control (no planned outdoor physical play). The independent variable was outdoor physical activity play, and the dependent variables were cortisol level measured four times during each condition and heart rate measured during the investigator contact time. Each participant was assessed twice under both conditions with cortisol measures averaged for each sample time when more than one saliva sample was available (Watamura et al., 2004). In total, 374 samples were collected, 12 of which (3.21%) contained insufficient volume to assay. Additionally, duplicate assays were assessed when samples were large enough, and these duplicates correlated well ($r$ = .96, $p < .01$). However, if the difference between duplicate values was greater than or equal to 40%, the decision was made to drop the sample (Kalman & Grahn, 2004). Of the remaining 362 samples, nineteen (5.25%) failed this criterion and were not included in the analyses. When possible, duplicate values were averaged for the sample; samples for duplicate trial days were then averaged as described above.

The combination of insufficient samples and duplicate assays greater than or equal to 40% resulted in two unusable sample values (i.e., child 07 at 3:30 p.m. under the play condition and child 19 at 11:30 a.m. under the control condition). Children with missing data for a sample time were included in the analyses for sample times when sufficient material was provided. To identify outliers, the data were standardized (*z* scores) which indicated two values measured at more than three standard deviations from the mean (i.e., child 02 at 3:30 p.m. and child 17 at 11:30 a.m., both under the play condition). These four scores were entered as blank in the analyses, in order to be interpreted as missing data by SPSS.

Raw heart rate data were voluminous with a data point every 15 seconds, from 8:30 a.m. until the records from the Actiheart™ monitors were downloaded (approximately 4:30 p.m.), for each day of participation for each child. Heart rate data from the period of investigator contact and nap time were analyzed for this study. Investigator contact time was defined using the daily record for the control condition, and using a combination of the daily record and activity count chart which is incorporated in the Actiheart™ software for the play condition. The mean of the heart rates for the contact period was calculated. To calculate resting heart rate, nap time was defined using the activity count chart (i.e., the flatline of activity from lunch time to early afternoon). These data points were then transferred to OriginPro® v7.0383 (OriginLab Corporation) and plotted. The plot was used to identify the 20 minute period where heart rate was consistently the lowest during the nap.

## Cortisol Data

Raw cortisol data for each child were reduced as explained above, and mean levels, along with graphed figures, are found in Appendix G.

Group means for cortisol measured at the four different sampling times throughout the day under both control and play conditions are presented in Figure 9. Please note that all raw data points were included in this graphic representation, and values may differ from other reported means due to the interpretation of missing data.

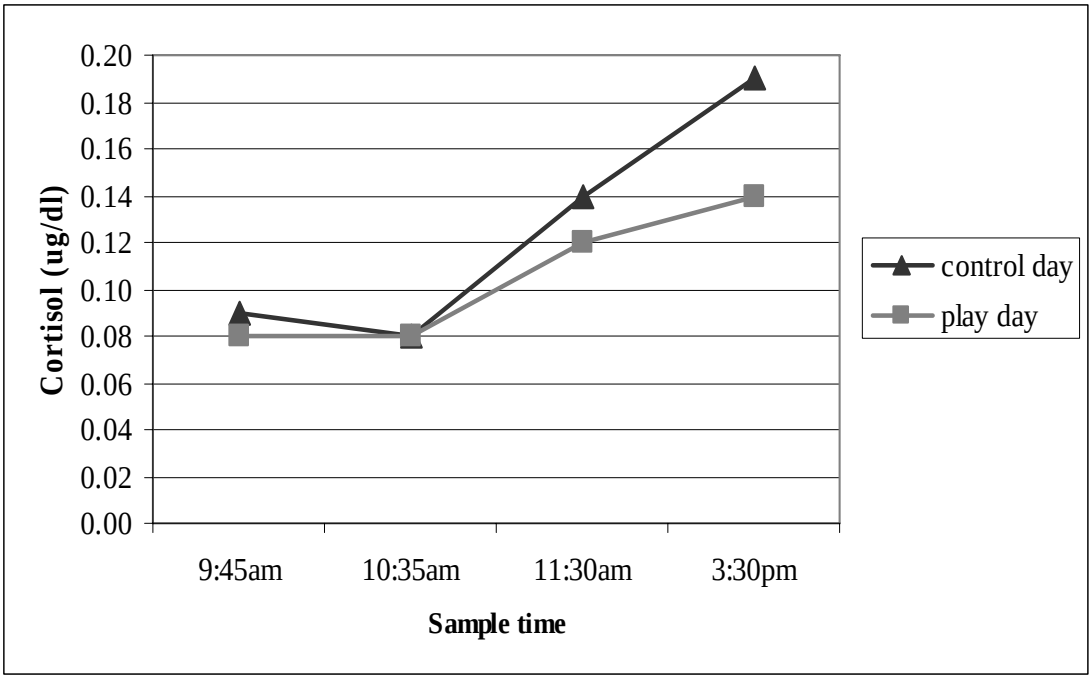


*Figure 9.* Mean ($N$ = 22) cortisol levels (µg/dl) over sample times for control and play conditions.

## Heart Rate Data

Raw heart rate data for each child was reduced as explained above, and mean levels, along with the cutoffs for calculating PAHR-50, are located in Appendix H.

Group means for HR measured during the investigator contact time under both control and play conditions are given in Table 2. Also included are mean RHR, and PAHR-50 (refer to definitions in Chapter I for the formulas used in calculating RHR and PAHR-50). Mean cutoff points for calculating PAHR-50 were 137 bpms for the control condition, and 140 bpms for the play condition.

| | HR | Time @ PAHR-50 | RHR |
|---|---|---|---|
| Control day | 122 | 3.8 | 91 |
| Play day | 152 | 22.7 | 93 |

*Table 2.* Mean HR (bpms) and time at PAHR-50 (mins) during contact sessions, and RHR (bpms) calculated from nap.

Figure 10 illustrates the number of children whose mean HR during the investigator contact time was above the cutoff value for PAHR-50. It also indicates the number of children whose PAHR-50 was either less than 10 or 20 minutes, and those with PAHR-50 greater than 20 minutes. Numbers are given for both control and play conditions.

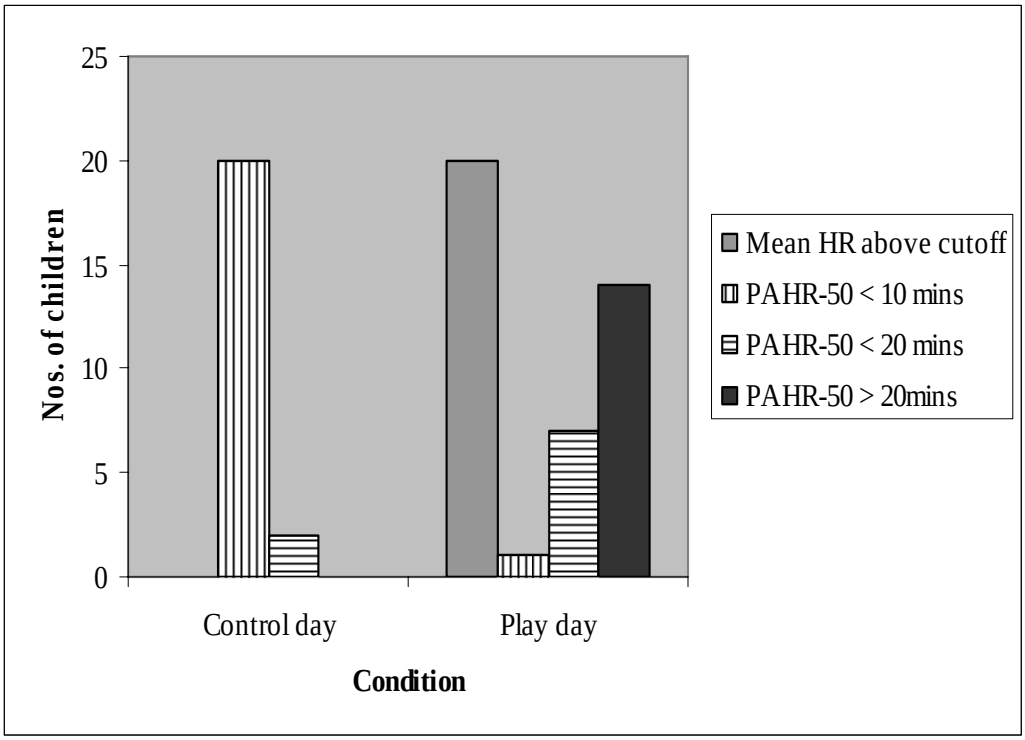


*Figure 10.* Number of children ($N$ = 22) with mean HR above PAHR-50 cutoff value, and PAHR-50 < 10 or 20 minutes, or > 20minutes.

## Hypotheses Testing

The analyses performed to investigate the hypotheses for the study included within-subjects repeated measures ANOVA, one sample *t* test, and regression. Alpha was set at .05 for hypotheses 1, 2, 3, and an ANOVA conducted in support of hypothesis 4. Alpha was set less stringently, at .1, for the regression test on hypothesis 4 to allow for the small sample size (Stevens, 2002). This increased the likelihood of finding a relationship for the play condition between HR and mid-afternoon cortisol levels (Type I error). Analyses were conducted on a complete set of data ($N = 22$) except for those using variables C11 ($n = 21$), P11 ($n = 21$), and P3 ($n = 20$).

A 2 (Condition) x 4 (Sample Time) factorial ANOVA with repeated measures on both factors revealed a significant main effect for both condition, $F(1, 18) = 8.89$, $p < .01$, and sample time, $F(3, 16) = 7.21$, $p < .01$. There was also a significant interaction between condition and sample time, $F(3, 16) = 5.94$, $p < .01$. The effect size was extremely strong ($\eta_p^2 = .53$), indicating that the interaction explained 53% of the variance observed. Therefore, appropriate follow-up tests were performed in order to investigate the specific relationships stated in the hypotheses.

*Hypothesis 1*

This hypothesis stated that physical activity play in the morning would result in lower cortisol levels mid-afternoon (P3) than at mid-afternoon on days without physical activity play (C3).

A one-way repeated measures ANOVA was conducted to investigate cortisol levels at C3 ($M = .19$, $SD = .11$) and P3 ($M = .13$, $SD = .08$). Cortisol levels were significantly lower at 3:30 p.m. under the play condition than they were at 3:30 p.m. for the control condition, $F(1, 19) = 11.20$, $p < .01$. Given the effect size ($\eta_p^2 = .37$),

this result can be said to be of very strong practical significance with the play condition explaining 37% of the decrease in cortisol levels in the afternoon.

To provide further support and authenticate the difference in cortisol levels at mid-afternoon, a one-way repeated measures ANOVA was conducted to investigate cortisol levels at C9 ($M$ = .09, $SD$ = .07) and P9 ($M$ = .09, $SD$ = .05). There was no difference in cortisol levels at the first sample time, 9:45 a.m., between the play and control conditions, $F$ (1, 21) = .25, $p$ = .63. Thus, lower levels of cortisol at mid-afternoon under the play condition were not due to levels being lower at the start of the day when compared to the control condition. For a review see Figure 9 which depicts the toddlers' average cortisol patterning at 9:45 a.m., 10:35 a.m., 11:30 a.m., and 3:30 p.m. for the play and control conditions.

*Hypothesis 2*

It was hypothesized that toddlers would show elevated cortisol levels immediately after physical activity (P10) as compared to levels prior to physical activity (P9), and in a similar manner to older children and adults.

To illuminate the cortisol patterning over the physical play session, the pre-activity ($M$ = .09, $SD$ = .05) and post-activity ($M$ = .08, $SD$ = .07) means for toddlers were compared using a repeated measures ANOVA with two levels. The results indicated that no rise in cortisol level was seen over the course of the physical play session in this study, $F$ (1, 21) = .40, $p$ = .53, and in fact, on average, the scores decreased slightly.

Four one-sample $t$ tests were performed to compare pre- and post-activity cortisol levels for the toddlers with pre- and post-exercise cortisol levels for 10-year-old boys (del Corral et al., 1994) and active adult males (Jacks, Sowash, Anning, McGloughlin, & Andres, 2002). The toddlers' pre-activity levels were significantly

different to the test value of .20 µg/dl for adults, $t$ (21) = -10.96, $p$ < .01, but not the test value of .08 µg/dl for older boys ($p$ = .63). Post-activity levels were significantly different between the toddlers and both the adults and older boys, $t$ (21) = -18.71, $p$ < .01, and $t$ (21) = -3.74, $p$ = .01, respectively. Test values for the post-activity comparison were .35 µg/dl for the adults, and .13 µg/dl for the 10-year-old boys. These results highlight the immaturity of the toddlers' HPA axis in regards to circulating levels of cortisol, and response to a physiological stressor.

*Hypothesis 3*

This hypothesis stated that post-activity cortisol levels (P10) would return to pre-activity levels (P9) within an hour (P11).

Owing to the results of testing hypothesis 2, it was unnecessary to investigate a recovery to pre-activity cortisol levels since no cortisol response to the physical play was observed. However, a one-way repeated measures ANOVA was conducted to examine any changes in cortisol level that did occur in the hour following the physical play session. Cortisol values for P10 ($M$ = .07, $SD$ = .07) and P11 ($M$ = .10, $SD$ = .06) were entered, and a significant increase was seen between 10:35 a.m. and 11:30 a.m., $F$ (1, 20) = 8.16, $p$ = .01. A similar significant increase between these times was also seen on the control day, $F$ (1, 20) = 9.49, $p$ < .01.

*Hypothesis 4*

Finally, it was hypothesized that heart rate during physical activity play would inversely correlate to mid-afternoon cortisol levels (P3).

Initially, a one-way repeated measures ANOVA was conducted to investigate HR during the investigator contact time for both the control condition ($M$ = 122, $SD$ = 7) and the play condition ($M$ = 152, $SD$ = 10). This analysis indicated that HR was

significantly higher, $F$ (1, 21) = 163.84, $p < .01$, during the physical play session, and that the effect size for this result was extremely strong ($\eta_p^2 = .89$).

The result of a regression to investigate the relationship between HR during the physical play session and cortisol measured at 3:30pm was not significant, $F$ (1, 18) = .80, $p = .38$, even at the more liberal alpha level. There was a weak to moderate inverse relationship existing for this study, but the analysis confirmed that HR explained only 4.2% ($r = -.21$) of the decrease in cortisol on the afternoon of the play session and could not, therefore, reliably be used as a predictor of this event.

# CHAPTER V

# DISCUSSION

The purpose of this study was to determine the effects of an acute bout of physical play on atypical cortisol levels observed in toddlers attending full-time daycare, and to examine their cortisol response to and recovery from this form of acute physical activity. Participants for the study were 22 African American toddlers ($M$ = 34 months, $SD$ = 6 months) who attended full-time daycare in Alabama. The sample was composed of boys ($n$ = 10) and girls ($n$ = 12), and all participants were classified as low SES. Saliva was collected from participants four times a day under both control and play conditions so that the influence of vigorous physical activity on cortisol levels could be investigated.

Chapter V presents a discussion of the results for the study in line with current literature on the topic, and consists of the following sections: (a) hypotheses findings, (b) cortisol patterns, (c) summary, (d) conclusions, and (e) considerations for future research.

## Hypotheses Findings

### *Hypothesis 1*

The main objective of this study was to determine whether participating in vigorous physical activity would affect the atypical cortisol levels reported in toddlers

who attend daycare full-time. It was hypothesized that physical play in the morning would result in lower cortisol levels mid-afternoon (3:30 p.m.) than at mid-afternoon on days without physical play. The results showed that cortisol levels in the toddlers were lower in the mid-afternoon when they had engaged in physical play in the morning, and that this effect was not due to the marginally lower mid-morning (9:45 a.m.) levels observed before the play condition. Analysis indicated that values at this time were not significantly different from those at mid-morning for the control condition. Thus, the hypothesis was supported. However, it should be noted that, even under the play condition mid-afternoon levels were elevated over mid-morning by .06 µg/dl, meaning the toddlers were still exhibiting an atypical cortisol pattern. It had been hoped that cortisol production would be reduced enough for there to be no change between mid-morning and mid-afternoon levels which would indicate a more typical pattern for this age group. Figure 11 illustrates a typical pattern at home for toddlers.

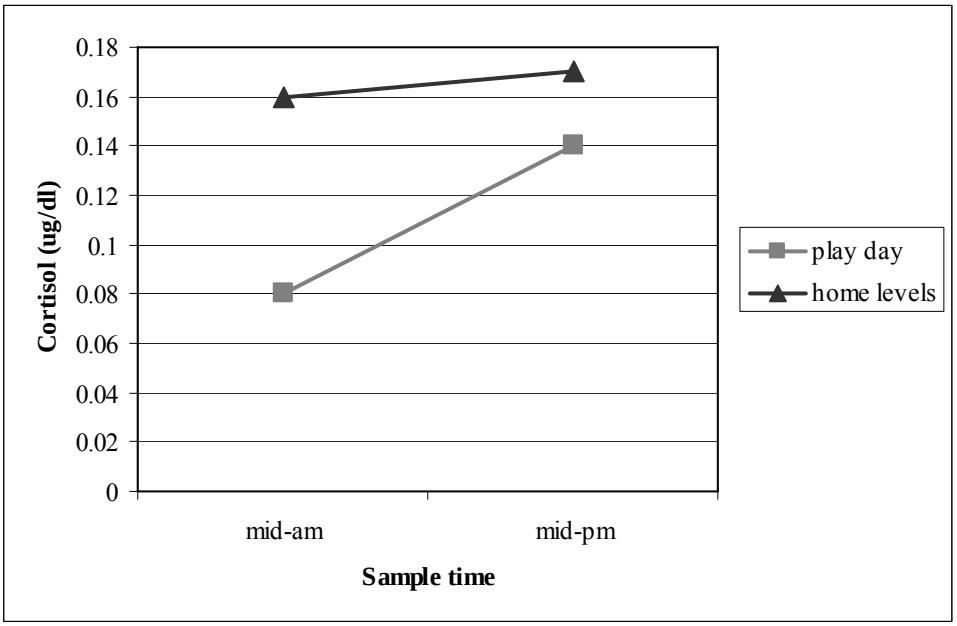


*Figure 11.* Comparison of cortisol values between the play condition in the present study and baseline (home) levels found by Watamura et al., 2003.

Studies in the child development field continue to investigate explanations for the rise in cortisol at daycare, and suggest associations with self-regulatory abilities and temperament, however no one has yet conveyed how to lower these context-specific cortisol elevations considering the realities of the situation. This study indicates that physical activity might provide a method for lowering these elevations, and the literature reviewed earlier in Chapter II alludes to several mechanisms, physiological and/or psychological, which may be responsible for this.

One possible explanation for the results is that β-endorphin production is increased with vigorous physical activity, and is understood to have the same precursor, corticotropin releasing hormone (CRH), as cortisol (Harte, Eifert, & Smith, 1995; Sforzo, 1989; Steinberg, Sykes, & LeBoutillier, 1995). Perhaps this study provides further support for the idea that increased levels of this opioid following the physical play session may be involved somehow in reducing cortisol release as part of their homeostatic 'job' (Grossman, Bouloux, & Price, 1984; Steinberg et al., 1995). Cortisol is not the only inhibitor which acts upon CRH; CRH is also the focus of negative feedback from other substances, one of which is β-endorphin (Miller & O'Callaghan, 2002). The review by Drolet and colleagues (2001) highlighted the action of endogenous opioids in regulating (and possibly terminating) the stress response, although it would appear that this mechanism is still not fully understood. Collecting regular blood samples in a non-clinical situation has been achieved with toddlers (B. Donzella, personal communication, November 29, 2004) but nonetheless it would be difficult to collect the substantial number of samples necessary to examine β-endorphin response to physical activity.

It also seems possible that the relationship between cortisol and affect may play a role. Exercise has been shown to increase positive affect in both adults (Daley

& Welch, 2004) and children (Williamson, Dewey, & Steinberg, 2001), and higher positive affect has been correlated with lower cortisol levels (Rudolph & McAuley, 1998). It could be suggested that this mechanism also finds support in the results, if the same effects were shown to hold true for toddlers. The biological pathways by which physical activity enhances mood as well as the association of either higher positive affect and/or lower negative affect with decreased cortisol levels (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005) are still under consideration. To assess the likelihood of this association may prove difficult in toddlers, and may need to be investigated via an older population initially.

Another option to explain the lower mid-afternoon cortisol on physical play days returns to the connection with naptime. Although Watamura, Sebanc, and Gunnar (2002) found that no variables associated with napping (e.g., quality of rest taken) were responsible for afternoon cortisol elevations, immediate post-rest saliva samples indicated a decline in cortisol levels from pre-rest samples. Adults have been shown to have lower cortisol during the night after exercise (Hackney & Viru, 1999), so conceivably the 3:30 p.m. levels in the present study could be a product of a similar relationship between the play session and naptime. It may be possible that the toddlers rest better (i.e., actual quality of sleep is improved) after physical activity due to the additional energy expenditure, or perhaps they are just more relaxed having had the opportunity to move freely.

One caveat should be stated regarding the elevations in cortisol observed in this and previous studies cited. The expected afternoon range for children 2½ years to 5½ years of age is .08 – .66 µg/dl (Salimetrics, LLC, 2004). Thus, values for both the play (.14 µg/dl) and control (.19 µg/dl) conditions in this study are within 'normal' limits, and any assumption of risk must be restrained.

*Hypothesis 2*

It was important to examine whether the hypothalamic-pituitary-adrenal (HPA) axis in toddlers responded to the outdoor play session as a physiological stressor by raising cortisol levels. The hypothesis stated that cortisol levels immediately after the physical play (10:35 a.m.) would be higher than those prior to the physical play (9:45 a.m.), and that this patterning would be similar to that seen in older children and adults. There was no change in cortisol levels pre- to post physical play, failing to support hypothesis 2.

As there is no previous literature on the HPA axis response to physical activity in this age group this unanticipated result is still of interest. The lack of significance ($p$ = .53) when comparing the mean pre- and post-activity cortisol levels was such that even increasing the sample number would be unlikely to result in a difference. This cortisol patterning across moderate to high intensity physical activity has not been shown before in the literature for other age groups. It should be mentioned that the PAHR-50 estimates suggest the children were engaged in moderate to high intensity physical activity during the outdoor play session, so this result cannot be explained by the fact that they did not participate in enough physical play to elicit a response.

The question arises as to whether any toddlers show a peak response to physical activity as a physiological stressor, or whether no response is developmentally appropriate. Is it possible that the temporal pattern of their response is quicker or slower than seen in an older population, and peak values existed but were missed due to the sampling times used? It is believed that children below the age of four years have an immature HPA system (Gunnar & Donzella, 2002; Watamura, Donzella, Kertes, & Gunnar, 2004) and that changes in cortisol

production are seen as development progresses, so perhaps a response might be observed as these children transition to preschool age.

Recently, it has been reported that adults' cortisol response to acute stress is lowered if the individual concerned is also under chronic stress (Matthews, Gump, & Owens, 2001), and suggestions have been made that children from low-income families may exhibit different stress responses because of exposure to lifestyle stressors (Keenan, Gunthorpe, & Young, 2002). If this is the case, one could conclude that the recurring daily stress of attending full-time daycare affects the cortisol reaction to physical activity as an acute stressor. Hyporesponsiveness to a stressor is considered maladaptive (Gunnar et al., 2001; Gunnar & Donzella, 2002), however, some toddlers in this study did display a cortisol response showing the hypothesized typical pattern following the physical play session. On average, in this study, however, the response was blunted and deciding whether this equates to hypocortisolism necessitates proof of stress reactivity elsewhere in the system (Gunnar & Vazquez, 2001). It can be concluded that the autonomic stress response was initiated based upon recorded increases in HR, suggesting the toddlers in this study may have exhibited hyporesponsiveness to physical activity as a stressor.

Additional support for the different patterning seen across physical activity in this age group was supplied by comparing the toddlers' pre- and post-physical activity cortisol levels with pre- and post-exercise levels for older children and adults (del Corral, Mahon, Duncan, Howe, & Craig, 1994; Jacks, Sowash, Anning, McGloughlin, & Andres, 2002). It was reasonable to expect and find that toddlers were significantly different from adults at both samples since it is known that baseline circulating cortisol levels are higher for adults. However, the difference was even greater at the post-activity sample highlighting the lack of a mature-like

response from the toddlers to this type of stressor. The results also showed that the toddlers had similar pre-activity levels to 10-year old boys, but were significantly lower at the post-activity sample. This suggests that by the age of 10 years children (at least, boys) are beginning to exhibit a more mature response pattern even if the actual levels of cortisol are still much lower than those observed in adults.

*Hypothesis 3*

It was hypothesized that post-physical activity cortisol levels (10:35 a.m.) would recover to pre-physical activity levels (9:45 a.m.) within an hour from termination of the stressor (11:30 a.m.). However, since no immediate elevation in cortisol was seen following the physical play, this analysis was not conducted.

It was decided to statistically analyze cortisol measured post-physical play (10:35 a.m.) and before lunch (11:30 a.m.) because graphs indicated that a surprising change did occur. In fact, cortisol levels were significantly elevated at the 11:30 a.m. sample. To check whether this rise was due to the physical play session the same analysis was run on data from the control condition. A similar rise at 11:30 a.m. was also seen on the control days suggesting that it was not due to any delayed response to the physical activity.

This increase in cortisol between 10:35 a.m. and 11:30 a.m. was unexpected as, on the whole, the children were engaged in quiet indoor activities during this time under both conditions. If this result had occurred only under the play condition, then earlier suggestions of a slower response to the physiological stressor might have been supported; cortisol levels at 11:30 a.m. might still have been recovering from a peak rise following physical activity. The analysis for the control condition nonetheless indicated that the rise also occurred on the control day. One possibility is that the sampling time immediately before lunch introduced a confound because of the open

plan design of the daycare facility. The anticipation of food is a possible cause for a rise in cortisol levels (Follenius, Brandenberger, & Hietter, 1982), and the result for these analyses could well have been due to the smell of lunch triggering this response in the toddlers who were noticeably hungry by this time.

*Hypothesis 4*

The engagement of the toddlers in the physical activity play was important, not only from the long-term view of the effectiveness of the intervention on the well-being of the children, but also to give an indication of possible physiological explanations for the lowering of cortisol levels. Results showed that mean heart rates under the play condition were much higher than under the control condition. Graphing the data (see Figure 10) suggested that the toddlers were also engaged in high intensity physical activity for a greater period of time under the play condition than the control condition, although this relationship was not formally analyzed. The hypothesis that heart rate during physical play would inversely correlate to mid-afternoon cortisol levels, however, was not supported. Although a weak to moderate relationship was seen, with an inclination for higher HR to correlate to lower cortisol mid-afternoon, this relationship was not significant ($\alpha = .10$). Low power for this analysis may explain the nonsignificant results.

Despite the lack of a relationship between HR and cortisol for this study, these results indicating significantly elevated HR during physical play are promising for highlighting both the sedentary nature of a typical day in full-time care, and the need for incorporating an intervention to promote physical activity amongst toddlers at daycare.

The baseline cortisol patterning between mid-morning and mid-afternoon for the study control day is considered to be atypical, and is similar to that reported in previous research with toddlers attending full-time daycare. See Figure 12 for a representation of these patterns. A typical 'unstressed' pattern for toddlers would show no significant change between mid-morning and mid-afternoon values (Watamura et al., 2004) For review of typical patterns see Figure 11.

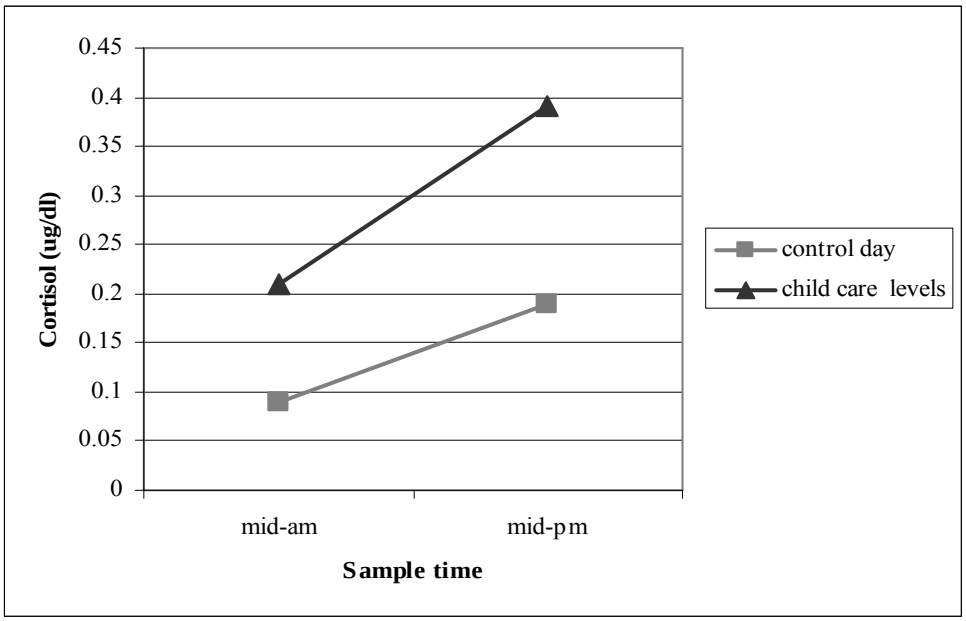


*Figure 12.* Comparison of baseline cortisol values between the control day in this study and daycare levels reported by Watamura et al., 2003.

Expected ranges for morning and afternoon cortisol are .06 - .70 and .08 - .66 respectively (Salimetrics, LLC, 2004). All mean data under both conditions for this study fall within these ranges, although the levels observed are on the low ends of the norms. However, the population data that were examined to create the expected ranges was predominantly Caucasian (M. Curran, personal communication, September 15, 2005). Therefore, at this time it is not possible to say whether levels for the study participants are low because of their ethnic background or because they

are a chronically stressed population exhibiting hyporesponsiveness to daycare stress. The latter may be more probable when adult research showing no difference in baseline cortisol levels between Caucasians and African Americans is taken into account (Masi, Rickett, Hawkley, & Cacioppo, 2004). As there are no data available on cortisol response following physical activity in Caucasian toddlers, it is not possible to deduce whether ethnic differences may occur though it is known that in adults, catecholamine responses to exercise do differ (Walker et al., 1992).

## Summary

The results of this study have shown that on days when toddlers engage in physical play in the morning, their mid-afternoon cortisol levels are lower than on days they do not. Conclusions cannot be drawn as to the mechanisms involved, but it is suspected that increases in β-endorphin and/or positive affect may play a role. Support for this latter suggestion can be found in a paper by Rudolph and McAuley (1998) who concluded that positive affect may be a mechanism for dampening cortisol response. This result cannot be generalized to other populations (e.g., SES, ethnic groups, age ranges) at this time.

Low-income, African American toddlers in this study did not show a cortisol response to physical activity at the 10:30 a.m. sampling. The lack of reactivity to the physical play session was contrary to reviewed literature which indicated a peak response approximately 20 minutes from the onset of a physiological stressor, and a return to baseline levels within an hour from the end of the stressor. Further study is necessary to be able to conclude whether this result is due to a slower temporal pattern of cortisol release in this age group, whether it is indicative of hypocortisolism, or whether typically no response occurs to this type of stressor in

toddlers. There was a significant increase in cortisol levels by 11:30 a.m. for both conditions, but it is possible that the 11:30 a.m. rise was due only to anticipation of lunch being served. Additional saliva sampling times would give a clearer picture.

Finally, results also indicated a trend for higher heart rate during physical play in the morning to be related to lower cortisol levels at mid-afternoon. Although, not significant, this result indirectly supports the earlier supposition regarding β-endorphin. It is known that higher heart rates translate to more vigorous physical activity, which in turn would lead to increased levels of β-endorphin (Powers & Howley, 2001). This conclusion would be premature at this point since other physiological responses connected to higher heart rate may be responsible for the lower cortisol levels.

The developmental literature emphasizes the risks which may be involved with either very high levels of cortisol release due to extreme acute stress, or regular lower elevations (de Kloet, 2004; Nelson & Carver, 1998). Levels observed in this study were within expected normal ranges for toddlers, although demonstrating an atypical pattern, thus it would be unwise to assume long-term developmental complications. It may even be possible that the increases in cortisol that toddlers have been shown to exhibit over a day in full-time center-based care constitute adaptation, and not risk. A healthy response in the HPA axis to 'stress' includes the release and inhibition of cortisol and other stress hormones (de Kloet, 2004). The literature suggests that children exhibiting an atypical pattern at daycare do not show this pattern on days at home, suggesting the feedback loop remains effective. Another consideration is that perhaps the elevations observed thusfar enable these children to adapt and cope more efficiently in the future. This would certainly be supported by Selye's theory of general adaptation syndrome (as cited in Edwards, 1984), which

suggests a learned stress response, and Gunnar and Cheathams' (2003) suspicion that the HPA system can be reorganized when perturbations are not excessive. Daycare toddlers may show habituation and attenuation of their cortisol response as preschoolers attending daycare full-time.

Longer exposures to cortisol may, however, incur risk, and it then becomes necessary to understand what problems may be faced. Very little is currently known about the effect cortisol has on brain development in young children. Areas involved in memory and attention are developing rapidly at this age (Gunnar, Bruce, & Donzella, 2001; Nelson & Carver, 1998), and have a large number of cortisol receptors. Animal studies would implicate glucocorticoids in teratogenic activity within the developing brain, but it appears that extreme levels are required to have this effect (Sapolsky, 2003).

Much more is understood about the health risks involved with elevated levels of cortisol which have been linked with central (i.e., visceral) adiposity, cardiovascular disease, and type II diabetes (Miller & O'Callaghan, 2002), although at this time more severe elevations than have been observed at daycare are implicated in these threats. For this investigator, as others, the more insidious problem may be that of the increase in allostatic load that these toddlers are undergoing. Whether they are within normal ranges or not, the fact remains that toddlers' cortisol patterns at daycare are atypical, and that the overall amount of cortisol they are exposed to is greater than on days at home. Multiply this by the number of days a year they attend their daycare and this may be where the issue lies. For example, lowered immune levels are an accepted part of attendance in daycare, but are caused when the HPA axis is repeatedly activated. It may be the accumulation of these daily alterations in

basal cortisol that provides a warning for future health risk (Gunnar, Bruce, & Donzella, 2001), and increases susceptibility to predisposed disease (de Kloet, 2004).

Thus, the physical play intervention may provide a two-pronged approach to start managing childhood obesity, heart disease, and diabetes. Firstly, the results of this study support those of Parish, St. Onge, Rudisill, Weimar, and Wall (2005) showing increases in physical activity during the mastery motivational climate utilized. Secondly, an acute bout of physical activity has been shown to reduce elevations in cortisol which is an acknowledged risk factor for all the above. The findings discussed here answer a call for interventions which address stress related illness (Duhault, 2002), and provide much needed physiological information on low-income African American toddlers. More specifically, this study was conducted to begin the process of understanding how atypical mid-afternoon cortisol increases can be reduced (Watamura, et al., 2003).

## Conclusions

The following conclusions can be drawn from the data collected during this dissertation:

- Low-income toddlers in this study exhibited a significant elevation in cortisol from mid-morning to mid-afternoon. These data support the pattern observed in earlier studies of middle-income children attending full-time daycare.
- Results indicate a relationship between physical play in the morning and lower elevations of cortisol in the afternoon. Further research is needed to be able to state conclusively the mechanism behind this relationship.

- Vigorous physical play did not elicit an elevated cortisol response as expected. Additional research is required to ascertain the reasons for the blunted response observed.
- A significant increase in cortisol level was seen at the 11:30 a.m. sample over the 10:30 a.m. sample supporting a similar rise recorded by Watamura and colleagues (2002). Future research is necessary to determine if this elevation is part of the overall rising pattern for the day, or whether it is a typical event for children of this age.
- The mastery motivational climate employed significantly raised heart rates for the toddlers during the physical play session when compared to the control day.

## Considerations for Future Research

The findings reported in this dissertation generate new questions to be investigated. These are listed below.

- The mid-afternoon cortisol levels observed were still elevated over those at mid-morning. Would a second, early afternoon physical play session avoid this, by lowering the mid-afternoon values further?
- Would chronic physical activity, that is physical play on a daily basis, afford different cortisol responses? For example, would one regular morning physical play session attenuate mid-afternoon cortisol so that no significant increase was seen from mid-morning?
- What are home levels like for this population? Are their typical daily patterns and/or exposure to cortisol different from those reported for higher SES

toddlers? For example, do they exhibit the same pattern of cortisol release during the day but at higher or lower values?

- Is it possible to distinguish the meaning of the lack of cortisol response to vigorous physical activity that was observed by repeating the study with Caucasian toddlers of low SES? Whether or not different ethnic populations exhibit a similar blunted response might help to determine the reason for this pattern.
- Would lower mid-afternoon cortisol values following physical play in the morning be observed in other populations, such as preschoolers, or children from middle-income SES?
- Is it possible to investigate the concept of adaptation by sampling preschool and kindergarten age children and comparing those who attended full-time daycare as toddlers with those who did not? Would this then indicate that the afternoon cortisol elevations are actually a beneficial adaptation, attenuating the HPA response to daycare as a stressor?
- Alternatively, would preschoolers who regularly participated in the physical play intervention as toddlers exhibit adapted cortisol responses to physical activity in comparison to those who did not? That is, would there be attenuation or increase in the peak cortisol response following physical activity for those preschoolers who are more physically fit?

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# APPENDICES

# APPENDIX A

# PARENTAL CONSENT FORMS

HUMAN SUBJECTS
OFFICE OF RESEARCH
PROJECT #04-210EP0412
APPROVED 12-17-04 TO 12-16-05

# Auburn University

Auburn University, Alabama 36849-5323

Department of Health and Human Performance
2050 Beard-Eaves-Memorial Coliseum

Telephone: (334) 844-4483
Fax: (334) 844-1467

## INFORMED CONSENT FORM

**The effects of physical activity play on the stress levels of toddlers**

Your toddler is invited to participate in a research study to be conducted by Dr. Mary E. Rudisill and colleagues. Your child currently participates in the Motor Development Program at ADC-Ridgecrest, and this new study will be conducted within this program. The purpose of the study is to examine the effect of physical activity on the levels of cortisol (a stress hormone) in toddlers attending full-time daycare. Your child was selected as a possible participant because he/she is in the age group of interest. Unfortunately, children taking regular steroid/asthma medication are unable to be included in the data collection, since these medications can influence saliva cortisol results. However, these children may participate alongside their peers in the research activities. All children attending ADC-Ridgecrest will participate twice a week in the Motor Development Program as usual.

Specifically, I am asking your approval to collect saliva samples from your child four times per day on several occasions. Dr. Mary Rudisill, Ms. Sarah Wall, and Ms. Loraine Parish will visit the daycare to collect saliva samples from the children. An explanation of the procedure follows:

The procedure begins with children being given a small amount of Kool-Aid (less than a teaspoon) to stimulate saliva flow in the mouth. Then they will be given a dental cotton roll to put into their mouth. The cotton roll will be removed when it is soaked with saliva. The samples will be frozen and sent to a laboratory and analyzed. At no time will children be left unattended while the cotton roll is in their mouth. Your toddler's samples will be coded and kept confidential.

In addition, if you provided your approval earlier this year, your child may occasionally be wearing a heart rate monitor and be video taped to monitor exercise patterns during the Motor Development Program as well. To attach the monitors, we will wipe the chest area with alcohol and put two pads in place to connect the monitor. Skin-safe tape will be used to secure the monitor to the skin. Physical activity sessions will be videotaped to confirm data output from the heart rate monitors.

There are no foreseeable risks or discomforts, above those that might be expected during physical activity play, associated with collection of this data. Please note, any child who expresses a desire to not participate in saliva sampling on any occasion will be allowed to stop immediately. We plan to use information obtained from sampling in any way thought best for education and publication. I plan to present the results of this study at a scientific conference and publish the results in an appropriate journal. All data will be collected and stored in a confidential way (code lists will be kept in a locked cabinet), and the children's results will be reported anonymously at all times.

By signing this form, you will be approving saliva collection and cortisol analysis to use with classroom data already being collected (heart rate and video data). Your child's participation in the data collection will in no way affect your child's standing in school. At the conclusion of the study, a summary of results will be made available to interested parents/guardians and educators. Should you have any questions or desire further information, please call Dr. Mary Rudisill at (334) 844 1458. For more information regarding your rights as a subject you may contact the Office of Research Programs: Mr. Chip Burson, Executive Director at (334) 844 5966, [bursoen@auburn.edu]; or Dr. Peter Grandjean, IRB Chair at (334) 844 1462 [grandpw@auburn.edu]. You will be provided of a copy of this form to keep.

**HAVING READ THE INFORMATION PROVIDED YOU MUST DECIDE WHETHER OR NOT TO ALLOW YOUR CHILD TO PARTICIPATE. YOUR SIGNATURE INDICATES YOUR WILLINGNESS TO ALLOW YOUR CHILD'S PARTICIPATION IN THE STUDY.**

Child's Name (print)_____

Parent/Guardian Signature_____ Date_____

Investigator Signature_____ Date_____

# APPENDIX B

## SAMPLE RECORD LOG

Record of Sampling

| Subid | HR # | Date | Time | Condition | Notes |
|---|---|---|---|---|---|
| 0931 | 09 03 03 05 01 | 5/3 | 9:55am | play 3 | Aaargh!!! (MER) |
| 1031 | 10 03 03 05 02 | | 9:48am | | good, little dry |
| 1131 | 11 03 03 05 03 | | 9:48am | | Great (MER) |
| 1231 | 12 03 03 05 04 | | 9:52am | | ok, but dry |
| 0932 | | | 11:01am | | sucked, chewed |
| 1032 | | | 10:48am | | Good |
| 1132 | | | 10:56am | | drenched in 30 secs |
| 1232 | | | 10:52am | | very dry |
| 0933 | | | 11:38am | | sucking, very dry |
| 1033 | | | 11:35am | | Great |
| 1133 | | | 11:32am | | drenched |
| 1233 | | | 11:43am | | helpful, but dry |
| 0934 | | | 3:33pm | | more cooperative |
| 1034 | | | 3:49pm | | Great |
| 1134 | | | 3:29pm | | HR monitor off |
| 1234 | | | 3:42pm | | very helpful, but dry |

# APPENDIX C

# ASSAY PROCEDURE

# High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit

Catalog No. 1-0102/1-0112 96-Well Kit Updated: 3/31/05

For Research Use Only, Not For Diagnostic Use

## Test Principle

A microtiter plate is coated with rabbit antibodies to cortisol. Cortisol in standards and unknowns compete with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical Density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present (2).

## Special Feature

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Cortisol values from samples with a pH $\leq$ 3.5 or $\geq$ 9.0 may be artificially inflated or lowered (1).

## Precautions

1. Stop Solution is a solution of sulfuric acid. This solution is caustic; use with care.
2. This kit uses break-apart microtiter strips. Unused wells must be stored at 4°C in the sealed foil pouch and used in the frame provided.
3. Do not mix components from different lots of kits.
4. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
5. See Material Safety Data at the end of procedure.
6. As for all quantitative assays for salivary analytes, we recommend that samples be screened for possible blood contamination (3, 4). This can be efficiently and economically accomplished using Salimetrics Blood Contamination EIA Kit (Cat no. 1-1302/1-1312).
7. Routine calibration of pipettes is critical for the best possible assay performance.
8. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
9. When running multiple plates or multiple sets of strips, a standard curve should be run with each individual plate and/or strips.

10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 70ºF (21.1ºC). Higher or lower temperatures will cause an increase or decrease in OD values, respectively.

Storage

All components of this kit are stable at 2-8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. Anti-Cortisol Coated Plate: A ready to use microtiter plate pre-coated with antibodies in a resealable foil pouch.
2. Cortisol Standards: Six vials, 500 µL each, labeled A-F, containing cortisol concentrations of 1.8, 0.600, 0.200, 0.067, 0.022, and 0.007 µg/dL, in a synthetic saliva matrix with a non-mercury preservative. (Values in nmol/L are 49.66, 16.55, 5.52, 1.84, 0.61, and 0.20 nmol/L respectively.)
3. Wash Buffer: A 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute the wash buffer concentrate 10 fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized $H_20$). (*If precipitate has formed in the concentrated wash buffer, it may be heated to 60°C for 15 minutes. Cool to room temperature before use in assay.)
4. Assay Diluent: A phosphate buffered solution containing a pH indicator and a non-mercury preservative.
5. Enzyme Conjugate: A solution of cortisol labeled with horseradish peroxidase.
6. Tetramethylbenzidine (TMB): A non-toxic ready to use solution.
7. Stop Solution: A solution of sulfuric acid in distilled water. (USA customers only). Stop solution is provided in powdered form to customers outside the USA. Reconstitute the powdered stop solution with 12.5 mL of deionized water. Let sit for 10 minutes before use.
8. Non-specific Binding Wells: These wells do not contain anti-cortisol antibody. In order to support multiple use, a strip of NSB wells is included with this kit. They are located in the foil pouch. Wells may be broken off and inserted where needed.

Note: The quantity of reagent provided with break-apart kits is sufficient for three individual runs. The volume of the diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.

Materials Needed But Not Supplied

- Precision pipette to deliver 25 µL
- Precision multichannel pipette to deliver 50 µL, and 200 µL
- Vortex
- Plate rotator (if unavailable, tap to mix)
- Plate reader with a 450 nm filter

- Log-linear graph paper or computer software for data reduction
- Deionized water
- Reagent reservoirs
- One disposable tube capable of holding 24 mL
- Pipette tips
- Serological pipette to deliver up to 24 mL

Specimen Collection
The preferred saliva collection method (5,6) is to use plain (non-citric acid) cotton Salivettes (Sarstedt). Freeze all saliva samples prior to assay in order to precipitate mucins. Thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Pipette clear sample into appropriate wells.

Procedure
Bring all reagents to room temperature.

Step 1: Determine your plate layout. Here is a suggested layout.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1.80 Std | 1.80 Std | Control H | Control H | | | | | | | | |
| B | .600 Std | .600 Std | Control L | Control L | | | | | | | | |
| C | .200 Std | .200 Std | Sample 1 | Sample 1 | | | | | | | | |
| D | .067 Std | .067 Std | Sample 2 | Sample 2 | | | | | | | | |
| E | .022 Std | .022 Std | Sample 3 | Sample 3 | | | | | | | | |
| F | .007 Std | .007 Std | Sample 4 | Sample 4 | | | | | | | | |
| G | Zero | Zero | Sample 5 | Sample 5 | | | | | | | | |
| H | Nsb | Nsb | Sample 6 | Sample 6 | | | | | | | | |

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSB's included in the foil pouch. Place in H-1, 2. Alternatively, NSB's may be placed wherever you choose on the plate. Reseal the zip-lock and refrigerate the pouch at 4°C. *Caution: Extra NSB wells should not be used for determination of calibrators or unknowns.*

Step 3:

- Pipette 24 mL of assay diluent into a disposable tube. Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards and unknowns into appropriate wells. Standards and samples should be assayed in duplicate.
- Pipette 25 µL of assay diluent into 2 wells to serve as the zero.
- Pipette 25 µL of assay diluent into each NSB well.

Step 5: Make a 1:1,600 dilution of the conjugate, by adding 15 µL of the conjugate to the 24 mL of assay diluent prepared in Step 3, (full plate only). Immediately mix the diluted conjugate solution and pipette 200 µL into each well using a multichannel pipette.

Step 6: Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 55 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle or by pipetting 300 µL of wash buffer into each well, and then discarding the liquid by inverting the plate over a sink. After each wash, the plate should be thoroughly blotted on paper towels before being turned upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Caution: DO NOT mix at speeds over 600 rpms.
- Wash off bottom of plate with a water-moistened lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution (correction at 492 to 620 is desirable).

Calculations

1. Compute the average Optical Density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells from the average OD of the zero, standards, and unknowns.

3. Calculate the percent bound (B/BO) for each standard and unknown by dividing the average OD (B) by the average OD for the zero (BO).
4. If calculating the results by hand, plot B/BO on the vertical axis against the log of the concentration on the horizontal axis for each calibrator and draw a straight line through the points. Determine the concentrations of the unknowns by interpolation.
5. If using software capable of logistics, use a 4 parameter sigmoid minus curve fit. Otherwise, use log-linear regression.

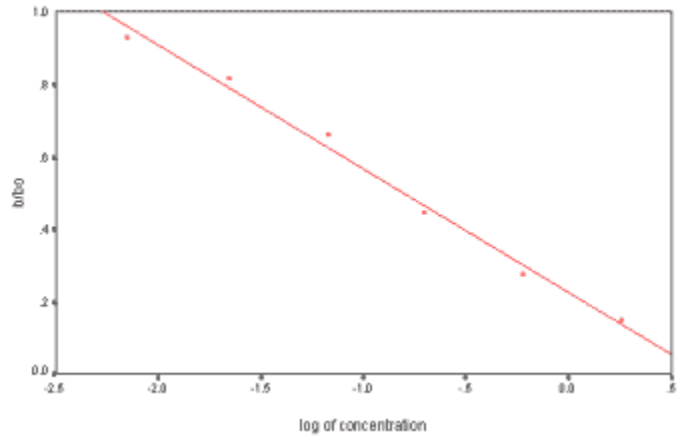
Typical Results

The following charts and graphs are for illustration only and SHOULD NOT be used to calculate results from another assay.

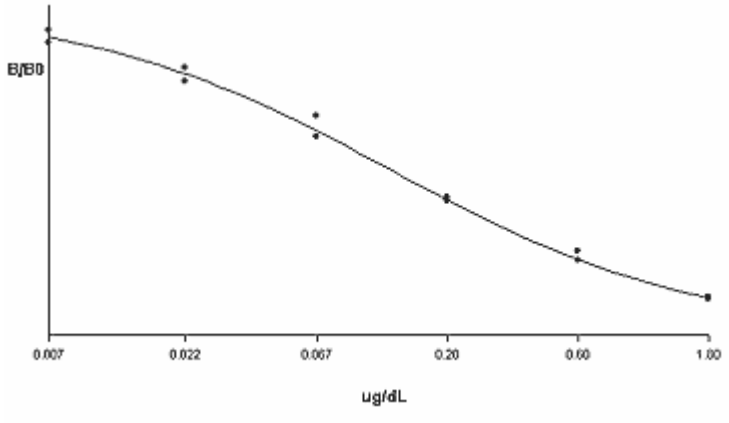
| Well | Sample | Average OD | B | B/Bo | Cortisol (µg/dL) |
|---|---|---|---|---|---|
| A1,A2 | S1 | 0.229 | 0.205 | 0.1207 | 1.613 |
| B1,B2 | S2 | 0.419 | 0.395 | 0.2326 | 0.757 |
| C1,C2 | S3 | 0.737 | 0.713 | 0.4199 | 0.214 |
| D1,D2 | S4 | 1.090 | 1.066 | 0.6278 | 0.052 |
| E1,E2 | S5 | 1.330 | 1.306 | 0.7691 | 0.020 |
| F1,F2 | S6 | 1.561 | 1.537 | 0.9052 | 0.008 |
| G1,G2 | B0 | 1.722 | 1.698 | NA | |
| H1,H2 | NSB | 0.024 | NA | NA | |

Example: Standard Curves

Log-Linear Regression




Cortisol 4-Parameter Sigmoid Minus Curve Fit




Material Safety Data*

Hazardous Ingredients
Stop Solution is a solution of sulfuric acid. This solution is caustic; use with care. We recommend the procedures listed below for all kit reagents.

Handling
Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

# APPENDIX D

## SAMPLE DAYCARE SCHEDULE

Sample Daycare Schedule

| | |
|---|---|
| 7:15 – 8:20 a.m. | Arrival, greeting, & free play |
| 8:20 – 8:30 a.m. | Bathroom, & breakfast preparation |
| 8:30 – 9:00 a.m. | Breakfast, washup, & teethbrushing |
| 9:00 – 9:10 a.m. | Free play |
| 9:10 – 9:30 a.m. | Small group activity |
| 9:30 – 10:00 a.m. | Outside play |
| 10:00 – 10:45 a.m. | Learning center |
| 10:45 – 11:00 a.m. | Small group activity |
| 11:00 – 11:20 a.m. | Outside play |
| 11:20 – 11:30 a.m. | Bathroom, & lunch preparation |
| 11:30 a.m. – 12:00 p.m | Lunch, bathroom, & cleanup |
| 12:00 – 2:00 p.m. | Naptime |
| 2:00 – 2:15 p.m. | Wake up, & bathroom |
| 2:15 – 2:30 p.m. | Snack, & washup |
| 2:30 – 3:15 p.m. | Teacher-initiated activity |
| 3:15 – 5:15 p.m. | Free play, & departure |

**Morning activity times may be rotated to accommodate small group activities

# APPENDIX E

# DATA COLLECTION SCHEDULE

Actual Collection Schedule

| Week Ending | Monday | Tuesday | Wednesday | Thursday | Friday | Notes |
|---|---|---|---|---|---|---|
| 29th April | E | C | C | | E | |
| 6th May | | E | C | E | C | |
| 13th May | C | C | E | E | | |
| 20th May | | C | E | C | E | Schools out |
| 27th May | C | E | | E | C | |
| 3rd June | closed | X | C | C | X | |
| 10th June | X | X | NASPSPA | | | |
| 17th June | | C | X | X | E | Accredited |
| 24th June | C | E | E | C | E | Fri = m/up |
| 1st July | | E | X | | closed | |

C = control day

E = play day

X = cancelled

# APPENDIX F

# SAMPLE LESSON PLAN

Physical Play Activities

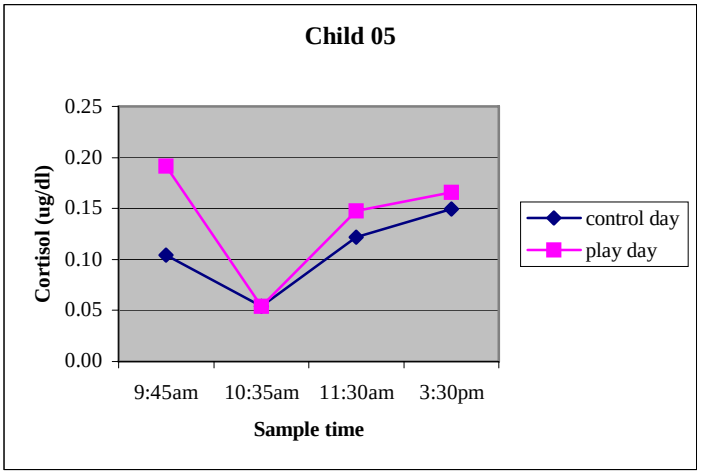
| | |
|---|---|
| Run - | running road made of alphabet squares |
| | leaping sticks |
| Jump - | colored sacks |
| Balance - | stilt cups |
| | wobble cushions |
| | colored turtles with cones and rubber rings |
| | scooters |
| Throw & Catch - | large target |
| | various size, weight, and textured balls |
| | beanbags |
| Strike - | hockey sticks |
| | various size and weight balls |
| Kick - | large goal net |
| | various size and weight balls |
| Climb - | cargo net |

# APPENDIX G

## MEAN CORTISOL DATA PER CHILD

Child 05 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.104 | 0.054 | 0.122 | 0.149 |
| play day | 0.191 | 0.054 | 0.147 | 0.166 |

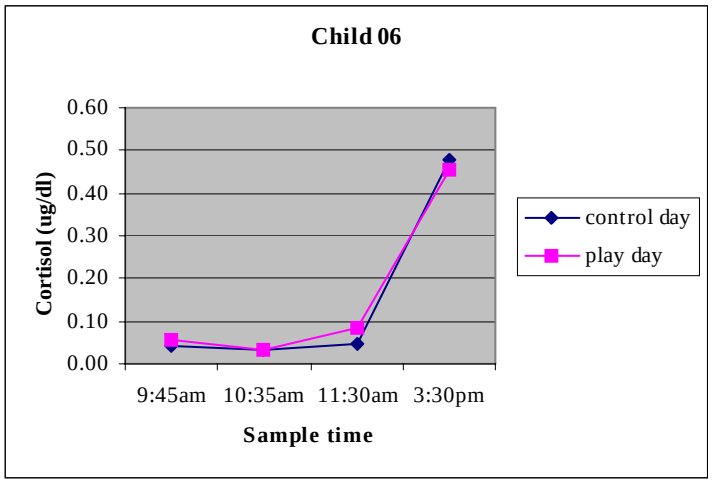



| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 234 | 0511 | 4/26 | 9:45am | 0.120 | 0.116 | 0.118 |
| 235 | 0512 | | 10:40am | 0.059 | 0.055 | 0.057 |
| 236 | 0513 | | 11:30am | 0.141 | 0.124 | 0.132 |
| 237 | 0514 | | 3:30pm | 0.143 | 0.158 | 0.151 |
| 238 | 0521 | 4/27 | 9:45am | 0.090 | 0.092 | 0.091 |
| 239 | 0522 | | 10:40am | 0.054 | 0.050 | 0.052 |
| 240 | 0523 | | 11:30am | 0.100 | 0.121 | 0.111 |
| 241 | 0524 | | 3:30pm | 0.136 | 0.160 | 0.148 |
| 242 | 0531 | 4/25 | 9:45am | 0.069 | 0.102 | 0.086 |
| 243 | 0532 | | 10:40am | 0.058 | 0.064 | 0.061 |
| 244 | 0533 | | 11:30am | 0.158 | 0.182 | 0.170 |
| 245 | 0534 | | 3:30pm | 0.140 | 0.166 | 0.153 |
| 246 | 0531 | 5/24 | 9:45am | 0.301 | 0.293 | 0.297 |
| 247 | 0532 | | 10:40am | 0.060 | 0.066 | 0.063 |
| 248 | 0533 | | 11:30am | 0.117 | 0.123 | 0.120 |
| 249 | 0534 | | 3:30pm | 0.742 | 0.753 | 0.748 |
| 250 | 0541 | 4/29 | 9:45am | 0 | 0 | 0 |
| 251 | 0542 | | 10:40am | 0.038 | 0.039 | 0.038 |
| 252 | 0543 | | 11:30am | 0.175 | 0.130 | 0.152 |
| 253 | 0544 | | 3:30pm | 0.164 | 0.193 | 0.178 |

Child 06 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.040 | 0.034 | 0.045 | 0.476 |
| play day | 0.058 | 0.032 | 0.082 | 0.457* |

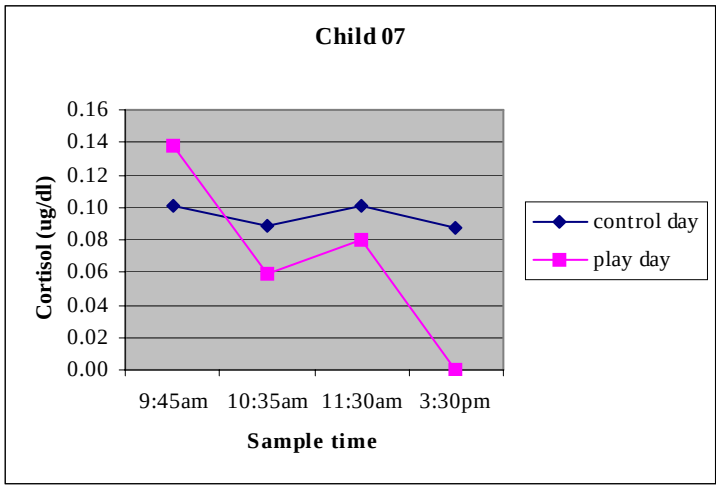
* outlier




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 133 | 0611 | 4/26 | 9:45am | 0.041 | 0.039 | 0.040 |
| 134 | 0612 | | 10:40am | 0.027 | 0.027 | 0.027 |
| 135 | 0613 | | 11:30am | 0.044 | 0.045 | 0.045 |
| 136 | 0614 | | 3:30pm | 0.594 | 0.641 | 0.618 |
| 137 | 0621 | 4/27 | 9:45am | 0.053 | 0.033 | 0.043 |
| 138 | 0622 | | 10:40am | 0.048 | 0.036 | 0.042 |
| 139 | 0623 | | 11:30am | 0 | 0 | 0 |
| 140 | 0624 | | 3:30pm | 0.352 | 0.318 | 0.335 |
| 141 | 0631 | 4/25 | 9:45am | 0.033 | 0.029 | 0.031 |
| 142 | 0632 | | 10:40am | 0.021 | 0.031 | 0.026 |
| 143 | 0633 | | 11:30am | 0.074 | 0.069 | 0.071 |
| 144 | 0634 | | 3:30pm | 0.616 | 0.565 | 0.591 |
| 145 | 0641 | 4/29 | 9:45am | 0.083 | 0.087 | 0.085 |
| 146 | 0642 | | 10:40am | 0.038 | 0.039 | 0.039 |
| 147 | 0643 | | 11:30am | 0.102 | 0.085 | 0.093 |
| 148 | 0644 | | 3:30pm | 0.342 | 0.305 | 0.323 |

Child 07 – Mean cortisol values within sample time (µg/dl)

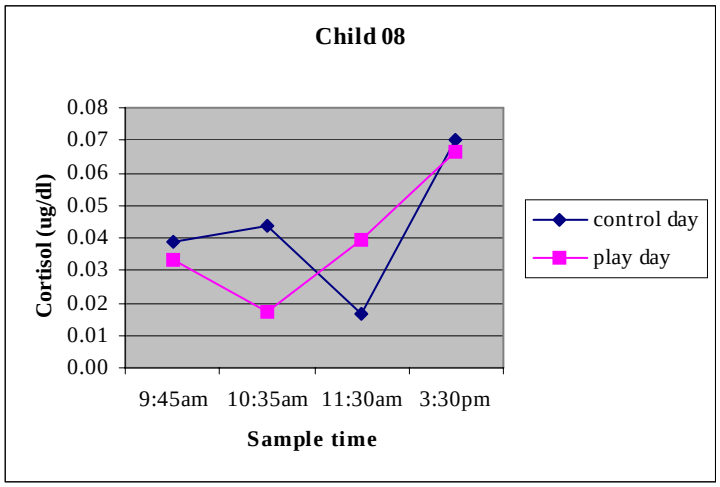
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.101 | 0.089 | 0.101 | 0.088 |
| play day | 0.138 | 0.059 | 0.080 | 0.000 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 5 | 0711 | 4/26 | 9:45am | 0.113 | 0.025 | 0.069 |
| 13 | 0712 | | 10:40am | 0.098 | 0 | 0.098 |
| 9 | 0713 | | 11:30am | 0.127 | 0.15 | 0.139 |
| 2 | 0714 | | 3:30pm | 0.112 | 0.033 | 0.073 |
| 10 | 0721 | 4/27 | 9:45am | 0.095 | 0.107 | 0.101 |
| 7 | 0722 | | 10:40am | 0.084 | 0.075 | 0.080 |
| 16 | 0723 | | 11:30am | 0.065 | 0.063 | 0.064 |
| 11 | 0724 | | 3:30pm | 0.078 | 0.098 | 0.088 |
| 14 | 0731 | 4/25 | 9:45am | 0.138 | 0 | 0.138 |
| 12 | 0732 | | 10:40am | 0.059 | 0.059 | 0.059 |
| 3 | 0733 | | 11:30am | 0 | 0.038 | 0.038 |
| 4 | 0734 | | 3:30pm | 0.145 | 0.068 | 0.107 |
| 8 | 0741 | 4/29 | 9:45am | 0 | 0 | 0 |
| 6 | 0742 | | 10:40am | 0 | 0 | 0 |
| 15 | 0743 | | 11:30am | 0.123 | 0.119 | 0.121 |
| 1 | 0744 | | 3:30pm | 0.247 | 0.039 | 0.143 |

Child 08 – Mean cortisol values within sample time (µg/dl)

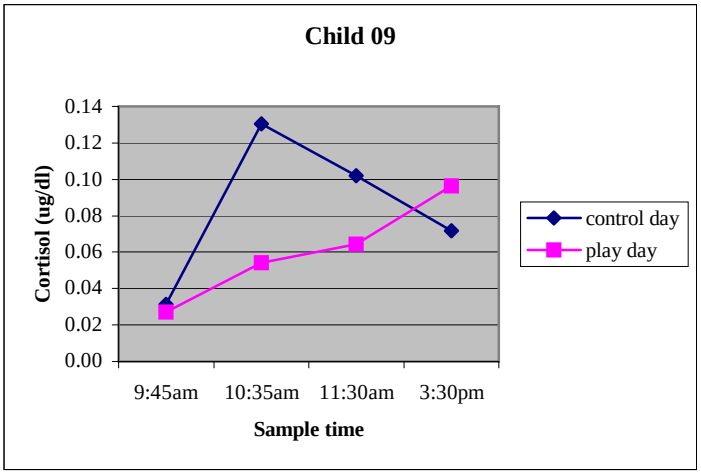
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.038 | 0.043 | 0.017 | 0.070 |
| play day | 0.033 | 0.017 | 0.039 | 0.067 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 97 | 0811 | 4/26 | 9:45am | 0.042 | 0.035 | 0.038 |
| 98 | 0812 | | 10:40am | 0.057 | 0 | 0.057 |
| 99 | 0813 | | 11:30am | 0 | 0 | 0 |
| 100 | 0814 | | 3:30pm | 0.088 | 0.106 | 0.097 |
| 101 | 0821 | 4/27 | 9:45am | 0.050 | 7.041 | 0.050 |
| 102 | 0822 | | 10:40am | 0.029 | 0.032 | 0.030 |
| 103 | 0823 | | 11:30am | 0.018 | 0.016 | 0.017 |
| 104 | 0824 | | 3:30pm | 0.048 | 0.039 | 0.043 |
| 105 | 0831 | 4/25 | 9:45am | 0.025 | 0.040 | 0.032 |
| 106 | 0832 | | 10:40am | 0.020 | 0.015 | 0.017 |
| 107 | 0833 | | 11:30am | 0.049 | 0.028 | 0.038 |
| 108 | 0834 | | 3:30pm | 0.058 | 0.035 | 0.046 |
| 109 | 0841 | 4/29 | 9:45am | 0.039 | 0.028 | 0.033 |
| 110 | 0842 | | 10:40am | 0.037 | 0.019 | 0.028 |
| 111 | 0843 | | 11:30am | 0.040 | 0.038 | 0.039 |
| 112 | 0844 | | 3:30pm | 0.070 | 0.063 | 0.067 |

Child 09 – Mean cortisol values within sample time (µg/dl)

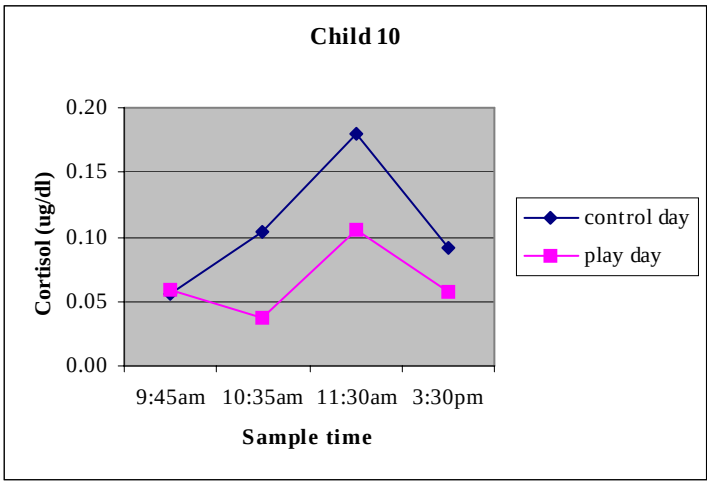
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.031 | 0.131 | 0.102 | 0.072 |
| play day | 0.027 | 0.054 | 0.064 | 0.096 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 65 | 0911 | 4/26 | 9:45am | 0.029 | 0.020 | 0.024 |
| 66 | 0912 | | 10:40am | 0.229 | 0.013 | 0.121 |
| 67 | 0913 | | 11:30am | 0.103 | 0.070 | 0.087 |
| 68 | 0914 | | 3:30pm | 0.047 | 0.031 | 0.039 |
| 69 | 0921 | 4/27 | 9:45am | 0.045 | 0.031 | 0.038 |
| 70 | 0922 | | 10:40am | 0.131 | 0 | 0.131 |
| 71 | 0923 | | 11:30am | 0.117 | 0 | 0.117 |
| 72 | 0924 | | 3:30pm | 0.079 | 0.064 | 0.072 |
| 73 | 0931 | 4/25 | 9:45am | 0.021 | 0.027 | 0.024 |
| 74 | 0932 | | 10:40am | 0.029 | 0.035 | 0.032 |
| 75 | 0933 | | 11:30am | 0.052 | 0 | 0.052 |
| 76 | 0934 | | 3:30pm | 0.034 | 0.040 | 0.037 |
| 77 | 0941 | 4/29 | 9:45am | 0.030 | 0.030 | 0.030 |
| 78 | 0942 | | 10:40am | 0.068 | 0.084 | 0.076 |
| 79 | 0943 | | 11:30am | 0.087 | 0.065 | 0.076 |
| 80 | 0944 | | 3:30pm | 0.168 | 0.144 | 0.156 |

Child 10 – Mean cortisol values within sample time (µg/dl)

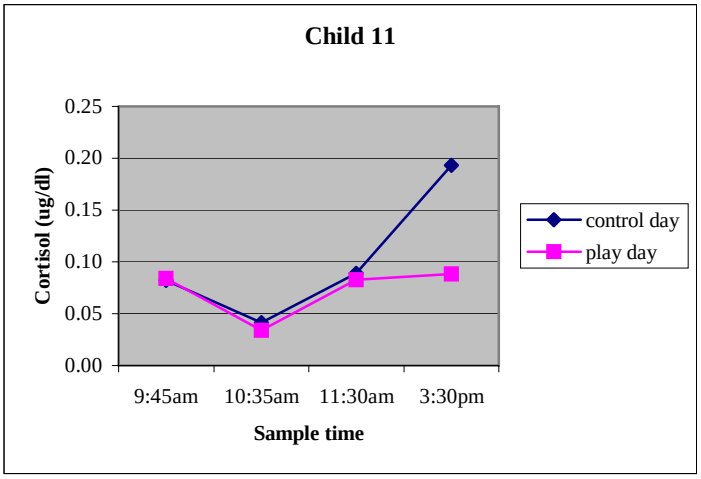
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.057 | 0.103 | 0.180 | 0.091 |
| play day | 0.059 | 0.037 | 0.106 | 0.057 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 81 | 1011 | 4/26 | 9:45am | 0.029 | 0.035 | 0.032 |
| 82 | 1012 | | 10:40am | 0.107 | 0.106 | 0.106 |
| 83 | 1013 | | 11:30am | 0.181 | 0 | 0.181 |
| 84 | 1014 | | 3:30pm | 0.074 | 0.063 | 0.069 |
| 85 | 1021 | 4/27 | 9:45am | 0.074 | 0.088 | 0.081 |
| 86 | 1022 | | 10:40am | 0.090 | 0.110 | 0.100 |
| 87 | 1023 | | 11:30am | 0.175 | 0.182 | 0.179 |
| 88 | 1024 | | 3:30pm | 0.130 | 0.099 | 0.114 |
| 89 | 1031 | 4/25 | 9:45am | 0.031 | 0.034 | 0.032 |
| 90 | 1032 | | 10:40am | 0.035 | 0.044 | 0.039 |
| 91 | 1033 | | 11:30am | 0.123 | 0.128 | 0.126 |
| 92 | 1034 | | 3:30pm | 0.052 | 0.056 | 0.054 |
| 93 | 1041 | 4/29 | 9:45am | 0.081 | 0.092 | 0.086 |
| 94 | 1042 | | 10:40am | 0.037 | 0.033 | 0.035 |
| 95 | 1043 | | 11:30am | 0.094 | 0.077 | 0.086 |
| 96 | 1044 | | 3:30pm | 0.066 | 0.053 | 0.059 |

Child 11 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.082 | 0.042 | 0.089 | 0.193 |
| play day | 0.084 | 0.034 | 0.082 | 0.088 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 164 | 1111 | 4/26 | 9:45am | 0.091 | 0.098 | 0.094 |
| 165 | 1112 | | 10:40am | 0.043 | 0.040 | 0.042 |
| 166 | 1113 | | 11:30am | 0.111 | 0.113 | 0.112 |
| 167 | 1114 | | 3:30pm | 0.065 | 0.068 | 0.066 |
| 168 | 1121 | 4/27 | 9:45am | 0.084 | 0.064 | 0.074 |
| 169 | 1122 | | 10:40am | 0.056 | 0.055 | 0.056 |
| 170 | 1123 | | 11:30am | 0.144 | 0.152 | 0.148 |
| 171 | 1124 | | 3:30pm | 0.114 | 0.128 | 0.121 |
| 172 | 1131 | 4/25 | 9:45am | 0.073 | 0.078 | 0.075 |
| 173 | 1132 | | 10:40am | 0.044 | 0.048 | 0.046 |
| 174 | 1133 | | 11:30am | 0.105 | 0.103 | 0.104 |
| 175 | 1134 | | 3:30pm | 0.083 | 0.111 | 0.097 |
| 176 | 1141 | 4/29 | 9:45am | 0.121 | 0.142 | 0.132 |
| 177 | 1142 | | 10:40am | 0.032 | 0.031 | 0.031 |
| 178 | 1143 | | 11:30am | 0.029 | 0.033 | 0.031 |
| 179 | 1144 | | 3:30pm | 0.091 | 0.103 | 0.097 |

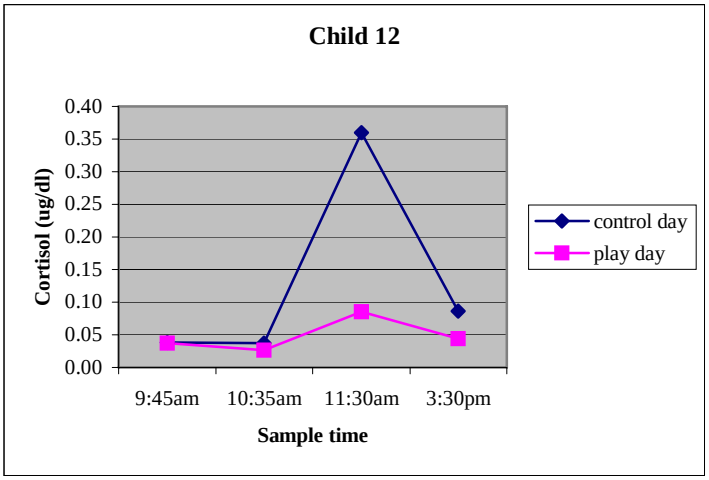
Child 11 data continues on page 110

Child 11 cont.

| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Mean |
|---|---|---|---|---|---|---|
| 180 | 1111 | 6/20 | 9:45am | 0.078 | 0.089 | 0.083 |
| 181 | 1112 | | 10:40am | 0.026 | 0.028 | 0.027 |
| 182 | 1113 | | 11:30am | 0.064 | 0.074 | 0.069 |
| 183 | 1114 | | 3:30pm | 0.386 | 0.370 | 0.378 |
| 184 | 1121 | 6/23 | 9:45am | 0.076 | 0.075 | 0.075 |
| 185 | 1122 | | 10:40am | 0.041 | 0.042 | 0.041 |
| 186 | 1123 | | 11:30am | 0.025 | 0.027 | 0.026 |
| 187 | 1124 | | 3:30pm | 0.214 | 0.202 | 0.208 |
| 188 | 1131 | 6/21 | 9:45am | 0.061 | 0.067 | 0.064 |
| 189 | 1132 | | 10:40am | 0.032 | 0.030 | 0.031 |
| 190 | 1133 | | 11:30am | 0.114 | 0.098 | 0.106 |
| 191 | 1134 | | 3:30pm | 0.062 | 0.065 | 0.064 |
| 192 | 1141 | 6/22 | 9:45am | 0.068 | 0.064 | 0.066 |
| 193 | 1142 | | 10:40am | 0.029 | 0.026 | 0.027 |
| 194 | 1143 | | 11:30am | 0.096 | 0.081 | 0.089 |
| 195 | 1144 | | 3:30pm | 0.095 | 0.098 | 0.096 |

Child 12 – Mean cortisol values within sample time (µg/dl)

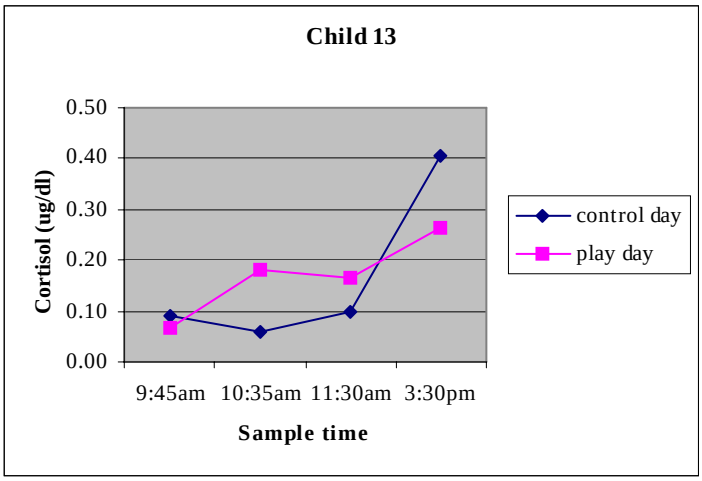
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.038 | 0.038 | 0.360 | 0.087 |
| play day | 0.038 | 0.027 | 0.085 | 0.045 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 28 | 1211 | 4/26 | 9:45am | 0.039 | 0.036 | 0.038 |
| 30 | 1212 | | 10:40am | 0.037 | 0.038 | 0.038 |
| 25 | 1213 | | 11:30am | 0 | 0.360 | 0.360 |
| 26 | 1214 | | 3:30pm | 0.085 | 0.081 | 0.083 |
| 22 | 1221 | 4/27 | 9:45am | 0 | 0.039 | 0.039 |
| 20 | 1222 | | 10:40am | 0.192 | 0.032 | 0.112 |
| 24 | 1223 | | 11:30am | 0 | 0 | 0 |
| 23 | 1224 | | 3:30pm | 0.097 | 0.084 | 0.091 |
| 29 | 1231 | 4/25 | 9:45am | 0.039 | 0.036 | 0.038 |
| 31 | 1232 | | 10:40am | 0.026 | 0.027 | 0.027 |
| 21 | 1233 | | 11:30am | 0.130 | 0 | 0.130 |
| 27 | 1234 | | 3:30pm | 0.045 | 0.044 | 0.045 |
| 18 | 1241 | 4/29 | 9:45am | 0.128 | 0.043 | 0.086 |
| 19 | 1242 | | 10:40am | 0.056 | 0.033 | 0.045 |
| 32 | 1243 | | 11:30am | 0 | 0.040 | 0.040 |
| 17 | 1244 | | 3:30pm | 0 | 0 | 0 |

Child 13 – Mean cortisol values within sample time (µg/dl)

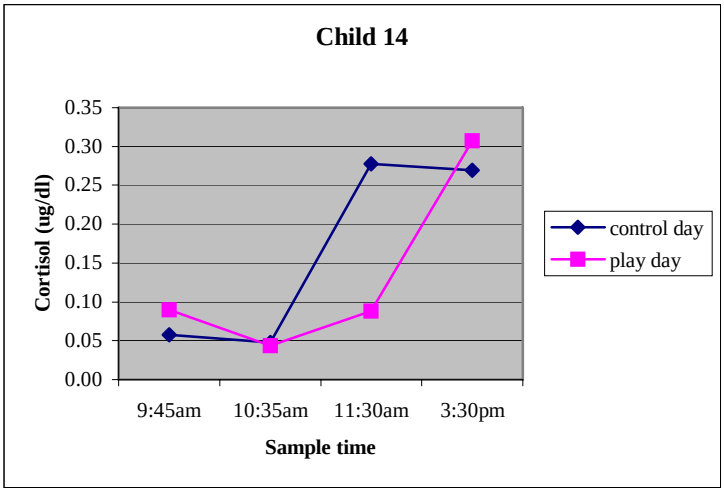
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.089 | 0.058 | 0.098 | 0.405 |
| play day | 0.065 | 0.179 | 0.163 | 0.264 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 302 | 1311 | 4/26 | 9:45am | 0.104 | 0.157 | 0.131 |
| 303 | 1312 | | 10:40am | 0.077 | 0 | 0.077 |
| 304 | 1313 | | 11:30am | 0.088 | 0.081 | 0.085 |
| 305 | 1314 | | 3:30pm | 0.431 | 0.454 | 0.442 |
| 306 | 1321 | 4/27 | 9:45am | 0.092 | 0.087 | 0.089 |
| 307 | 1322 | | 10:40am | 0.045 | 0.031 | 0.038 |
| 308 | 1323 | | 11:30am | 0.102 | 0.123 | 0.112 |
| 309 | 1324 | | 3:30pm | 0.387 | 0.348 | 0.367 |
| 310 | 1331 | 4/25 | 9:45am | 0.065 | 0.062 | 0.063 |
| 311 | 1332 | | 10:40am | 0.088 | 0.086 | 0.087 |
| 312 | 1333 | | 11:30am | 0.198 | 0 | 0.198 |
| 313 | 1334 | | 3:30pm | 0.262 | 0.276 | 0.269 |
| 314 | 1341 | 4/29 | 9:45am | 0.067 | 0.067 | 0.067 |
| 315 | 1342 | | 10:40am | 0.267 | 0.277 | 0.272 |
| 316 | 1343 | | 11:30am | 0.150 | 0.108 | 0.129 |
| 317 | 1344 | | 3:30pm | 0.306 | 0.213 | 0.259 |

Child 14 – Mean cortisol values within sample time (µg/dl)

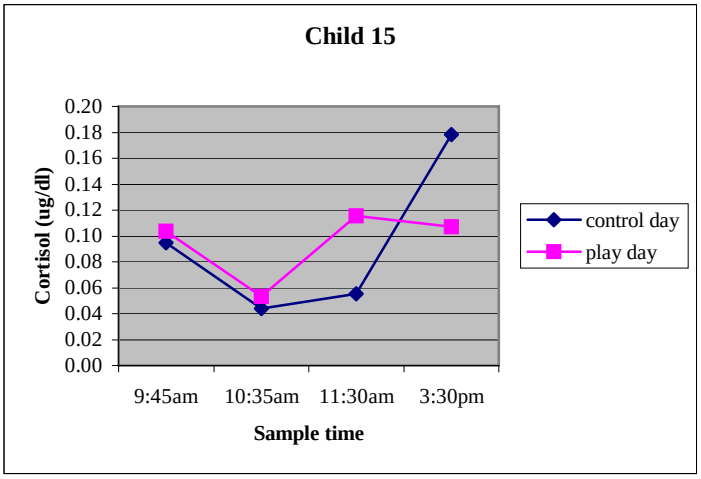
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.057 | 0.047 | 0.277 | 0.269 |
| play day | 0.089 | 0.043 | 0.088 | 0.307 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 149 | 1411 | 4/26 | 9:45am | 0.064 | 0.059 | 0.062 |
| 150 | 1412 | | 10:40am | 0.043 | 0.039 | 0.041 |
| 151 | 1413 | | 11:30am | 0.134 | 0.132 | 0.133 |
| 152 | 1414 | | 3:30pm | 0.294 | 0.273 | 0.283 |
| 153 | 1421 | 4/27 | 9:45am | 0.053 | 0 | 0.053 |
| 154 | 1422 | | 10:40am | 0.056 | 0.051 | 0.053 |
| 155 | 1423 | | 11:30am | 0.433 | 0.409 | 0.421 |
| 156 | 1424 | | 3:30pm | 0.264 | 0.247 | 0.255 |
| 157 | 1431 | 4/25 | 9:45am | 0.077 | 0.067 | 0.072 |
| 158 | 1432 | | 10:40am | 0.035 | 0.028 | 0.032 |
| 159 | 1433 | | 11:30am | 0.054 | 0 | 0.054 |
| 160 | 1434 | | 3:30pm | 0.337 | 0.330 | 0.333 |
| 161 | 1441 | 4/29 | 9:45am | 0.118 | 0.096 | 0.107 |
| 162 | 1442 | | 10:40am | 0.060 | 0.050 | 0.055 |
| 163 | 1443 | | 11:30am | 0.130 | 0.115 | 0.122 |
| 164 | 1444 | | 3:30pm | 0.297 | 0.265 | 0.281 |

Child 15 – Mean cortisol values within sample time (µg/dl)

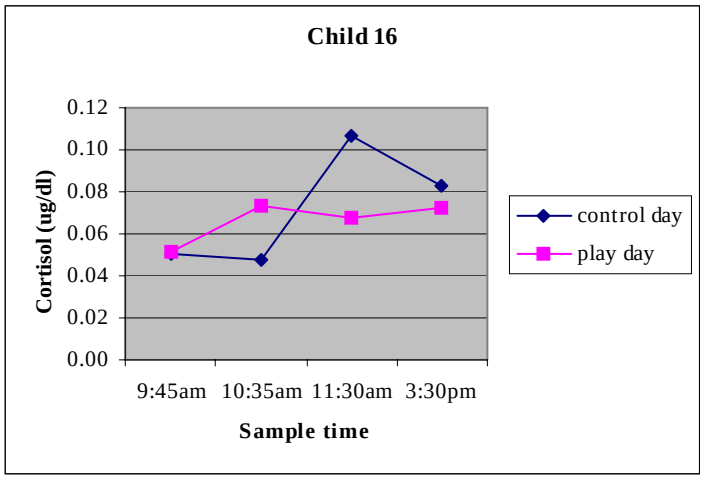
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.095 | 0.044 | 0.055 | 0.178 |
| play day | 0.104 | 0.053 | 0.116 | 0.107 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 254 | 1511 | 4/26 | 9:45am | 0.106 | 0.114 | 0.110 |
| 255 | 1512 | | 10:40am | 0.050 | 0.058 | 0.054 |
| 256 | 1513 | | 11:30am | 0.064 | 0.076 | 0.070 |
| 257 | 1514 | | 3:30pm | 0.262 | 0.231 | 0.247 |
| 258 | 1521 | 4/27 | 9:45am | 0.082 | 0.077 | 0.080 |
| 259 | 1522 | | 10:40am | 0.031 | 0.036 | 0.033 |
| 260 | 1523 | | 11:30am | 0.040 | 0.042 | 0.041 |
| 261 | 1524 | | 3:30pm | 0.108 | 0.111 | 0.110 |
| 262 | 1531 | 4/25 | 9:45am | 0.106 | 0.109 | 0.108 |
| 263 | 1532 | | 10:40am | 0.053 | 0.052 | 0.053 |
| 264 | 1533 | | 11:30am | 0.126 | 0.139 | 0.132 |
| 265 | 1534 | | 3:30pm | 0.080 | 0.085 | 0.082 |
| 266 | 1541 | 4/29 | 9:45am | 0.092 | 0.108 | 0.100 |
| 267 | 1542 | | 10:40am | 0.053 | 0.055 | 0.054 |
| 268 | 1543 | | 11:30am | 0.094 | 0.104 | 0.099 |
| 269 | 1544 | | 3:30pm | 0.126 | 0.137 | 0.132 |

Child 16 – Mean cortisol values within sample time (µg/dl)

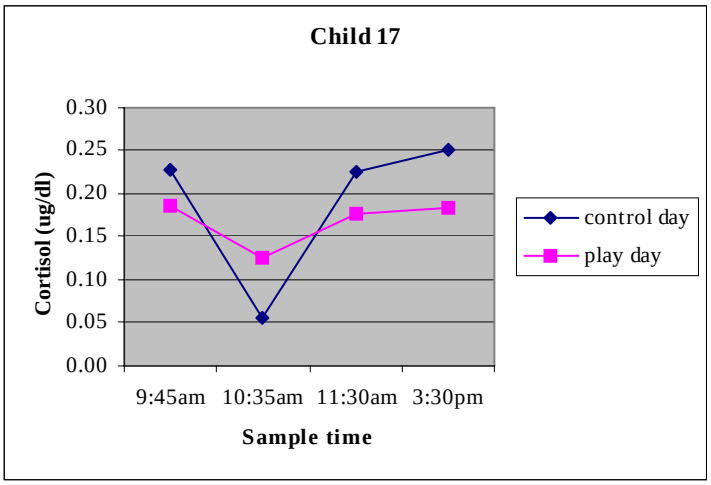
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.051 | 0.047 | 0.107 | 0.082 |
| play day | 0.051 | 0.073 | 0.067 | 0.073 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 196 | 1611 | 4/26 | 9:45am | 0.039 | 0.038 | 0.039 |
| 197 | 1612 | | 10:40am | 0.036 | 0.036 | 0.036 |
| 198 | 1613 | | 11:30am | 0.059 | 0.052 | 0.055 |
| 199 | 1614 | | 3:30pm | 0.097 | 0.099 | 0.098 |
| 200 | 1621 | 4/27 | 9:45am | 0.065 | 0.061 | 0.063 |
| 201 | 1622 | | 10:40am | 0.059 | 0.059 | 0.059 |
| 202 | 1623 | | 11:30am | 0.159 | 0 | 0.159 |
| 203 | 1624 | | 3:30pm | 0.069 | 0.065 | 0.067 |
| 204 | 1641 | 4/25 | 9:45am | 0.054 | 0.051 | 0.052 |
| 205 | 1642 | | 10:40am | 0.100 | 0.096 | 0.098 |
| 206 | 1643 | | 11:30am | 0.075 | 0.078 | 0.077 |
| 207 | 1644 | | 3:30pm | 0.071 | 0.063 | 0.067 |
| 208 | 1631 | 4/29 | 9:45am | 0.058 | 0.049 | 0.053 |
| 209 | 1632 | | 10:40am | 0.072 | 0.079 | 0.076 |
| 210 | 1633 | | 11:30am | 0.066 | 0.073 | 0.069 |
| 211 | 1634 | | 3:30pm | 0.080 | 0.097 | 0.089 |
| 212 | 1641 | 5/20 | 9:45am | 0.046 | 0.050 | 0.048 |
| 213 | 1642 | | 10:40am | 0.044 | 0.047 | 0.045 |
| 214 | 1643 | | 11:30am | 0.056 | 0 | 0.056 |
| 215 | 1644 | | 3:30pm | 0.064 | 0.061 | 0.062 |

Child 17 – Mean cortisol values within sample time (µg/dl)

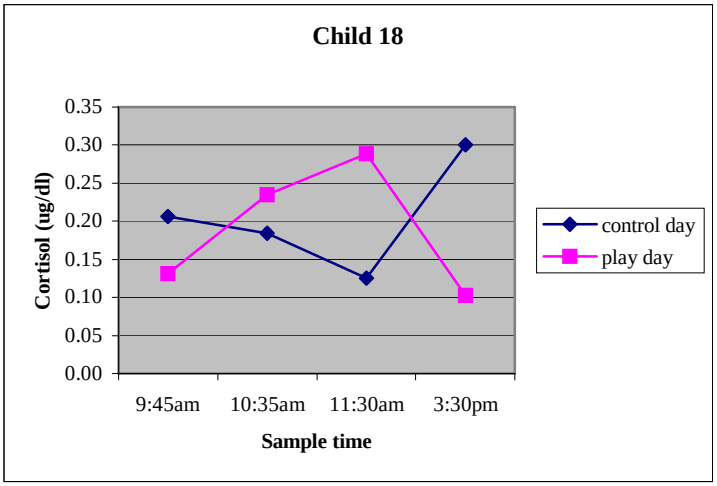
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.228 | 0.057 | 0.225 | 0.252 |
| play day | 0.187 | 0.125 | 0.177 | 0.184 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 45 | 1711 | 4/26 | 9:45am | 0.220 | 0.193 | 0.207 |
| 42 | 1712 | | 10:40am | 0.062 | 0.051 | 0.057 |
| 41 | 1713 | | 11:30am | 0.339 | 0.318 | 0.329 |
| 44 | 1714 | | 3:30pm | 0 | 0 | 0 |
| 33 | 1721 | 4/27 | 9:45am | 0.270 | 0.227 | 0.249 |
| 43 | 1722 | | 10:40am | 0 | 0 | 0 |
| 46 | 1723 | | 11:30am | 0.125 | 0.119 | 0.122 |
| 35 | 1724 | | 3:30pm | 0 | 0.252 | 0.252 |
| 40 | 1731 | 4/25 | 9:45am | 0.288 | 0.267 | 0.278 |
| 39 | 1732 | | 10:40am | 0 | 0 | 0 |
| 47 | 1733 | | 11:30am | 0.154 | 0.128 | 0.141 |
| 36 | 1734 | | 3:30pm | 0.175 | 0.220 | 0.198 |
| 37 | 1741 | 4/29 | 9:45am | 0.102 | 0.090 | 0.096 |
| 38 | 1742 | | 10:40am | 0.125 | 0 | 0.125 |
| 34 | 1743 | | 11:30am | 0.239 | 0.188 | 0.214 |
| 48 | 1744 | | 3:30pm | 0.171 | 0 | 0.171 |

Child 18 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.206 | 0.184 | 0.126 | 0.300 |
| play day | 0.131 | 0.235 | 0.289 | 0.103 |

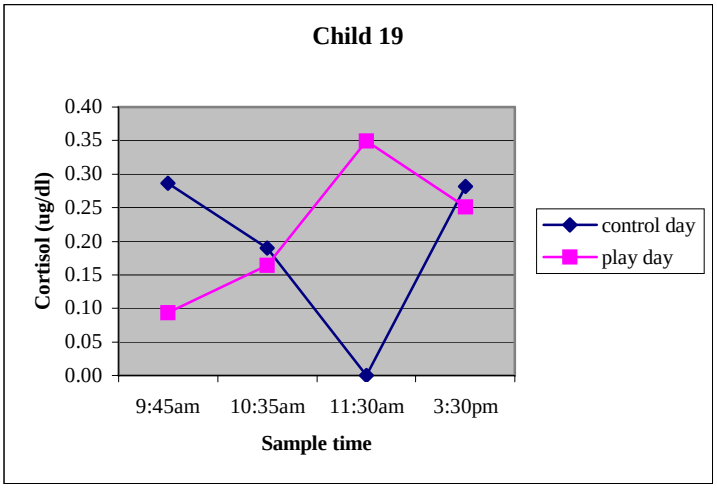



| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 318 | 1811 | 4/26 | 9:45am | 0.299 | 0.214 | 0.257 |
| 319 | 1812 | | 10:40am | 0.184 | 0 | 0.184 |
| 320 | 1813 | | 11:30am | 0.140 | 0 | 0.140 |
| 321 | 1814 | | 3:30pm | 0.189 | 0.122 | 0.156 |
| 322 | 1821 | 4/27 | 9:45am | 0.156 | 0 | 0.156 |
| 323 | 1822 | | 10:40am | 0.078 | 0.051 | 0.065 |
| 324 | 1823 | | 11:30am | 0.104 | 0.118 | 0.111 |
| 325 | 1824 | | 3:30pm | 0.284 | 0.317 | 0.300 |
| 326 | 1831 | 4/25 | 9:45am | 0.082 | 0.083 | 0.082 |
| 327 | 1832 | | 10:40am | 0.121 | 0.110 | 0.116 |
| 328 | 1833 | | 11:30am | 0.391 | 0.411 | 0.401 |
| 329 | 1834 | | 3:30pm | 0.113 | 0.093 | 0.103 |
| 330 | 1841 | 4/29 | 9:45am | 0.173 | 0.188 | 0.180 |
| 331 | 1842 | | 10:40am | 0.354 | 0 | 0.354 |
| 332 | 1843 | | 11:30am | 0.179 | 0.174 | 0.177 |

Child 19 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.286 | 0.190 | 0.000 | 0.282 |
| play day | 0.094 | 0.164 | 0.349* | 0.251 |

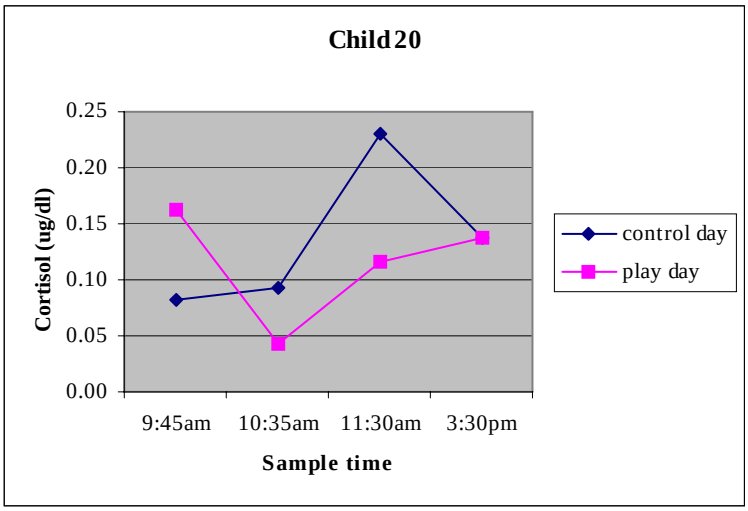
* outlier




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 55 | 1911 | 4/26 | 9:45am | 0.921 | 0.891 | 0.906 |
| 56 | 1912 | | 10:40am | 0.198 | 0.160 | 0.179 |
| 50 | 1913 | | 11:30am | 0 | 0 | 0 |
| 53 | 1914 | | 3:30pm | 0.367 | 0.374 | 0.371 |
| 61 | 1921 | 4/27 | 9:45am | 0.286 | 0 | 0.286 |
| 63 | 1922 | | 10:40am | 0.203 | 0.200 | 0.202 |
| 49 | 1923 | | 11:30am | 0 | 0 | 0 |
| 54 | 1924 | | 3:30pm | 0.193 | 0 | 0.193 |
| 52 | 1931 | 4/25 | 9:45am | 0.107 | 0.117 | 0.112 |
| 59 | 1932 | | 10:40am | 0.243 | 0 | 0.243 |
| 60 | 1933 | | 11:30am | 0.349 | 0 | 0.349 |
| 57 | 1934 | | 3:30pm | 0.410 | 0.390 | 0.400 |
| 51 | 1941 | 4/29 | 9:45am | 0.083 | 0.069 | 0.076 |
| 58 | 1942 | | 10:40am | 0.085 | 0.085 | 0.085 |
| 62 | 1943 | | 11:30am | 0 | 0 | 0 |
| 64 | 1944 | | 3:30pm | 0.105 | 0.100 | 0.103 |

Child 20 – Mean cortisol values within sample time (µg/dl)

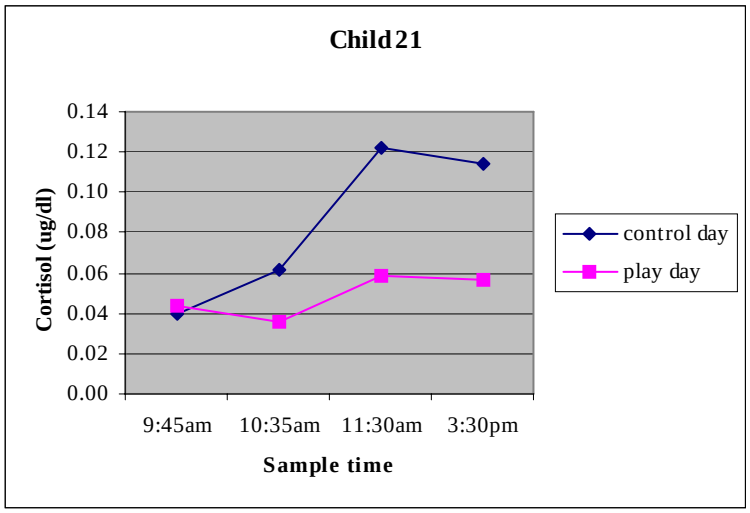
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.082 | 0.093 | 0.231 | 0.138 |
| play day | 0.162 | 0.042 | 0.117 | 0.138 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 333 | 2011 | 4/26 | 9:45am | 0.093 | 0.094 | 0.093 |
| 334 | 2012 | | 10:40am | 0.135 | 0.141 | 0.138 |
| 335 | 2013 | | 11:30am | 0.276 | 0.227 | 0.251 |
| 336 | 2014 | | 3:30pm | 0.173 | 0.188 | 0.180 |
| 337 | 2021 | 4/27 | 9:45am | 0.071 | 0 | 0.071 |
| 338 | 2022 | | 10:40am | 0.043 | 0.055 | 0.049 |
| 339 | 2023 | | 11:30am | 0.220 | 0.202 | 0.211 |
| 340 | 2024 | | 3:30pm | 0.100 | 0.093 | 0.096 |
| 341 | 2031 | 4/25 | 9:45am | 0.093 | 0.088 | 0.090 |
| 342 | 2032 | | 10:40am | 0.042 | 0 | 0.042 |
| 343 | 2033 | | 11:30am | 0.129 | 0.118 | 0.123 |
| 344 | 2034 | | 3:30pm | 0.182 | 0.207 | 0.195 |
| 345 | 2041 | 4/29 | 9:45am | 0.240 | 0.227 | 0.234 |
| 346 | 2042 | | 10:40am | 0 | 0 | 0 |
| 347 | 2043 | | 11:30am | 0.101 | 0.119 | 0.110 |
| 348 | 2044 | | 3:30pm | 0.077 | 0.086 | 0.082 |

Child 21 – Mean cortisol values within sample time (µg/dl)

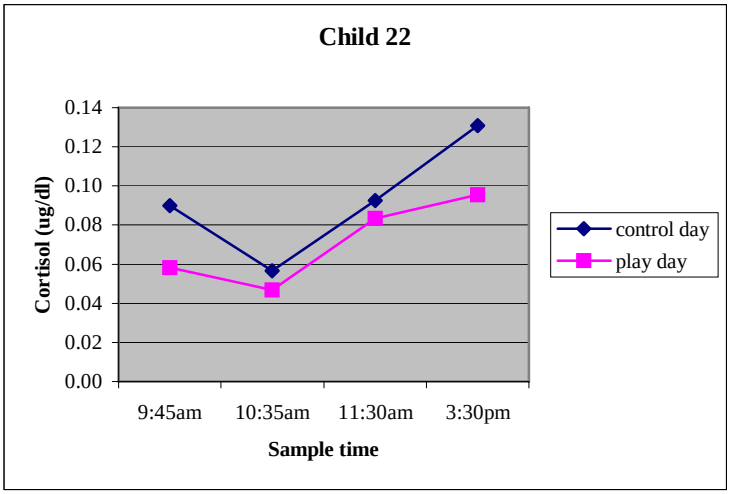
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.040 | 0.061 | 0.122 | 0.115 |
| play day | 0.044 | 0.036 | 0.058 | 0.057 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 349 | 2121 | 4/26 | 9:45am | 0.049 | 0.045 | 0.047 |
| 350 | 2122 | | 10:40am | 0.055 | 0.054 | 0.054 |
| 351 | 2123 | | 11:30am | 0.156 | 0.111 | 0.134 |
| 352 | 2124 | | 3:30pm | 0.158 | 0.138 | 0.148 |
| 353 | 2121 | 4/27 | 9:45am | 0.036 | 0.028 | 0.032 |
| 354 | 2122 | | 10:40am | 0.067 | 0.071 | 0.069 |
| 355 | 2123 | | 11:30am | 0.110 | 0.113 | 0.111 |
| 356 | 2124 | | 3:30pm | 0.075 | 0.088 | 0.081 |
| 357 | 2141 | 4/25 | 9:45am | 0.044 | 0.043 | 0.044 |
| 358 | 2142 | | 10:40am | 0.036 | 0 | 0.036 |
| 359 | 2143 | | 11:30am | 0.058 | 0 | 0.058 |
| 360 | 2144 | | 3:30pm | 0.061 | 0.052 | 0.057 |

Child 22 – Mean cortisol values within sample time (µg/dl)

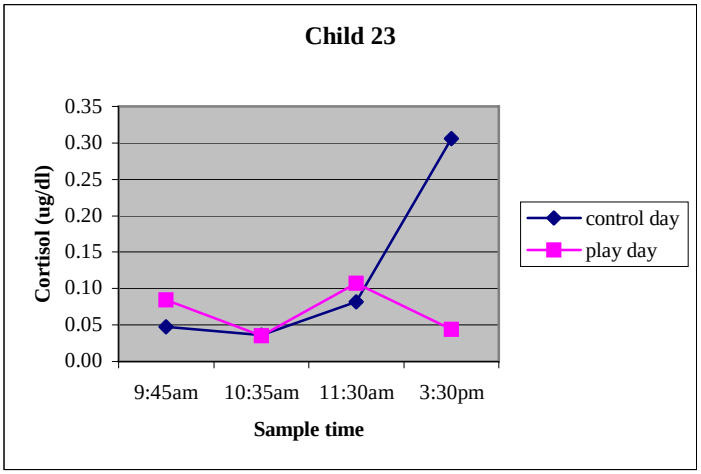
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.090 | 0.057 | 0.092 | 0.131 |
| play day | 0.058 | 0.047 | 0.084 | 0.096 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 270 | 2211 | 4/26 | 9:45am | 0.110 | 0.098 | 0.104 |
| 271 | 2212 | | 10:40am | 0.063 | 0.065 | 0.064 |
| 272 | 2213 | | 11:30am | 0.096 | 0.096 | 0.096 |
| 273 | 2214 | | 3:30pm | 0.163 | 0.159 | 0.161 |
| 274 | 2211 | 4/27 | 9:45am | 0.077 | 0.074 | 0.076 |
| 275 | 2212 | | 10:40am | 0.053 | 0.046 | 0.050 |
| 276 | 2213 | | 11:30am | 0.090 | 0.089 | 0.089 |
| 277 | 2214 | | 3:30pm | 0.099 | 0.102 | 0.101 |
| 278 | 2231 | 4/25 | 9:45am | 0.064 | 0.057 | 0.060 |
| 279 | 2232 | | 10:40am | 0.057 | 0.056 | 0.057 |
| 280 | 2233 | | 11:30am | 0.127 | 0.127 | 0.127 |
| 281 | 2234 | | 3:30pm | 0.124 | 0.121 | 0.122 |
| 282 | 2241 | 4/29 | 9:45am | 0.061 | 0.051 | 0.056 |
| 283 | 2242 | | 10:40am | 0.036 | 0.038 | 0.037 |
| 284 | 2243 | | 11:30am | 0.041 | 0.039 | 0.040 |
| 285 | 2244 | | 3:30pm | 0.069 | 0.069 | 0.069 |

Child 23 – Mean cortisol values within sample time (µg/dl)

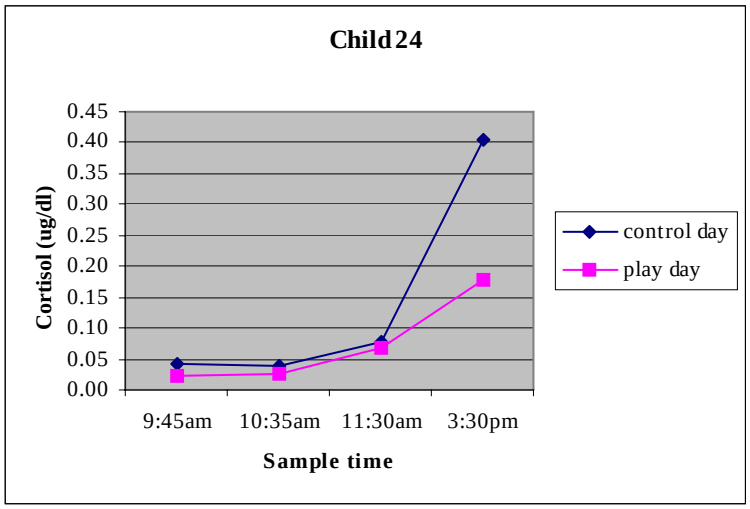
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.047 | 0.036 | 0.082 | 0.306 |
| play day | 0.085 | 0.036 | 0.108 | 0.044 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 113 | 2311 | 4/26 | 9:45am | 0.043 | 0.037 | 0.040 |
| 114 | 2312 | | 10:40am | 0.034 | 0.032 | 0.033 |
| 115 | 2313 | | 11:30am | 0.063 | 0.054 | 0.058 |
| 116 | 2314 | | 3:30pm | 0.176 | 0.158 | 0.167 |
| 117 | 2311 | 4/27 | 9:45am | 0.031 | 0.034 | 0.033 |
| 118 | 2312 | | 10:40am | 0.020 | 0.026 | 0.023 |
| 119 | 2313 | | 11:30am | 0.130 | 0.171 | 0.150 |
| 120 | 2314 | | 3:30pm | 0.562 | 0.756 | 0.659 |
| 121 | 2321 | 4/25 | 9:45am | 0.067 | 0.070 | 0.068 |
| 122 | 2322 | | 10:40am | 0.053 | 0.052 | 0.053 |
| 123 | 2323 | | 11:30am | 0.038 | 0.036 | 0.037 |
| 124 | 2324 | | 3:30pm | 0.091 | 0.095 | 0.093 |
| 125 | 2331 | 4/29 | 9:45am | 0.053 | 0.047 | 0.050 |
| 126 | 2332 | | 10:40am | 0.042 | 0.032 | 0.037 |
| 127 | 2333 | | 11:30am | 0.183 | 0.180 | 0.181 |
| 128 | 2334 | | 3:30pm | 0.044 | 0.053 | 0.048 |
| 129 | 2341 | 6/22 | 9:45am | 0.122 | 0.116 | 0.119 |
| 130 | 2342 | | 10:40am | 0.034 | 0.034 | 0.034 |
| 131 | 2343 | | 11:30am | 0.033 | 0.035 | 0.034 |
| 132 | 2344 | | 3:30pm | 0.038 | 0.043 | 0.041 |

Child 24 – Mean cortisol values within sample time (µg/dl)

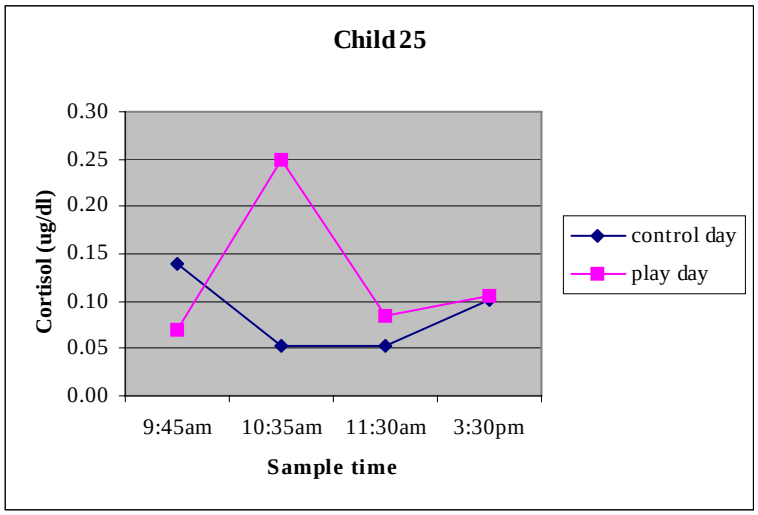
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.042 | 0.040 | 0.077 | 0.405 |
| play day | 0.023 | 0.026 | 0.067 | 0.179 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 216 | 2411 | 4/26 | 9:45am | 0.043 | 0.037 | 0.040 |
| 217 | 2412 | | 10:40am | 0.042 | 0.040 | 0.041 |
| 218 | 2413 | | 11:30am | 0.086 | 0.088 | 0.087 |
| 219 | 2414 | | 3:30pm | 0.220 | 0.212 | 0.216 |
| 220 | 2421 | 4/27 | 9:45am | 0.045 | 0.045 | 0.045 |
| 221 | 2422 | | 10:40am | 0.038 | 0.040 | 0.039 |
| 222 | 2423 | | 11:30am | 0.068 | 0.066 | 0.067 |
| 223 | 2424 | | 3:30pm | 0.572 | 0.614 | 0.593 |
| 224 | 2431 | 4/25 | 9:45am | 0.019 | 0.020 | 0.019 |
| 225 | 2432 | | 10:40am | 0.027 | 0.029 | 0.028 |
| 226 | 2433 | | 11:30am | 0.047 | 0.050 | 0.048 |
| 227 | 2434 | | 3:30pm | 0.194 | 0.199 | 0.197 |
| 228 | 2441 | 4/29 | 9:45am | 0.030 | 0.025 | 0.028 |
| 229 | 2442 | | 10:40am | 0.023 | 0.023 | 0.023 |
| 230 | 2443 | | 11:30am | 0.087 | 0.087 | 0.087 |
| 231 | 2444 | | 3:30pm | 0.163 | 0.159 | 0.161 |

Child 25 – Mean cortisol values within sample time (µg/dl)

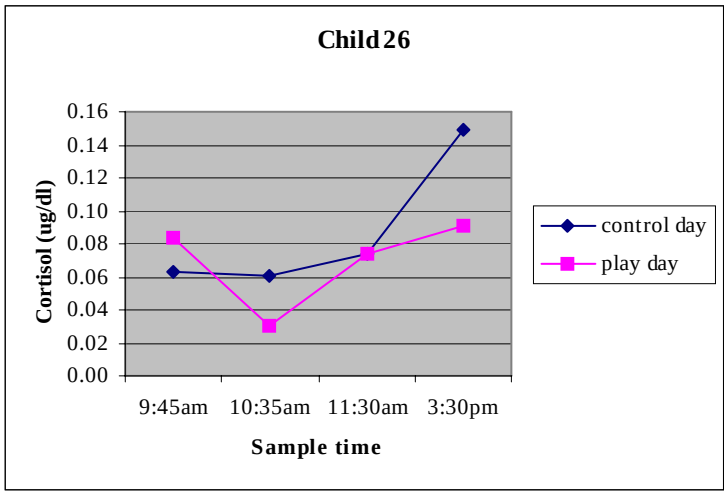
| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.140 | 0.053 | 0.052 | 0.102 |
| play day | 0.070 | 0.249 | 0.084 | 0.105 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 361 | 2511 | 4/26 | 9:45am | 0.133 | 0.130 | 0.132 |
| 362 | 2512 | | 10:40am | 0.044 | 0.047 | 0.045 |
| 363 | 2513 | | 11:30am | 0.056 | 0.058 | 0.057 |
| 364 | 2514 | | 3:30pm | 0.050 | 0.047 | 0.048 |
| 365 | 2521 | 4/27 | 9:45am | 0.145 | 0.153 | 0.149 |
| 366 | 2522 | | 10:40am | 0.050 | 0.069 | 0.060 |
| 367 | 2523 | | 11:30am | 0.050 | 0.044 | 0.047 |
| 368 | 2524 | | 3:30pm | 0.156 | 0.154 | 0.155 |
| 369 | 2531 | 4/25 | 9:45am | 0.045 | 0.033 | 0.039 |
| 370 | 2532 | | 10:40am | 0.062 | 0.051 | 0.056 |
| 371 | 2533 | | 11:30am | 0.062 | 0.037 | 0.049 |
| 372 | 2534 | | 3:30pm | 0.164 | 0.159 | 0.161 |
| 373 | 2541 | 4/29 | 9:45am | 0.108 | 0.095 | 0.102 |
| 374 | 2542 | | 10:40am | 0.471 | 0.412 | 0.442 |
| 375 | 2543 | | 11:30am | 0.089 | 0.079 | 0.084 |
| 376 | 2544 | | 3:30pm | 0.048 | 0.051 | 0.049 |

Child 26 – Mean cortisol values within sample time (µg/dl)

| | 9:45am | 10:35am | 11:30am | 3:30pm |
|---|---|---|---|---|
| control day | 0.063 | 0.061 | 0.074 | 0.149 |
| play day | 0.083 | 0.030 | 0.074 | 0.091 |




| Sample | Subid | Date | Time | Raw Cort 1 | Raw Cort 2 | Raw Mean |
|---|---|---|---|---|---|---|
| 286 | 2611 | 4/26 | 9:45am | 0.066 | 0.071 | 0.068 |
| 287 | 2612 | | 10:40am | 0.078 | 0.077 | 0.077 |
| 288 | 2613 | | 11:30am | 0.079 | 0.079 | 0.079 |
| 289 | 2614 | | 3:30pm | 0.111 | 0.099 | 0.105 |
| 290 | 2621 | 4/27 | 9:45am | 0.059 | 0.057 | 0.058 |
| 291 | 2622 | | 10:40am | 0.041 | 0.046 | 0.044 |
| 292 | 2623 | | 11:30am | 0.066 | 0.071 | 0.069 |
| 293 | 2624 | | 3:30pm | 0.187 | 0.200 | 0.194 |
| 294 | 2631 | 4/25 | 9:45am | 0.088 | 0.130 | 0.109 |
| 295 | 2632 | | 10:40am | 0.031 | 0.029 | 0.030 |
| 296 | 2633 | | 11:30am | 0.073 | 0.075 | 0.074 |
| 297 | 2634 | | 3:30pm | 0.136 | 0.140 | 0.138 |
| 298 | 2641 | 4/29 | 9:45am | 0.057 | 0.059 | 0.058 |
| 299 | 2642 | | 10:40am | 0.027 | 0.031 | 0.029 |
| 300 | 2643 | | 11:30am | 0.008 | 0.009 | 0.009 |
| 301 | 2644 | | 3:30pm | 0.042 | 0.045 | 0.044 |

# APPENDIX H

## MEAN HEART RATE DATA PER CHILD

Child 05 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 127 | 96 | 0.5 |
| play day | 140 | 106 | 0.4 |

control cutoff PAHR 50 = 144

play cutoff PAHR 50 = 158

| Id # | Description | Raw Values |
|---|---|---|
| 05 01 26 04 01 | indoor control | 124 |
| | nap time | 94 |
| | Time>PAHR 50 | 1 |
| 05 02 27 04 01 | indoor control | 130 |
| | nap time | 98 |
| | Time>PAHR 50 | 0 |
| 05 03 24 05 03 | physical play | 142 |
| | nap time | 109 |
| | Time>PAHR 50 | 0 |
| 05 04 29 04 01 | physical play | 138 |
| | nap time | 102 |
| | Time>PAHR 50 | 1 |

Child 06 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 120 | 90 | 3.2 |
| play day | 142 | 83 | 25.9 |

control cutoff PAHR 50 = 136

play cutoff PAHR 50 = 124

| Id # | Description | Raw Values |
|---|---|---|
| 06 01 26 04 02 | indoor control | 109 |
| | nap time | 83 |
| | Time>PAHR 50 | 2 |
| 06 02 27 04 02 | indoor control | 131 |
| | nap time | 98 |
| | Time>PAHR 50 | 4 |
| 06 03 25 04 02 | physical play | 139 |
| | nap time | 84 |
| | Time>PAHR 50 | 22 |
| 06 04 29 04 02 | physical play | 146 |
| | nap time | 81 |
| | Time>PAHR 50 | 29 |

Child 07 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 120 | 95 | 2.0 |
| play day | 141 | 91 | 19.5 |

control cutoff PAHR 50 = 142

play cutoff PAHR 50 = 136

| Id # | Description | Raw Values |
|---|---|---|
| 07 01 26 04 03 | indoor control | 120 |
| | nap time | 92 |
| | Time>PAHR 50 | 2.5 |
| 07 02 27 04 03 | indoor control | 120 |
| | nap time | 98 |
| | Time>PAHR 50 | 1.5 |
| 07 03 25 04 03 | physical play | 137 |
| | nap time | 101 |
| | Time>PAHR 50 | 10.7 |
| 07 04 29 04 03 | physical play | 145 |
| | nap time | 81 |
| | Time>PAHR 50 | 28.3 |

Child 08 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 132 | 95 | 4.8 |
| play day | 149 | 98 | 15.9 |

control cutoff PAHR 50 = 143

play cutoff PAHR 50 = 147

| Id # | Description | Raw Values |
|---|---|---|
| 08 01 26 04 04 | indoor control | 128 |
| | nap time | 94 |
| | Time>PAHR 50 | 4.0 |
| 08 02 27 04 04 | indoor control | 137 |
| | nap time | 96 |
| | Time>PAHR 50 | 5.7 |
| 08 03 25 04 04 | physical play | 149 |
| | nap time | 93 |
| | Time>PAHR 50 | 23.8 |
| 08 04 29 04 04 | physical play | 148 |
| | nap time | 103 |
| | Time>PAHR 50 | 7.9 |

Child 09 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 119 | 84 | 7.7 |
| play day | 161 | 87 | 30.6 |

control cutoff PAHR 50 = 126

play cutoff PAHR 50 = 131

| Id # | Description | Raw Values |
|---|---|---|
| 09 01 04 05 01 | indoor control | 122 |
| | nap time | 84 |
| | Time>PAHR 50 | 7.7 |
| 09 02 06 05 01 | indoor control | 115 |
| | nap time | n/a |
| | Time>PAHR 50 | n/a |
| 09 03 03 05 01 | physical play | 162 |
| | nap time | 92 |
| | Time>PAHR 50 | 31.3 |
| 09 04 18 05 04 | physical play | 161 |
| | nap time | 83 |
| | Time>PAHR 50 | 30.0 |

Child 10 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 115 | 87 | 2.4 |
| play day | 149 | 92 | 19.8 |

control cutoff PAHR 50 = 130

play cutoff PAHR 50 = 137

| Id # | Description | Raw Values |
|---|---|---|
| 10 01 04 05 02 | indoor control | 118 |
| | nap time | 91 |
| | Time>PAHR 50 | 2.5 |
| 10 02 06 05 02 | indoor control | 112 |
| | nap time | 83 |
| | Time>PAHR 50 | 2.2 |
| 10 03 03 05 02 | physical play | 155 |
| | nap time | 93 |
| | Time>PAHR 50 | 21.1 |
| 10 04 05 05 02 | physical play | 144 |
| | nap time | 91 |
| | Time>PAHR 50 | 18.6 |

Child 11 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 121 | 97 | 1.4 |
| play day | 143 | 95 | 15.7 |

control cutoff PAHR 50 = 146

play cutoff PAHR 50 = 142

| Id # | Description | Raw Values |
|---|---|---|
| 11 01 04 05 03 | indoor control | 122 |
| | nap time | 90 |
| | Time>PAHR 50 | 2.7 |
| 00 01 20 06 04 | indoor control | 129 |
| | nap time | 95 |
| | Time>PAHR 50 | 2.2 |
| 11 02 06 05 03 | indoor control | 121 |
| | nap time | 116 |
| | Time>PAHR 50 | 0.0 |
| 11 02 23 06 04 | indoor control | 114 |
| | nap time | 88 |
| | Time>PAHR 50 | 0.8 |
| 11 03 03 05 03 | physical play | 155 |
| | nap time | 101 |
| | Time>PAHR 50 | 17.4 |
| 00 03 21 06 04 | physical play | 136 |
| | nap time | 93 |
| | Time>PAHR 50 | 12.7 |
| 11 04 05 05 03 | physical play | 139 |
| | nap time | 96 |
| | Time>PAHR 50 | 12.4 |
| 11 04 22 06 04 | physical play | 140 |
| | nap time | 89 |
| | Time>PAHR 50 | 20.3 |

Child 12 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 113 | 98 | 0.7 |
| play day | 156 | 104 | 16.6 |

control cutoff PAHR 50 = 147

play cutoff PAHR 50 = 155

| Id # | Description | Raw Values |
|---|---|---|
| 12 01 04 05 04 | indoor control | 115 |
| | nap time | 99 |
| | Time>PAHR 50 | 0.0 |
| 12 02 06 05 04 | indoor control | 112 |
| | nap time | 97 |
| | Time>PAHR 50 | 1.5 |
| 12 03 03 05 04 | physical play | 144 |
| | nap time | 103 |
| | Time>PAHR 50 | 9.9 |
| 12 04 05 05 04 | physical play | 168 |
| | nap time | 104 |
| | Time>PAHR 50 | 23.3 |

Child 13 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 119 | 89 | 1.1 |
| play day | 151 | 95 | 21.1 |

control cutoff PAHR 50 = 134

play cutoff PAHR 50 = 143

| Id # | Description | Raw Values |
|---|---|---|
| 13 01 09 05 01 | indoor control | 116 |
| | nap time | 88 |
| | Time>PAHR 50 | 1.0 |
| 13 02 10 05 01 | indoor control | 121 |
| | nap time | 90 |
| | Time>PAHR 50 | 1.2 |
| 13 03 11 05 01 | physical play | 149 |
| | nap time | 98 |
| | Time>PAHR 50 | 16.9 |
| 13 04 12 05 01 | physical play | 153 |
| | nap time | 92 |
| | Time>PAHR 50 | 25.3 |

Child 14 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 114 | 92 | 0.5 |
| play day | 154 | 95 | 22.8 |

control cutoff PAHR 50 = 138

play cutoff PAHR 50 = 142

| Id # | Description | Raw Values |
|---|---|---|
| 14 01 09 05 02 | indoor control | 117 |
| | nap time | n/a |
| | Time>PAHR 50 | 0.0 |
| 14 02 10 05 02 | indoor control | 110 |
| | nap time | 92 |
| | Time>PAHR 50 | 0.5 |
| 14 03 11 05 02 | physical play | 148 |
| | nap time | 91 |
| | Time>PAHR 50 | 25.3 |
| 14 04 12 05 02 | physical play | 160 |
| | nap time | 98 |
| | Time>PAHR 50 | 20.3 |

Child 15 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 135 | 101 | 2.6 |
| play day | 173 | 100 | 25.8 |

control cutoff PAHR 50 = 151

play cutoff PAHR 50 = 150

| Id # | Description | Raw Values |
|---|---|---|
| 15 01 09 05 03 | indoor control | 131 |
| | nap time | 96 |
| | Time>PAHR 50 | 3.0 |
| 15 02 10 05 03 | indoor control | 139 |
| | nap time | 105 |
| | Time>PAHR 50 | 2.2 |
| 15 03 11 05 03 | physical play | 167 |
| | nap time | 98 |
| | Time>PAHR 50 | 24.3 |
| 15 04 12 05 03 | physical play | 180 |
| | nap time | 102 |
| | Time>PAHR 50 | 27.3 |

Child 16 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 113 | 89 | 1.6 |
| play day | 144 | 91 | 23.1 |

control cutoff PAHR 50 = 133

play cutoff PAHR 50 = 137

| Id # | Description | Raw Values |
|---|---|---|
| 16 01 09 05 04 | indoor control | 116 |
| | nap time | 86 |
| | Time>PAHR 50 | 2.0 |
| 16 02 10 05 04 | indoor control | 110 |
| | nap time | 91 |
| | Time>PAHR 50 | 1.2 |
| 16 03 18 05 03 | physical play | 143 |
| | nap time | 89 |
| | Time>PAHR 50 | 23.8 |
| 16 04 12 05 04 | physical play | 141 |
| | nap time | 92 |
| | Time>PAHR 50 | 18.4 |
| 16 04 20 05 03 | physical play | 149 |
| | nap time | 92 |
| | Time>PAHR 50 | 27.0 |

Child 17 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 134 | 98 | 1.9 |
| play day | 149 | 98 | 17.1 |

control cutoff PAHR 50 = 146

play cutoff PAHR 50 = 146

| Id # | Description | Raw Values |
|---|---|---|
| 17 01 17 05 01 | indoor control | 132 |
| | nap time | 95 |
| | Time>PAHR 50 | 3.0 |
| 17 02 19 05 01 | indoor control | 135 |
| | nap time | 100 |
| | Time>PAHR 50 | 0.8 |
| 17 03 18 05 01 | physical play | 147 |
| | nap time | 95 |
| | Time>PAHR 50 | 19.4 |
| 17 04 20 05 01 | physical play | 150 |
| | nap time | 100 |
| | Time>PAHR 50 | 14.9 |

Child 18 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 119 | 84 | 7.8 |
| play day | 167 | 87 | 32.6 |

control cutoff PAHR 50 = 126

play cutoff PAHR 50 = 130

| Id # | Description | Raw Values |
|---|---|---|
| 18 01 17 05 02 | indoor control | 119 |
| | nap time | 80 |
| | Time>PAHR 50 | 10.2 |
| 18 02 19 05 02 | indoor control | 119 |
| | nap time | 88 |
| | Time>PAHR 50 | 5.5 |
| 18 03 18 05 02 | physical play | 162 |
| | nap time | 87 |
| | Time>PAHR 50 | 32.0 |
| 18 04 20 05 02 | physical play | 173 |
| | nap time | 86 |
| | Time>PAHR 50 | 33.3 |

Child 19 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 125 | 95 | 2.0 |
| play day | 140 | 97 | 13.2 |

control cutoff PAHR 50 = 143

play cutoff PAHR 50 = 146

| Id # | Description | Raw Values |
|---|---|---|
| 19 01 23 05 01 | indoor control | 127 |
| | nap time | 91 |
| | Time>PAHR 50 | 4.0 |
| 19 02 27 05 01 | indoor control | 124 |
| | nap time | 99 |
| | Time>PAHR 50 | 0.0 |
| 19 03 24 05 01 | physical play | 137 |
| | nap time | n/a |
| | Time>PAHR 50 | 0.0 |
| 19 04 26 05 01 | physical play | 143 |
| | nap time | 97 |
| | Time>PAHR 50 | 13.2 |

Child 20 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 126 | 85 | 10.7 |
| play day | 151 | 91 | 30.0 |

control cutoff PAHR 50 = 128

play cutoff PAHR 50 = 137

| Id # | Description | Raw Values |
|---|---|---|
| 20 01 23 05 02 | indoor control | 119 |
| | nap time | 86 |
| | Time>PAHR 50 | 3.7 |
| 20 02 27 05 02 | indoor control | 133 |
| | nap time | 84 |
| | Time>PAHR 50 | 17.6 |
| 20 03 24 05 02 | physical play | 151 |
| | nap time | 94 |
| | Time>PAHR 50 | 29.7 |
| 20 04 26 05 02 | physical play | 151 |
| | nap time | 88 |
| | Time>PAHR 50 | 30.3 |

Child 21 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 114 | 80 | 6.2 |
| play day | 141 | 86 | 25.8 |

control cutoff PAHR 50 = 120

play cutoff PAHR 50 = 129

| Id # | Description | Raw Values |
|---|---|---|
| 21 02 27 05 03 | indoor control | 114 |
| | nap time | 78 |
| | Time>PAHR 50 | 9.2 |
| 21 02 27 06 01 | indoor control | 114 |
| | nap time | 82 |
| | Time>PAHR 50 | 3.2 |
| 21 04 26 05 03 | physical play | 141 |
| | nap time | 86 |
| | Time>PAHR 50 | 25.8 |

Child 22 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 119 | 89 | 7.2 |
| play day | 164 | 94 | 29.0 |

control cutoff PAHR 50 = 134

play cutoff PAHR 50 = 141

| Id # | Description | Raw Values |
|---|---|---|
| 22 01 01 06 01 | indoor control | 124 |
| | nap time | 84 |
| | Time>PAHR 50 | 8.9 |
| 22 01 20 06 01 | indoor control | 115 |
| | nap time | 94 |
| | Time>PAHR 50 | 5.5 |
| 22 03 21 06 01 | physical play | 156 |
| | nap time | 96 |
| | Time>PAHR 50 | 27.6 |
| 22 04 22 06 01 | physical play | 173 |
| | nap time | 92 |
| | Time>PAHR 50 | 30.5 |

Child 23 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 136 | 93 | 12.4 |
| play day | 170 | 97 | 30.4 |

control cutoff PAHR 50 = 140

play cutoff PAHR 50 = 145

| Id # | Description | Raw Values |
|---|---|---|
| 23 01 01 06 02 | indoor control | 138 |
| | nap time | 90 |
| | Time>PAHR 50 | 18.8 |
| 23 01 20 06 02 | indoor control | 137 |
| | nap time | 89 |
| | Time>PAHR 50 | 18.1 |
| 23 02 23 06 02 | indoor control | 135 |
| | nap time | 101 |
| | Time>PAHR 50 | 0.2 |
| 23 03 21 06 02 | physical play | 167 |
| | nap time | 98 |
| | Time>PAHR 50 | 29.3 |
| 23 04 22 06 02 | physical play | 173 |
| | nap time | 95 |
| | Time>PAHR 50 | 31.5 |

Child 24 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 119 | 91 | 2.9 |
| play day | 159 | 90 | 27.5 |

control cutoff PAHR 50 = 136

play cutoff PAHR 50 = 134

| Id # | Description | Raw Values |
|---|---|---|
| 24 01 14 06 01 | indoor control | 119 |
| | nap time | 93 |
| | Time>PAHR 50 | 1.0 |
| 24 02 27 06 02 | indoor control | 119 |
| | nap time | 88 |
| | Time>PAHR 50 | 4.7 |
| 24 03 17 06 01 | physical play | 165 |
| | nap time | 90 |
| | Time>PAHR 50 | 28.8 |
| 24 04 24 06 02 | physical play | 153 |
| | nap time | 89 |
| | Time>PAHR 50 | 26.3 |

Child 25 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 121 | 89 | 3.5 |
| play day | 145 | 84 | 28.3 |

control cutoff PAHR 50 = 134

play cutoff PAHR 50 = 126

| Id # | Description | Raw Values |
|---|---|---|
| 25 01 14 06 02 | indoor control | 123 |
| | nap time | 91 |
| | Time>PAHR 50 | 2.5 |
| 25 02 27 06 03 | indoor control | 120 |
| | nap time | 87 |
| | Time>PAHR 50 | 4.5 |
| 25 03 17 06 02 | physical play | 146 |
| | nap time | 83 |
| | Time>PAHR 50 | 29.8 |
| 25 04 24 06 03 | physical play | 144 |
| | nap time | 85 |
| | Time>PAHR 50 | 26.8 |

Child 26 – Mean values during investigator contact and nap time

| | HR (bpms) | RHR (bpms) | Time>PAHR 50 (mins) |
|---|---|---|---|
| control day | 114 | 87 | 1.2 |
| play day | 162 | 91 | 27.7 |

control cutoff PAHR 50 = 131

play cutoff PAHR 50 = 136

| Id # | Description | Raw Values |
|---|---|---|
| 26 01 14 06 03 | indoor control | 111 |
| | nap time | 89 |
| | Time>PAHR 50 | 0.0 |
| 26 02 27 06 04 | indoor control | 116 |
| | nap time | 85 |
| | Time>PAHR 50 | 2.5 |
| 26 03 17 06 03 | physical play | 163 |
| | nap time | 89 |
| | Time>PAHR 50 | 28.6 |
| 26 04 24 06 04 | physical play | 161 |
| | nap time | 92 |
| | Time>PAHR 50 | 26.8 |