THE EFFECTS OF PLAY AND SOCIOECONOMIC STATUS ON HAIR CORTISOL IN CHILDREN

By

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Abstract

Hair cortisol concentration can serve as a biomarker of an individual's chronic stress. High levels of cortisol in children have been found to reflect an increased risk of developing cardiometabolic disease and depressive symptoms later in life. The purpose of my study is to evaluate the effect of unstructured play and socioeconomic status on hair cortisol in 3rd through 5th-grade children. The LiiNK project is working to improve the physical and mental health of children by implementing 60 minutes of outdoor unstructured play, or recess, in schools daily. The study plan was to work with the LiiNK project to evaluate if a significant difference exists in the hair cortisol of children who receive increased unstructured play at a LiiNK intervention school versus children who receive 30 minutes of unstructured play at a control school. Additionally, my study aimed to evaluate if there is a significant difference in hair cortisol levels according to socioeconomic status in children, with a student's free or reduced lunch status serving as an indicator of socioeconomic status.

Hair samples from 100 students were included in this study and an EIA kit was used to measure the cortisol concentrations in the extracted hair samples. Due to a small sample size of participants from the intervention school, comparisons of unstructured play were unable to be determined. Analysis of socioeconomic status at the control school partially supported my hypothesis, with the hair cortisol of students on free or reduced lunch being significantly lower than students not on free or reduced lunch in the fall semester, but not the spring semester. There was a significant increase in the hair cortisol of students receiving free or reduced lunch from the fall to the spring semester.

Introduction

Exercise and activity play a large role in the development of children and adolescents. There is a plethora of positive impacts that activity has on children, including reducing the risk of heart disease, diabetes, high blood pressure, and obesity (Janssen & LeBlanc, 2010). Additionally, physical activity has been found to have a positive impact on mental health with improvements in symptoms of anxiety and depression (Hu et al., 2023). These positive benefits are important determinants of future participation in physical activity and health promotion as an adult (Kohl & Cook, 2013). The recommended amount of physical activity that children should receive for optimal health is 60 minutes per day, however, only 24% of children 6-17 years old receive 60 minutes of physical activity every day (U.S. Department of Health and Human Services, 2011).

Unstructured play is a type of physical activity that is free or imaginative play (Lee, 2020). In many schools, recess is a perfect way to allow children to experience unstructured play, where children are free to play, move, and socialize outside in a way that isn't governed by strict guidelines. Unstructured play has been found to increase physical activity, help develop cognitive skills, and improve socialization skills (Lee et al., 2020). Unstructured play is a useful strategy not only to increase the amount of physical activity children receive, but unstructured play also increases the enjoyment of physical activity and the motivation to participate in physical activity (Lee et al., 2020). Further research is needed to continue to determine how unstructured play impacts mental health and stress, as this evidence is limited in current research.

Cortisol is a hormone involved in stress response, and it is an important biomarker that reflects the risk for chronic psychosocial stress, depressive symptoms, and cardiometabolic disease later in life (Li et al., 2023; Vliegenthart et al., 2016). Additionally, elevated levels of hair cortisol have been found to correlate with an increase in body mass index in children, a factor of cardiovascular disease and diabetes developing in youth (Chu et al., 2017). Cortisol has also been found to correlate with depression, and the age at which a teenager is diagnosed with depression has been found to have a correlation to cortisol reactivity (Colich et al., 2015). As evidenced by previous research, it is important to develop further research regarding cortisol to improve the health and well-being of children for generations to come.

There is conflicting evidence regarding the effect of physical activity on physiological stress, as evidenced by cortisol levels, however, it has been found that intense cardiovascular physical activity like running may raise cortisol levels, and motor activity like playing catch may lower cortisol levels (Wegner et al., 2019). Therefore, further research is indicated to evaluate the effect of physical activity on cortisol.

It is important to study cortisol and how unstructured play affects cortisol in children of different socioeconomic statuses. Socioeconomic status (SES) is a term that refers to a person's social standing due to their education, income, and occupation (American Psychological Association, 2023). Few studies have been conducted to evaluate an association of daily unstructured play on cortisol with an analysis of SES, leaving room for further research.

The purpose of this study is to evaluate the effects of unstructured play and socioeconomic status on the physiological stress of 3rd-5th grade children. My research questions seek to answer the following:

1. Is there a significant difference in the hair cortisol levels of 3rd through 5th-grade children involved in 60 minutes per day of unstructured play compared to 30 minutes of unstructured play?
2. Is there a significant difference in hair cortisol levels according to socioeconomic status in 3rd through 5th-grade children?

Theoretical Framework

The Whole Child Framework is used to support this research. The Whole Child approach addresses the cognitive, physical, social, and emotional needs of students (ASCD, n.d.). Schools can choose to implement the Whole Child approach into classrooms to develop the whole child, rather than focusing solely on the performance of students on academic tests. The Whole Child Framework is used in school to benefit the child's emotional health, physical health, and self-sufficiency.

Review of Literature by Themes

A review of research from within the past 10 years identified with search terms ‘stress’, ‘cortisol’, ‘hair’, ‘children’, ‘socioeconomic status’, physical activity’, and ‘unstructured play’ excluding studies of adults over 18 from a variety of databases yielded systematic reviews, meta-analyses, and experimental research.

Cortisol and Chronic Stress in Children

To understand the correlation of cortisol to play, it is important to first understand cortisol in children. In a systematic review by Li et al. (2023), chronic stress, measured

by stress due to life events, was found to be positively correlated with hair cortisol concentration in children (Li et al., 2023). This review involved 1455 participants from 13 studies of 5 countries. Cortisol can serve as a valid biomarker to measure stress in children, despite varying strengths of correlation depending on characteristics and measurements of stress (Li et al., 2023).

In a study by Xu et al. (2019), interactions of hair cortisol on stress, anxiety, and depressive symptoms were evaluated in 324 adolescents (Xu et al., 2019). Hair cortisol was found to be significantly correlated to anxiety and depressive symptoms along with perceived stress (Xu et al., 2019). This emphasizes the significance that cortisol has in the mental health of children and signifies why monitoring and controlling cortisol levels is important to the health of children.

Unstructured Play and Chronic Stress

A research study by Martikainen et al. (2013) studies the effect of physical activity on cortisol in children. This study utilized 258 8-year-olds in Finland to study salivary cortisol in children participating in various levels of physical activity, and how they respond to stress (Martikainen et al., 2013). In this study, it was discovered that children who underwent more physical activity had a lower rise in cortisol in response to a stressor, which may imply that children who undergo more physical activity are able to handle stressful situations more effectively (Martikainen et al., 2013). It should be acknowledged that the Martikainen et al. (2013) study utilized salivary cortisol rather than hair cortisol, and salivary cortisol reflects a more acute change in cortisol concentration than hair does, indicating the need for further hair cortisol research.

A dissertation by Kirby (2022) examined the influence of daily unstructured play on chronic and perceived stress and obesity in elementary children compared to children receiving standard recess. In this study, the control schools engaged in 30 minutes of unstructured play while the intervention schools engaged in 45 minutes of unstructured play daily (Kirby, 2022). In this study, chronic stress was measured by using hair cortisol concentrations (Kirby, 2022). It was found that 45 minutes of unstructured outdoor play was significantly correlated with below-average levels of hair cortisol (Kirby, 2022). Further research is needed to evaluate the correlation of longer durations of unstructured play, such as 60 minutes, on cortisol.

Socioeconomic Status and Hair Cortisol

Studies such as the systematic review by Gray et al. (2018) have identified evidence that suggests an inverse correlation between socioeconomic status and hair cortisol. Most studies that have studied socioeconomic status reference caregiver education and income as an indication of socioeconomic status. Articles included in this systematic review reported contrasting findings regarding a correlation. Two out of the eight articles included in this systematic review reported a significant inverse relationship between hair cortisol and parental education, five reported no significant relationship, and one reported a significant positive relationship. Grey et al. (2018) suggested that this may be because there are inconsistent degrees of socioeconomic status differences between comparison groups (Gray et al., 2018). Therefore, it is suggested that further quality evidence is needed regarding the comparison of SES and hair cortisol.

Little research has been conducted regarding SES, unstructured play, and hair cortisol all in one study leaving room for further evaluation.

Levels of Evidence

The Joanna Briggs Institute Levels of Evidence tool was used to determine the level of each evidence source in the literature review (Joanna Briggs Institute, 2013). The following table outlines the level of each source.

Authors	Level of Evidence
Grey et al. (2018)	Level I
Farbo et al. (2022)	Level II
Kirby (2022)	Level II
Martikainen et al. (2013)	Level II
Li et al. (2023)	Level III
Xu et al. (2019)	Level III

Methodology

This project was a cross-sectional study as a part of a larger study called the LiiNK Project. My objective was to analyze children's hair cortisol concentration in relation to SES and length of unstructured recess within the LiiNK Project (Rhea, 2023). The LiiNK Project is an organization that is working to fight the problem of decreasing physical activity in schools by implementing longer recesses with unstructured play in schools. The LiiNK Project has also collected evidence with research regarding 60 minutes of daily unstructured recess for children and provides a program based on research-proven implementations to improve child development (Rhea, 2023). Schools that have adopted the LiiNK Project curriculum are called LiiNK Schools, and the LiiNK

curriculum includes four 15-minute outdoor recess periods per day in addition to character development lessons. All schools in this study that utilize 60 minutes of recess per day are LiiNK schools as the intervention group, whereas the schools that do 30 minutes of recess per day serve as the control group. Hair samples were obtained and analyzed in both the fall 2023 and spring 2024 semesters from all participating students.

Children from two elementary schools in Texas were involved in this study. The sample for this study are children 3rd through 5th grade who attend an intervention or a control school. All 3rd-5th grade children at these intervention and control schools were offered to participate in the study, and only children with parental consent, minor assent, and hair at least 1 inch in length were included in the study. A team of trained post-doctorate researchers, graduate students, and I were involved in data collection and analysis for this study.

After the schools were contacted and agreed to participate in LiiNK Project research, the parents of children at these schools were informed of the study. The parents were given consent packets, detailing that this study will have their child's chronic stress assessed through hair sampling, and the parents were given the choice to consent to their child's participation in the study (Kirby, 2022). If the child was given parental consent to participate, they were called to a room during P.E. class where the study was explained. The child was told that the sample of hair removed would be from under the top layer of hair, and the sample would show how much of a stress hormone they have, called cortisol. If the child expressed they didn't want to participate, denying their assent, no hair was collected and they were returned to class. If a parent of a child

at an intervention school chose for their child not to participate in the LiiNK research hair collection, the child still participated in the unstructured recess and other LiiNK curriculum at that school. The child is also allowed to participate in other aspects of LiiNK research without participation in the hair collection.

Once the child confirmed their assent, the gloved researcher parted the child's hair horizontally between the tops of the ears to obtain a sample with minimal sun exposure (Meyer et al., 2014). A hair sample of at least 3cm in length with 30-50 strands of hair was then cut with scissors as close to the scalp as possible. After the hair was cut, it was attached to a small sheet of aluminum foil and secured with tape. Then, the scalp end of the hair was labeled with a permanent marker, because this is the end that was used for cortisol analysis. The foil and hair were then folded and placed in an envelope that is immediately labeled with the child's unique study ID number and the date of collection before being sealed, maintaining confidentiality. The hair samples were stored in a container within a researcher's locked office at Texas Christian University until analysis. The hair samples were then transported to the Harris College of Nursing and Health Sciences Research lab where each sample was processed for cortisol concentrations to be analyzed.

The samples were cut to 3cm or shorter from the root of each sample. Each centimeter from the root represents about one month of hair growth, which allows the determination of chronic cortisol, dependent on the length of the hair sample. Then, screw-cap tubes to hold each hair sample were placed on the scale, zeroed, before the hair sample was added to the tube and weighed with the scale zeroed. Two steel beads were added to each tube, then each sample was pulverized with the beads for a minute

to powder the hair, allowing for increased surface area for cortisol extraction. Methanol was then added to each tube to and incubated for 24 hours, which extracts the cortisol. Then, the tubes were centrifuged and the supernatant of each sample including the cortisol was transferred to new microcentrifuge tubes. The extracted cortisol was then reconstituted in an assay buffer. A high-sensitivity enzyme immunoassay (EIA) kit was used to determine the concentration of cortisol in the samples with assay buffer. Once the samples were transferred to the EIA microplate and prepared for analysis, the plate was then read by a microplate reader software which determined the concentration of cortisol of each sample in pg/mg (Meyer et al., 2014).

In addition to collecting hair samples, demographic information was collected with parental consent, including the child's grade level and gender. The free-and-reduced lunch status of each student was also obtained from each school to serve as a measurement of SES, and this was stored with the other study data on a password-protected server. A parent is able to qualify their child for free-or-reduced if they fill out an application for their children, and the school district determines if the children qualify to receive free-or-reduced lunch based on socioeconomic factors. The application asks questions regarding the family's income, the number of students in the household, and other financial assistance received. The free-and-reduced lunch rates were used to analyze the correlation between SES and hair cortisol levels.

Data Analysis Plan

The original data analysis plan was to separately average the cortisol levels of the intervention schools and the control schools, and analyze the two means for a significant difference in cortisol levels using a t-test with Microsoft Excel to evaluate if

there a significant difference in the hair cortisol levels of 3rd through 5th grade children involved in 60 minutes per day of unstructured play compared to 30 minutes of recess activity.

Due to a very small sample size of the intervention school, I was unable to complete this analysis of cortisol regarding unstructured play. I was able to conduct statistical analysis in regard to SES.

The cortisol levels of children with and without free or reduced lunch were analyzed with an independent t-test through Microsoft Excel to determine if there is a significant difference in the means of hair cortisol in relation to SES in 3rd through 5th graders.

Results

Due to a very small sample size from the intervention school, analysis of hair cortisol between schools with less versus more unstructured play cannot be determined. Data was only available from the control school, in which all students receive an equal amount of unstructured play per day of 30 minutes. However, findings were able to be analyzed from the control school in regard to SES and hair cortisol. Hair cortisol values were determined in both the fall and spring semesters for these students. Data of 104 third through fifth graders from one school was analyzed with hair cortisol from the fall semester and the spring semester for each student. Four participants were excluded from data analysis due to hair cortisol values being above the expected range (outliers), with 100 participants remaining for analysis. The outliers were identified by using a box-and-whisker plot, with the participants from the four highest plots removed from my analysis (Figure 1). After the outliers were excluded, 30 third graders, 27 fourth graders,

and 43 fifth graders were included in fall and spring analysis. There were 48 females and 52 males.

Out of the 100 fall cortisol values, the lowest cortisol concentration was 1.58 pg/mg and the highest was 54.05 pg/mg. Out of the 100 spring cortisol values from the same participants, the lowest cortisol concentration was 0.78 pg/mg and the highest was 99.5 pg/mg (M=23.01, SD=21.94). For the fall sample, the mean hair cortisol was 18.90 pg/mg and the SD was 17.64 pg/mg. For the spring sample, the mean hair cortisol was 27.28 pg/mg and the SD was 24.92 pg/mg (Table 1). A paired samples t-test was performed to compare the fall and spring cortisol for differences. A significant difference in the fall versus spring hair cortisol values was found with p=0.0081.

Mean and correlation tests were also performed to evaluate if a significant difference in cortisol was present between genders and each grade level. No statistically significant difference in hair cortisol was found in the fall or spring semester when evaluating males to females through t-tests. When evaluating grade levels, 3rd graders had the greatest mean hair cortisol in both the fall and spring semesters, but no statistically significant difference between grade levels was found. An increase in mean hair cortisol was found in all three grade levels from the fall to spring semester (Table 2).

Of the 100 participants, 72 did not qualify for free or reduced lunch, and 28 did qualify for free or reduced lunch. The mean cortisol for students who did not qualify for free and reduced lunch was M=21.40 pg/mg in the fall (SD=18.96), and M=26.24 pg/mg in the spring (SD=24.93). The mean cortisol for students who did qualify for free and

reduced lunch was M=12.47 pg/mg in the fall (SD=11.71), and 29.81 pg/mg in the spring (SD=25.16) (Table 3).

Through conducting a paired samples t-test, no statistically significant difference was found in the hair cortisol of students who did not qualify for free or reduced lunch in the fall versus spring semester (p=0.18). A statistically significant difference was found however in the hair cortisol of students who did qualify for free or reduced lunch in the fall versus spring semesters (p=0.0062) (Table 4).

Through conducting an independent t-test, a statistically significant difference was found in the hair cortisol of students who did versus did not qualify for free or reduced lunch in the fall semester (p=0.022), and no statistically significant difference was found in the hair cortisol of students who did versus did not qualify for free or reduced lunch in the spring semester (p=0.52) (Table 5).

Discussion

Our findings partially support my hypothesis that there is a significant difference in the means of hair cortisol in relation to SES. It was found that students from a lower SES had a significantly lower cortisol level compared to students from a higher SES in the fall semester, but not in the spring semester. This is an interesting finding in the fall semester, which may suggest that students from a higher SES are more stressed than students from a lower SES in the fall. In the spring semester, students from a lower SES had a higher but not statistically significant difference in hair cortisol from students from a higher SES. Is it possible that students on free or reduced lunch start off the school year not very stressed at first, but the stress grows due to the increasing pressure of school and testing?

The inconclusiveness of a relationship between SES and hair cortisol is supported by Gray et al. (2018), in which the majority of articles included in this systematic review found no significant correlation between hair cortisol and SES. It is possible that the lack of significant difference found in my study may be due to the relatively minimal difference in lifestyle of the two groups. Every student included in this study attends the same elementary school, which may contribute to the difficulty in truly analyzing students from different socioeconomic statuses. As suggested by Gray et al. (2018), further research is needed on participants of more significantly different socioeconomic statuses, such as a study of participants from different countries, to have a more accurate analysis.

One significant factor that likely affected the spring cortisol values is that for many of the grade levels students had taken the STAAR test within a few days of the spring hair cortisol sampling. STAAR testing is a standardized test all 3rd through 5th-grade students take in the state of Texas to measure a student's learned content for a particular grade and subject (Texas Education Agency, 2024). STAAR testing happens April through May, and each student must take 2-3 STAAR tests each year. STAAR tests take months to prepare for and pose students at risk of repeating a grade level if a STAAR test is not passed.

There was found to be a statistically significant difference in the hair cortisol for all students in the fall versus the spring, which may support the evidence from the literature review that psychological stress has an effect on hair cortisol (Li et al., 2023). When looking into this further it was found that students from a lower SES had a high increase in cortisol from the fall to spring semester, students from a higher SES had a

minimal and non-significant increase in cortisol in the spring semester. From these results, it can be hypothesized that students from a lower SES experience more stress in response to the longitudinal progression of the school year compared to those from higher SES groups. What is not known is the specific school stressor, such as STAAR testing or other school events.

Despite the inconclusive evidence found regarding SES and hair cortisol, my study did identify a change over the school year in a physiological marker of stress. This data generates further research questions regarding lower SES students, who may be more prone to stressors. This is a significant finding and supports existing research of physiological stress and hair cortisol (Li et al, 2023). There are few research analyses of hair cortisol and standardized testing in school-age children, but one article by Heissel et al. (2018) describes their research study conducted with 93 children at New Orleans charter schools to study salivary cortisol during high-stakes testing weeks and weeks without testing. This study found that students on average had an increase in cortisol on days of a high-stakes exam. Additionally, this study found evidence that students of lower SES saw the greatest change in cortisol in response to testing, which reflects similar results found in my research. It was also found that students with greater variations in cortisol performed worse on high-stakes exams (Heissel et al., 2018). This leads to further questioning about whether cortisol may impact a student's ability to perform to their fullest potential on exams. This is a significant finding that requires further research, as no identified studies similar to this have been performed since.

There are several limitations to this study, including the unequal number of students from lower versus higher SES groups. All the students in this study attended

the same school, creating a lack of geographic diversity in addition to a relatively small sample size. Additionally, there were several outliers in the data set, with the highest being 307 pg/mg. It is inconclusive what caused these outliers, or, if these elevated values truly represent high levels of cortisol. Additionally, there are many other factors not recorded in this study that may affect hair cortisol, including family stressors, extracurricular physical activity, and more. This leaves room for further analysis regarding hair cortisol. Finally, with all students attending the same school, every student received the same amount of unstructured play time, so this research question was unable to be determined. This leaves room for further analysis in regard to unstructured play and hair cortisol.

Nursing Implications

While the evidence for the effects of unstructured play and socioeconomic status on hair cortisol is inconclusive, it may be assumed that the psychological stress of school, inclusive of high-stakes standardized testing, may contribute to increased physiological stress. This supports the importance of promoting positive mental health in order to manage cortisol levels and improve the overall well-being and physical health of school-age children.

For a nurse who works with pediatric patients, it is imperative to focus on not only the physical but psychological health of children. Helping children promote healthy mental health will prevent the progression of other conditions associated with chronic stress which is in alignment with the whole child framework. As nurses, we have the power to serve as advocates for improved mental health resources for children and should work to find ways to continue decreasing stress in children.

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Figures

Figure 1: Hair Cortisol Fall versus Spring with Outliers (pg/mg)

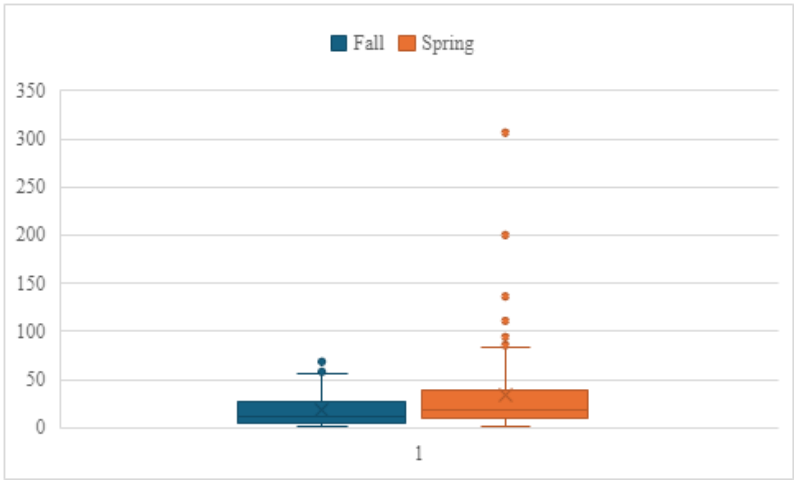

Table 1: Descriptive Statistics Fall and Spring

	N	Fall (pg/mg)	Spring (pg/mg)
Minimum	100	1.58	0.78
Maximum	100	54.05	99.5
Mean	100	18.90	27.28
Standard Deviation	100	17.64	24.92
T-Test (all Fall versus all Spring)	p=0.0081		

Table 2: Gender and Grade Level Hair Cortisol Statistics

	Male Mean (pg/mg)	Female Mean (pg/mg)	Male-Female T-Test	3rd Grade Mean (pg/mg)	4th Grade Mean (pg/mg)	5th Grade Mean (pg/mg)	3rd-4th Grade T-Test	4th-5th Grade T-Test	3rd-5th Grade T-Test
Fall	18.27	19.57	p=0.715	23.93	15.09	17.77	p=0.0655	p=0.504	p=0.167
Spring	27.48	26.98	p=0.920	34.27	27.01	22.47	p=0.320	p=0.395	p=0.0536

Table 3: Descriptive Statistics per SES

	N	Fall (pg/mg)	Spring (pg/mg)
Mean, No Free or Reduced Lunch	72	21.40 (SD=18.96)	26.24 (SD=24.93)
Mean, Yes Free or Reduced Lunch	28	12.47 (SD=11.71)	29.81 (SD=25.16)

Table 4: Pared T-Tests Comparing Fall and Spring Semester Cortisol

	Fall versus spring
No Free or Reduced Lunch	p=0.18
Yes Free or Reduced Lunch	p=0.0062

Table 5: Independent T-Tests Comparing Cortisol in regard to SES

	Fall	Spring
No versus Yes Free or Reduced Lunch	p=0.022	p=0.52