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No Change in Executive Function or Stress Hormones Following a Bout of Moderate Treadmill Exercise in Preadolescent Children

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ABSTRACT

International Journal of Exercise Science 13(5): 1650-1666, 2020. Several studies suggest that acute bouts of exercise improve executive function in preadolescent children. However, the mechanisms underlying these effects are not completely understood. Specifically, no studies have examined the relationship between the stress hormone response to exercise and improvements in executive function in preadolescent children. The purpose of this study was to examine the effects of a bout of moderate intensity exercise versus rest on working memory (List Sorting Working Memory Task) and selective inhibition/attention (Eriksen flanker task) in preadolescent children, as well as to investigate whether changes in stress hormones (salivary cortisol and alpha-amylase) could explain any differences in performance on these tasks. Twenty-four children completed both a 30-minute moderate intensity bout of treadmill walking and seated rest in a laboratory setting. Tests of executive function and salivary stress hormone analyses were completed before and after each condition. 2x2 Repeated Measures ANOVAs were used to test the effects of time, condition, and time*condition on all executive function and hormonal outcomes. Linear regression models were used to determine if changes in executive function measures were related to changes in stress hormones in the exercise condition. Likely due to methodological limitations, there were no effects of time, condition, nor an interactive effect on working memory, selective inhibition, salivary cortisol, or salivary alpha-amylase. However, there was a trend observed, where the magnitude of the increase in salivary alpha-amylase levels in the exercise condition marginally predicted the improvement in reaction time on the Eriksen flanker task. This suggests that exercise-induced changes in alpha-amylase may underlie improvements in executive function and highlights the need for additional research to more fully understand these relationships in preadolescent children.

KEY WORDS: Stress hormones, executive function, exercise, preadolescent children

INTRODUCTION

According to the Physical Activity Guidelines for Americans (26), children and adolescents ages 6-17 years are recommended to participate in 60 minutes or more of moderate-to-vigorous physical activity each day. However, only 21.6% of 6-19-year-old children and adolescents in the United States attain this recommended amount of activity on at least 5 days per week (16). Despite copious scientific evidence of positive associations between physical activity and academic achievement among school-aged children, there is an increasing trend in the United States to cut physical education programs in public schools – limiting programs to only $764 a year on average (15, 28).

Physical activity is a critical component of children's cognitive development, particularly given its positive impact on executive function. Executive function is characterized by a set of cognitive processes that regulate an individual's ability to organize purposeful goal-directed behaviors, think critically, and respond appropriately to environmental and contextual stimuli. It includes several components, such as inhibition, working memory, scheduling, planning, and shifting (3, 18, 20, 23). Findings from multiple studies have indicated that single bouts of acute, moderate intensity aerobic exercise improve executive function – particularly inhibition and working memory – in preadolescent children (6, 14). Inhibition refers to an individual's ability to act on their own choice rather than on impulse alone. Working memory is the ability to maintain information for a short period without having external cues or aids as guidance. While both inhibition and working memory have been shown to improve following acute bouts of aerobic physical activity in preadolescents (9, 10, 14, 19), the physiological mechanisms underlying these improvements are still in question.

There are several proposed mechanisms by which an acute bout of physical activity may improve executive function. For example, increased levels of brain oxygenation may occur following a bout of exercise due to enhanced cerebral blood flow, specifically to the prefrontal cortex (4). An increase in both oxygenated and deoxygenated hemoglobin have been observed in the prefrontal cortex during exercise and cognitive tasks (12, 30). While increased cerebral blood flow to the prefrontal cortex may be one factor driving acute improvements in executive function following a bout of exercise, several studies highlighted mechanisms such as enhanced nutrient transfer to the prefrontal cortex, greater neurotrophic factor growth, and arousal levels. Further, Hillman et al. (14) observed that neural activity of the P300 waveform significantly increased following a bout of aerobic exercise in preadolescent children. In event-related potential (ERP) research, in which electroencephalography (EEG) is used to measure the brain's electrical activity in response to stimuli, the P300 waveform is interpreted as the speed of stimulus classification; greater attention is reflected in greater P300 amplitude (31). Therefore, such findings by Hillman et al. (14) suggest that following exercise, a greater proportion of attentional resources are allocated towards environmental stimuli, thus improving executive function.

While these findings suggest that improved prefrontal cortex oxygenation and neural activity are key factors in enhancing executive function following exercise, they do not account for the potential mediating role of the hormonal response to physical stress. Cortisol, for example, is a glucocorticoid hormone released after stimulation of the adrenal cortex as part of the stress response of the hypothalamic pituitary adrenal (HPA) axis. While serum cortisol levels appear to drop in direct response to a bout of acute resistance exercise in a young adult male population (33), no studies have explored changes in cortisol levels in a preadolescent population following acute bouts of moderate aerobic exercise. Further, no studies have examined the relationship between changes in cortisol in response to exercise and improvements in executive function in this population.

The HPA-axis modulates the hormonal response to stress in conjunction with the sympathetic nervous system (SNS). With respect to salivary hormone analytes, alpha-amylase is an enzyme that, when released from the salivary glands, breaks down carbohydrates in response to prepare the body for stressful stimuli (13). High-intensity interval training has shown increases in salivary alpha-amylase among male adolescents in comparison to continuous moderate intensity exercise and has been closely linked with epinephrine and norepinephrine activity (7). Given their relationship with circulating levels of stress hormones, salivary cortisol and salivary alpha-amylase are accepted as surrogate markers of HPA-axis and SNS activity in response to physical and psychological stressors (14, 17, 36). The release of cortisol in particular may influence prefrontal cortex activity following exercise, as the prefrontal cortex hosts a high density of corticosteroid receptors in its pyramidal neurons (36). Additionally, at moderate levels of HPA-axis and SNS stimulation, neuroendocrine hormones are associated with greater synaptic activity in the prefrontal cortex that underlie improved executive function. Conversely, very high or low levels of HPA-axis and SNS activity are associated with reduced activity in these brain regions and impaired executive function (27). Given that moderate levels of physical stress may produce moderate stimulation of both the HPA-axis and the SNS, it is possible that moderate-intensity aerobic exercise acutely improves executive function by producing an optimal level of state arousal. Therefore, the objectives of this study were to examine 1) the effect of a single bout of aerobic exercise versus rest on executive function in preadolescent children and 2) whether the changes in stress hormone levels correlated with the improvement in executive function following exercise. It was hypothesized that 1) executive function scores would be higher following exercise compared to rest and 2) improvement in executive function would relate to the increase in stress hormone levels following exercise.

METHODS

Participants

This study included a convenience sample of 24 pre-adolescent participants between the ages of 7 and 12 years old (male: n = 13; female: n = 11). Participants were recruited via fliers, emails, and word-of-mouth communication from various elementary schools, community centers, and public events in the greater San Luis Obispo, CA area. Participants were deemed eligible for the study if they: 1) were between 7 and 12 years old, 2) could participate in 30 minutes of moderate aerobic exercise, and 3) spoke English as their first language. Participants were excluded from the study if they had: 1) any pre-existing heart conditions, 2) any pre-existing lung conditions, 3) any metabolic conditions, 4) emotional or mental disorders, 5) major medical condition(s) that prohibit(s) physical activity, and 6) reached puberty. All inclusion and exclusion criteria were screened via a telephone call with the child's primary caregiver. All participants and primary caregivers were informed of the risks, benefits, and requirements of the study via an informed assent form signed by every child and an informed consent form signed by every parent, respectively.

Protocol

This study utilized a randomized-crossover block design in which all participants completed two experimental conditions in random order on two separate visits to the Human Performance Laboratory at California Polytechnic State University, San Luis Obispo. Each visit was separated by a one-week washout period. During the first visit, participants completed one of two randomly assigned protocols: 1) a 30-minute bout of moderate intensity aerobic exercise or 2) a 30-minute bout of seated rest. During the second visit, participants completed the protocol in the opposite order. In order to control for the time of day, all primary caregivers were asked to schedule each visit within the same relative time frame. Primary caregivers were instructed to have their child refrain from any sort of physical activity or exercise for at least two hours prior to arrival. They were also asked to have their child fast and refrain from drinking any liquids other than water for at least two hours prior to testing. Additionally, caregivers were instructed to wait behind a medical curtain during each visit so as not to interfere or distract the child during testing procedures. The details of each protocol are described below. Upon completion of each visit, participants were compensated with a $20 gift card to the campus bookstore. All protocols were administered by a trained member of the research staff. All procedures were approved by the Institutional Review Board at California Polytechnic State University, San Luis Obispo. This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (24).

The resting condition consisted of 30 minutes of seated rest. This bout of rest was performed in a quiet corner of the laboratory, with only the participant and a trained member of the research staff present. In order to control for potential heightened levels of mental stimulation during the bout of rest, participants watched an age-appropriate documentary about dinosaurs. To maintain a consistent level of stimulation across conditions, participants watched the same television show during the 30-minute exercise condition. The exercise condition consisted of 30 minutes of treadmill walking at a work rate that elicited 60-70% of the participant's age-predicted maximum heart rate (calculated via the equation 220-age) per the generalized equation for predicting maximal heart rate in the ACSM guidelines (1). Before the exercise condition, the protocol was explained thoroughly and each participant was instructed to hold on to the treadmill handlebars as a safety precaution. Prior to the 30 minutes of moderate intensity treadmill walking, participants completed a warmup beginning at a speed of 1.5 miles per hour on a flat grade, and over three minutes gradually increased in speed and incline so that participants reached their individualized target heart rate by the end of the warmup. Heart rate was monitored using a Polar H9 Heart Rate Monitor (Polar Electro, Kempele, Finland) and was recorded at the end of each minute of the exercise session. If the participants' heart rate varied more than ± 5 bpm from the intended range, the speed and/or grade of the treadmill were altered accordingly until the heart rate returned to the prescribed zone. Throughout the entire protocol, participants' perceived exertion was monitored using the Children's Effort Rating Table (CERT) (35) and was recorded every third minute. If at any time the participant felt uncomfortable or could no longer continue the exercise due to any sort of pain or discomfort, they could immediately terminate the exercise protocol without loss of compensation.

During the first visit to the laboratory, the anthropometric characteristics of each participant were assessed. Height was assessed via stadiometer and was measured to the nearest 0.1 cm. Weight was assessed via digital scale and measured to the nearest 0.1 kg. Height and weight were subsequently used to calculate Body Mass Index (BMI). Each participant's BMI was calculated as kg/m^2 and used to determine their percentile rank based on their age and physiological sex using the Center for Disease Control growth charts (25). Primary caregivers additionally completed the Child Stress Disorders Checklist (CSDC) to measure acute stress and posttraumatic symptoms in children (29) by determining stress over a 2-year period using a points system, with a higher score correlating with a higher level of stress.

In order to compare the effect of both the exercise and resting protocols on executive function, participants completed two tests of executive function selected from the NIH Toolbox (HealthMeasures, College Park, Maryland) immediately prior to and after both conditions. Both tests were completed using the NIH Toolbox application on an iPad (Apple Inc., Cupertino, CA, USA). All tests were administered by trained members of the research staff.

To assess selective inhibition, participants completed a NIH-certified, modified Eriksen flanker task, which has been shown to have high test-retest reliability (ICC = .92), convergent validity (r = .64), and discriminant validity (r = .55) in individuals ages 8-15 (37). The test consists of a series of arrows presented on an iPad screen, with two arrows on the bottom of the screen pointing left and right. The participant is asked to focus only on the middle arrows and press the corresponding arrow for the direction the middle arrow is pointing using their index finger. The test includes both congruent and incongruent trials. Congruent trials display an array of arrows facing the same direction (i.e., >>>>>), while incongruent trials include an array of arrows where the middle arrow points in the opposite direction of the surrounding four arrows (i.e., >><>>). Before initiation of the trials, participants were led through four practice trials to become familiar with the protocol with a research assistant available to answer any additional questions. During this time, participants were asked to place their index finger on an area designated as "home base" to serve as a consistent starting and ending place for the participant's index finger both before and after their trial responses. Participants were additionally instructed to respond as quickly and accurately as possible to each trial. However, once the practice was complete the participant completed the task without assistance from the research assistant. Accuracy was measured as a percentage of correct responses out of 20, while reaction time (RT) was measured as an average of all 20 trials in milliseconds.

To assess working memory, participants completed a NIH-certified List Sorting Working Memory Test, an assessment found to have high test-retest reliability (ICC = .80), convergent validity (r = .57), and discriminant validity (r = .63) in individuals ages 8-15. This involved the participant sorting and naming recently presented stimuli in size order from smallest to largest (34). During the task, participants were presented visually and auditorily with a series of stimuli (either animals or food), followed by a blank screen. While each stimulus was read aloud in a pre-recorded computerized voice, a picture of the stimulus was simultaneously displayed on the iPad for two seconds. Subjects had to rely on working memory to remember and re-order each list of pictorial stimuli from smallest to largest in size (e.g., bunny, sheep, elephant) and responded orally once the blank screen following each series was displayed on the iPad. The test has two sections: 1) one-list series in which participants re-order by size one category of lists (i.e., animals only or food only) and 2) two-list series in which participants re-order two different categories of lists in size order from smallest to biggest (i.e., food and animals). Following a correct response, the presented list increased by one additional stimulus, thus making the task more difficult to remember and re-order. The greatest number of stimuli presented in a list was seven items. Following an incorrect response, participants received another chance to re-order the same number of different stimuli. If participants gave two incorrect responses consecutively, the test concluded. Before the start of each section of the test, participants were guided through two initial practice trials with a trained research assistant, who monitored the test and coded on the iPad whether the responses were correct or incorrect by following procedures listed in the NIH Toolbox Manual.

In order to compare the effects of exercise and rest on the stress hormone responses of the HPA-axis and the SNS, salivary cortisol (HPA) and alpha-amylase (SNS) levels were measured before and after each condition. Salivary samples were collected through the SalivaBio Oral Swab (SOS) Saliva Collection Method. Trained research assistants collected the sample via an oral swab of approximately 2mL of saliva, which is recommended for adults and children ages 6 and up. The swab was placed under the tongue for 1.5-2 minutes and then removed and placed into a swab tube for future analysis. Immediately after placing a swab in a tube, the sample was labeled and placed in a freezer kept at or below –20 degrees Celsius. Samples were later shipped to a third party laboratory for analysis of salivary cortisol and salivary alpha-amylase with no identifying information other than the participant number and timepoint. Samples were assayed at the Salimetrics' SalivaLab (Carlsbad, CA) using the Salimetrics Salivary Cortisol (Cat. No. 1-3002) and Salivary Alpha-Amylase (Cat. No. 1-1902) Kits, without modification to the manufacturers' protocols. The cortisol assay has a lower limit of sensitivity of 0.007 µg/dl, an average intra-assay coefficient of variation of 4.60%, and an average inter-assay coefficient of variation of 6.0%. The alpha-amylase assay has a lower limit sensitivity of 0.4 U/mL, an average intra-assay coefficient of variation of 5.47%, and an average inter-assay coefficient of variation of 4.70%. These standards meet the manufacturers' criteria for accuracy and repeatability in Salivary Bioscience, and exceed the applicable NIH guidelines for Enhancing Reproducibility through Rigor and Transparency.

Statistical Analysis

2x2 Repeated Measures ANOVAs were used to test the effect of time (pre/post), condition (rest/exercise), and time*condition with LSD tests for multiple comparisons to compare the mean differences between the exercise and rest conditions for the following measurement outcomes: salivary alpha amylase concentration, salivary cortisol concentration, score on the List Sorting Working Memory Test, and reaction time on the Eriksen flanker task. Raw scores for the List Sorting Working Memory Test were age-adjusted to control for changes in cognition between ages 7 and 12. Simple linear regression analyses were used to test relationships between any observed changes in executive function and changes in stress hormones due to exercise. Change scores were calculated for all variables in the exercise condition as Post-exercise minus

Pre-exercise. Given the lack of studies examining the effects of exercise on these executive function and hormonal measures in the population of interest, it was difficult to perform precise power analyses for this study. Using data from the development of the NIH Toolbox testing battery (34), it was determined that a minimum sample size of n = 21 would give us 80% power to detect a difference of 2.47 ± 0.13 in the uncorrected mean WM scores. Furthermore, using data from a previous study on cortisol responses to exercise in this population (21), it was determined that a minimum sample size of n = 39 would give us 80% power to detect a difference of 0.12 ± 0.13 µg/dl in the mean salivary cortisol concentrations. For all analyses, a significance level of $p < 0.05$ was set. All statistical analyses were conducted using JMP Pro version 14 (SAS Institute, Cary, NC).

RESULTS

In total, 24 participants (13 male, 11 female) completed all testing sessions. Overall, our population consisted of preadolescent children who were of normal weight status. Given the recorded characteristics of the exercise session, the bout of treadmill walking was indeed reflective of a moderate intensity aerobic exercise session. Table 1 below provides an overview of the mean demographic, anthropometric, and exercise bout characteristics of the population.

Table 1. Descriptive characteristics.

	Mean
Age (years)	9.29 ± 1.21
BMI Percentile	40.22 ± 23.93
Exercise HR	139.62 ± 7.11
%HR_{max}	66.27 ± 3.41
Exercise RPE	5.40 ± 1.21

Data are displayed as Mean ± SD. %HR_{max} reflects the relative intensity of the exercise bout using a maximal heart rate estimated via the equation HR_{max} = 220-age.

There were no effects of time (F = 3.39, p = 0.08), condition (F = 0.10, p = 0.75) nor an interactive effect of time and condition (F = 1.87, p = 0.18) on WM score (See Figure 1). With respect to attention/inhibitory control, there were no effects of time (F = 2.95, p = 0.10), condition (F = 4.02, p = 0.06), nor an interactive effect of time and condition (F = 0.27, p = 0.60) on RT (See Figure 2).

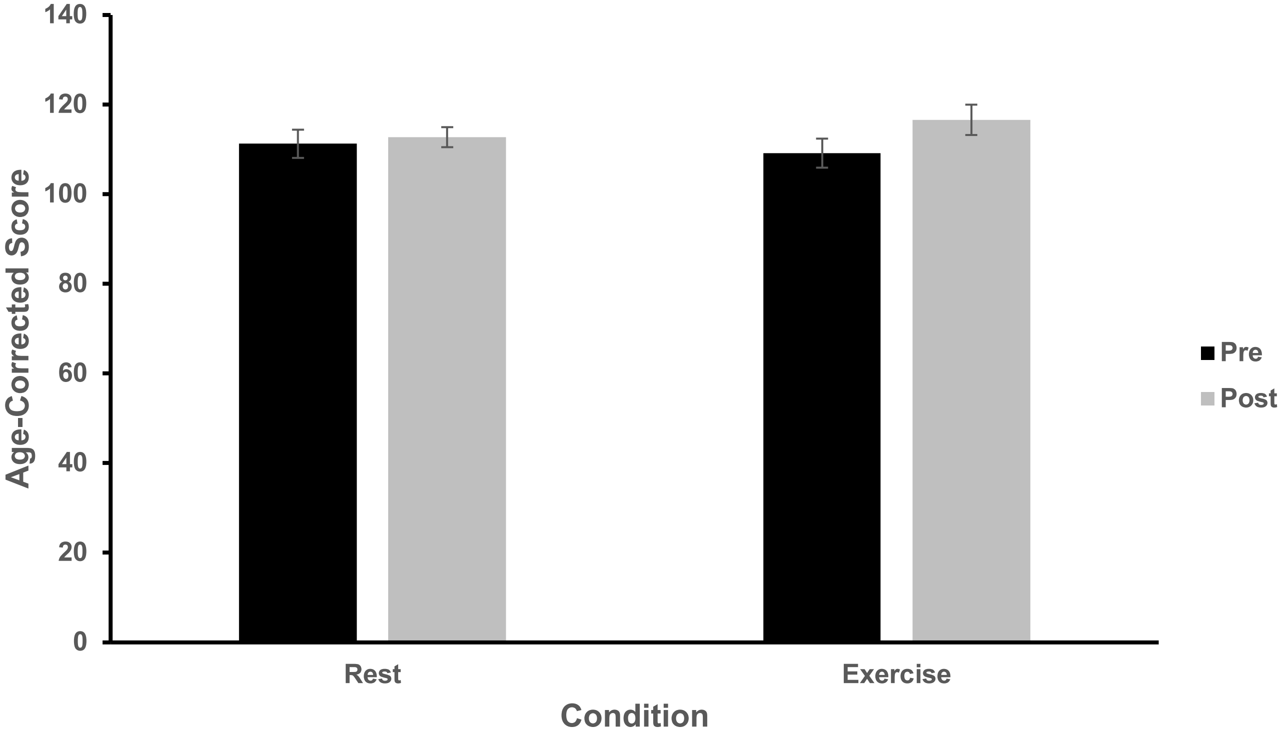

Figure 1. Comparison of WM scores across pre- and post-resting and exercise conditions. Data are displayed as Mean ± SEM.

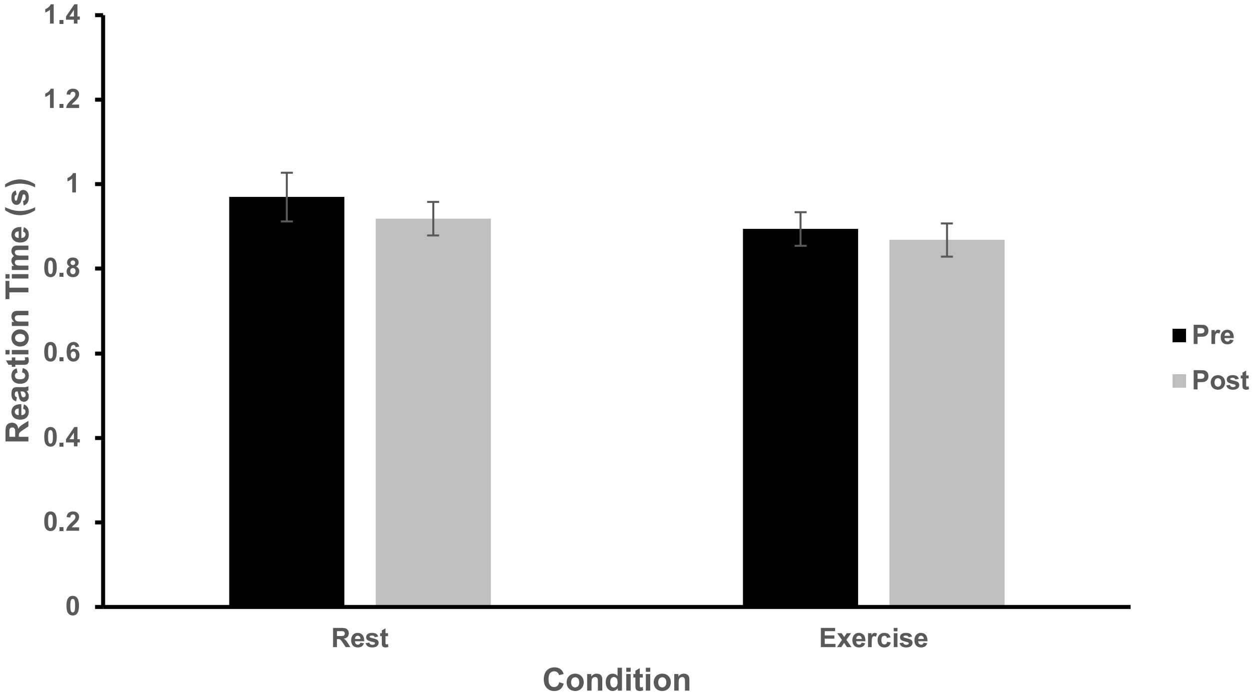

Figure 2. Mean comparison of Flanker Task Reaction Time across pre- and post-resting and exercise conditions. Data are displayed as Mean ± SEM.

There were no effects of time (F = 0.79, $p = 0.38$), condition (F = 0.42, $p = 0.44$) nor an interactive effect of time and condition (F = 0.63, $p = 0.43$) on mean cortisol concentrations (See Figure 3). Additionally, there were no effects of time (F = 2.78, $p = 0.11$), condition (F = 3.16, $p = 0.09$), nor an interactive effect of time and condition (F = 0.05, $p = 0.82$) on mean salivary alpha-amylase concentrations (See Figure 4). Descriptive data for both hormones by time and condition are displayed in Table 2.

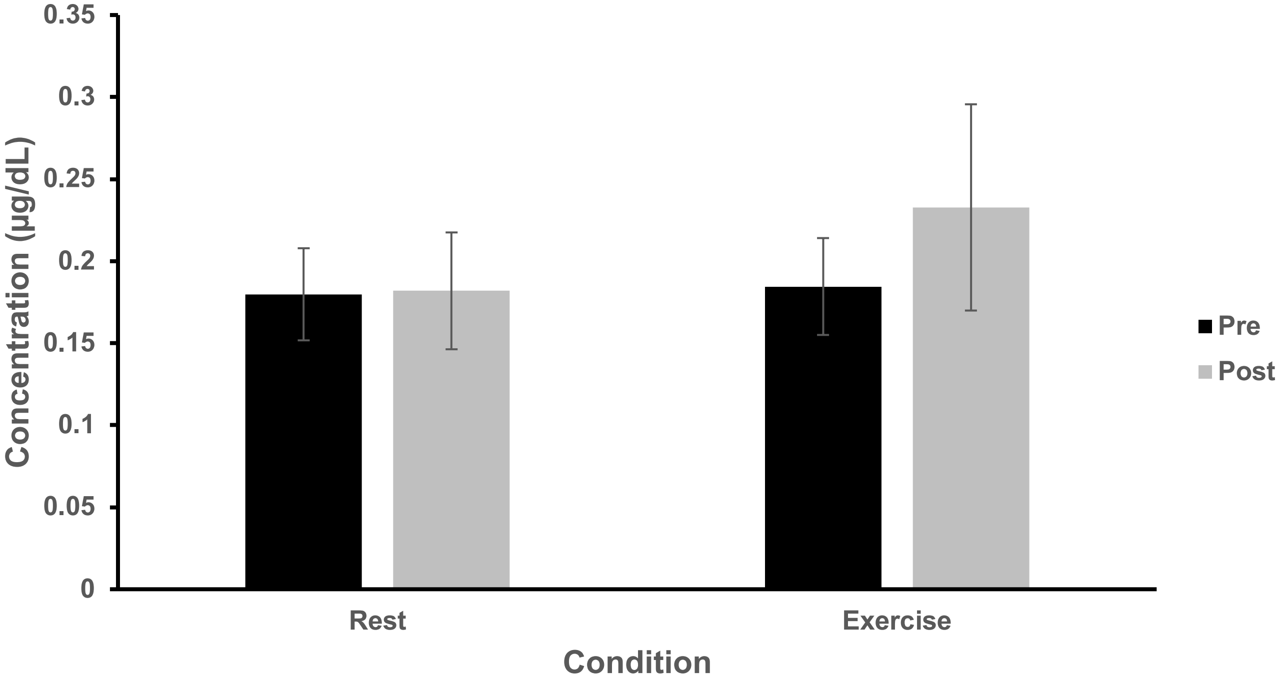

Figure 3. Mean comparison of cortisol concentrations across pre- and post-resting and exercise conditions. Data are displayed as Mean ± SEM.

Table 2. Salivary cortisol and alpha-amylase concentrations by time and condition.

	Pre	Post
Cortisol (µg/dL)		
Rest	0.17 ± 0.13 (0.05-0.61)	0.18 ± 0.17 (0.05-0.79)
Exercise	0.18 ± 0.14 (0.05-0.54)	0.23 ± 0.30 (0.05-1.30)
Alpha-Amylase (U/mL)		
Rest	101.8 ± 73.4 (24.9-296.3)	84.1 ± 50.7 (28.7-215.8)
Exercise	113.3 ± 76.3 (40.0-266.8)	99.2 ± 46.5 (33.5-222.9)

Data are displayed as Mean ± SD (Minimum-Maximum).

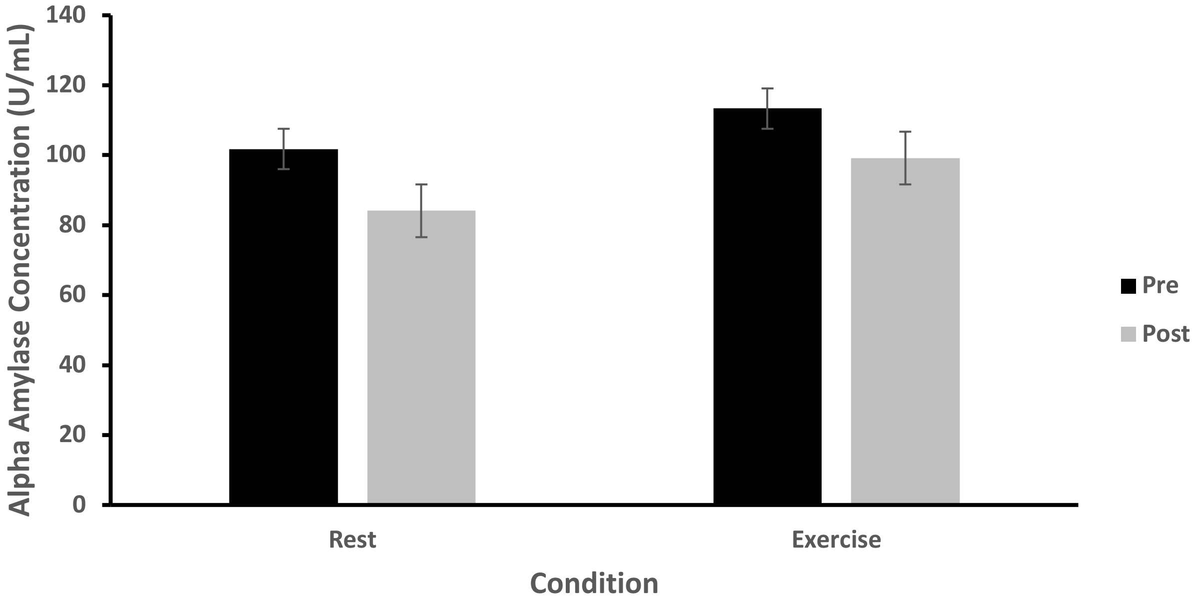

Figure 4. Mean comparison of salivary alpha-amylase (AA) concentrations across pre- and post-resting and exercise conditions. Data are displayed as Mean ± SEM.

The regression analyses did not reveal a significant relationship between changes in cortisol and WM score (F = 1.95, p = 0.18, $r^2 = 0.08$) or RT on the Eriksen flanker task (F = 1.07, p = 0.31, $r^2 = 0.05$) in the exercise condition. Additionally, there was no relationship between changes in alpha-amylase and WM score (F = 0.27 p = 0.61, $r^2 = 0.01$) in the exercise condition. While not statistically significant, there was an observed trend between changes in alpha-amylase and changes in RT in the exercise condition (F = 3.31, p = 0.08, $r^2 = 0.14$). Specifically, those who had a greater increase in alpha-amylase had a greater reduction in their RT on the Eriksen flanker task (See Figure 5).

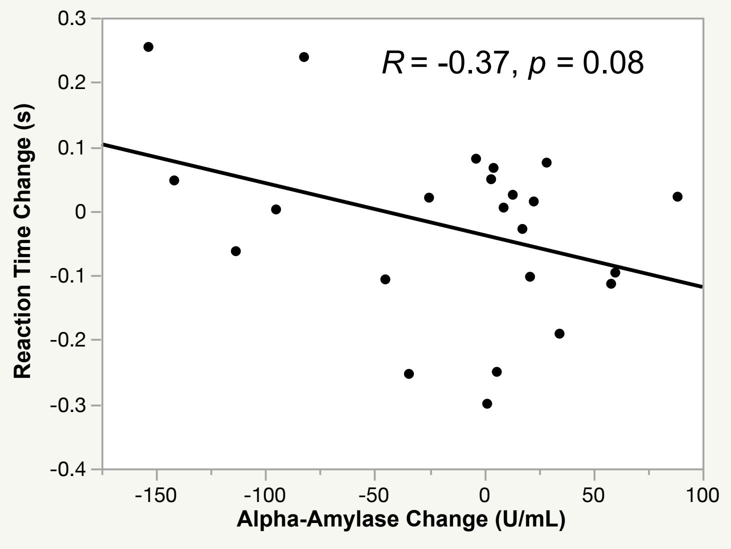

Figure 5. Exercise change in flanker task reaction time by change in alpha-amylase.

DISCUSSION

The purpose of this study was to examine whether a bout of aerobic exercise versus rest leads to improvements in executive function in preadolescent children, and if so, if these improvements correlate with changes in stress hormone levels pre-to-post exercise. It was hypothesized that a single bout of exercise would improve working memory and attention/inhibition compared to a bout of seated rest. Additionally, it was hypothesized that these improvements would be related to moderate levels of HPA-axis and SNS activation, as measured by salivary cortisol and alpha-amylase, respectively. The overall findings suggest that a 30-minute bout of treadmill walking neither improved executive functions nor influenced stress hormone responses in this population of preadolescent children. Second, our analyses did not reveal significant relationships between changes in stress hormones and changes in executive function in the exercise condition. However, there was a trend observed, where participants who had a greater increase in alpha-amylase concentration showed a greater reduction in RT on the Eriksen flanker task.

The finding that a single bout of moderate intensity aerobic exercise did not improve attention/inhibition is inconsistent with the current body of literature in this population. For example, Hillman et al. (14) found that a 20-minute bout of treadmill walking at 60% HRmax improved response accuracy and reaction time on a modified flanker task, similar to the one administered in our study. The inconsistency in findings could be explained by differences in the experimental protocols of the two studies. Specifically, Hillman et al. (14) administered tests of executive function immediately after both a bout of exercise and seated rest. In comparison, the current study administered tests immediately before and after both conditions, for a total of four repetitions across the study. It is possible that the decision to administer the tests both pre- and post-rest/exercise created a learning effect. For example, reaction time on the Eriksen flanker test decreased following 30 minutes of either exercise or rest, although this decrease was not statistically significant. This decision was made so we could compute a change score pre-to-post exercise and therefore determine whether changes in hormone levels related to changes in executive function. However, future studies may consider limiting the number of times tests of executive function are administered in order to avoid possible learning effects.

Our findings suggest that there was no improvement in working memory following a bout of moderate-intensity exercise in this population. Previous studies have noted improvements in working memory following an acute bout of exercise in preadolescents. For example, Chen et al. (8) found that a single bout of moderate intensity jogging improved performance on a modified visual “2-back” working memory task. The inconsistency in findings is possibly attributable to differences in the working memory tests employed. The “2-back” task used in the Chen et al. (8) study involves a series of changing letters and asks participants to identify whether the current letter is the same as the letter displayed two trials ago. When participants answered correctly, the amount of time it took to respond was recorded as the index of working memory. The current study used the List Sorting Working Memory Test (34), which involves ordering lists of objects based on pre-determined criteria, using the number of items successfully recalled as the index of working memory, with no recording of response time. It is possible that response time on a working memory task is a more sensitive measure of executive function in this population and would have yielded significant results if used in the current study. This is supported by the data, which suggest that working memory score improved slightly following the exercise condition, although this effect was not statistically significant. Studies seeking to further elucidate the effects of acute exercise on working memory in this population should consider using tasks that factor in both response accuracy and response time.

Very few studies have examined the acute impact of aerobic exercise on stress hormone responses in preadolescent children. With respect to HPA-axis stimulation, the findings suggest that exercise increased salivary cortisol concentrations compared to rest, although this effect was not statistically significant. The lack of significance could be due to the large variability in the post-exercise cortisol response compared with pre-exercise, post-exercise, and post-rest. This may indicate that preadolescent children vary substantially in their HPA-axis response to exercise, which would increase the number of participants needed to observe an effect. Indeed, our power analyses indicated that a larger sample size would have been required to detect a change in salivary cortisol. The only previous study examining the effect of an acute bout of aerobic exercise on cortisol release in preadolescent children also noted that while cortisol levels increased from resting, the effect was not statistically significant (21). Future studies seeking to examine salivary cortisol changes due to exercise in this population should use adequately powered sample sizes, which was challenging to attain in the present study given our within-subjects design (i.e., multiple visits of our children and their caregivers to the laboratory). Please note, however, that there is a precedent for using our chosen sample size (14, 21) and that the present study was adequately powered as far as detecting a change in working memory performance between groups.

With respect to SNS stimulation, this is the first study, to our knowledge, to examine the effects of exercise on salivary alpha-amylase concentrations in preadolescent children. The results suggest that neither rest nor exercise altered salivary alpha-amylase concentrations. However, the lack of effect could be attributable to insufficient intensity of the exercise bout. Indeed, it is well established that SNS input into both the sinoatrial node and the ventricular myocardium increases in order to enhance cardiac output during exercise. However, these effects are typically not observed until exercise intensity reaches a moderate intensity. During the transition from rest to light-moderate intensity exercise, the increase in cardiac output is achieved via parasympathetic nervous system withdrawal, although these phenomena have not been studied in preadolescent children (11). The ACSM guidelines categorize exercise between 64-76% of maximal heart rate as moderate intensity (1). On average, participants in this study exercised at around 66% of their age-predicted maximal heart rate (calculated utilizing the 220-age equation), which is toward the lower end of the moderate intensity range. However, the equation 208 – 0.7 * age (32) may be a more valid estimate of maximal heart rate in a preadolescent population (22). When examining the average percentage of age-predicted maximal heart rate achieved during the exercise condition using the latter equation (32), participants exercised at a significantly higher relative intensity compared to the 220-age estimation (69.30% ± 3.53 vs. 66.27% ± 3.41, t = 91.6, $p < 0.0001$). This suggests that the 220-age equation likely *underestimated* the relative intensity of the exercise condition, and curiously still did not result in a statistically significant difference in stress hormone levels between groups. While this is well within the 64-76% range, few research studies have examined whether this level of intensity is sufficient to elicit a strong enough release of salivary alpha amylase in preadolescent populations. In order to further our understanding of stress hormone responses to exercise in preadolescent children, future studies should seek to characterize the sympathetic nervous system responses to exercise across a broader range of intensities.

With respect to our second hypothesis, we did not observe any significant relationships between changes in stress hormones and changes in executive function in the exercise condition. However, while not statistically significant, our regression analyses revealed a trend where those with a greater increase in salivary-alpha amylase levels showed a greater reduction in RT on the Eriksen flanker task following exercise. It is possible that the bout of exercise was sufficient to elicit an SNS response in some of our participants, and that those participants experienced improvements in selective inhibition/attention. The fact that not all of our participants exhibited these effects may be related to the issues of exercise intensity discussed previously. Given that these effects were not observed in all of our participants, future studies should further examine these relationships. Specifically, researchers should test this hypothesis using an intensity of exercise that is sufficient to elicit a SNS response in the entire study population.

Although we cannot draw clear conclusions from our data regarding the relationships between exercise, stress hormones, and executive function, other studies have highlighted underlying mechanisms. Several studies have shown that aerobic exercise enhances prefrontal cortex oxygenation acutely (4,12,30). Specifically, a recent review of the literature suggests that light-moderate intensity exercise is associated with a 10-30% increase in cerebral blood flow, with the effects greater in younger compared to older individuals (5). This may indicate that cerebral blood flow and oxygenation are important mechanisms driving acute improvements in executive function following moderate aerobic exercise in preadolescent children. Additionally, work by Hillman et al. (14) suggests that acute improvements in executive function are related to increased neural activity and allocation of attentional resources in a population of preadolescent children. It is worth noting that the intensity of exercise in the aforementioned study was similar to that of this study. Given that we did not observe any changes in the HPA-axis or SNS, we cannot derive any concrete conclusions regarding relations between stress hormone levels and moderate-intensity exercise. Additionally, we did not measure prefrontal cortex oxygenation, blood flow, or neural activity. Therefore, while we cannot directly compare our findings to these previous studies, these are important avenues for future research.

There were several strong aspects of the study design employed. First, the participants completed each condition in a randomized crossover fashion, which allowed them to serve as their own controls. Second, participants completed both conditions at the same time of day in order to control for diurnal fluctuations in the stress hormones of interest. Lastly, the prescribed modality and intensity of the exercise condition were similar to those in other studies that have reported acute improvements in executive function in this population.

While there were strong aspects of this study, there were also several methodological limitations. One limitation was the potential learning effect induced by multiple administrations of the executive function tests, which, while necessary to compute a change score pre-to-post manipulation, may have hindered our ability to detect differences between conditions. Second, although the sample size was similar to that of other studies that observed improvements in executive function following exercise in this population, our sample size of n = 24 was underpowered with regard to detecting changes in stress hormones. However, our sample size was similar to those reported in studies of the effects of aerobic exercise on executive function and stress hormone responses in preadolescent children by Hillman (14) et al. and Mahon (21) et al., indicating a precedent for using these sample sizes in similar studies. Third, while the guardians of each participant were instructed to limit the child's physical activity in the hours prior to each laboratory visit, it is unknown if these recommendations were followed. If the participants engaged in physical activity in the hours prior to each visit, it is possible that this hindered our ability to see differences in executive function between the exercise and resting conditions. Fourth, it is possible that analyzing boys and girls as one group in this study does not account for possible differences in cognitive maturation (2). While we did not have any a priori hypotheses about gender, future work should consider investigating if these effects depend on gender, particularly for those participants approaching adolescence. Fifth, by controlling heart rate utilizing the ACSM age-predicted maximal heart rate (220-age) calculation, our estimation of moderate-intensity activity may have been inaccurate in this preadolescent population. However, note that using the equation 208 – 0.7 * age (32) showed that our estimate may have been an underestimation, therefore suggesting our exercise manipulation should have been sufficient. Sixth, using percentages of age-predicted maximal heart rate is not a gold standard method for estimating relative exercise intensity. While this method is convenient, it would be more precise to have participants perform a maximal oxygen uptake test (VO_{2max}) and prescribe the intensity of the exercise condition as a relative percentage of each participant's $VO_{2max.}$ The decision to use percentages of predicted maximal heart rate was made in order to avoid exposing participants to additional uncomfortable stimuli, which may have altered their stress-hormone responses in the exercise condition. However, the error associated with using percentages of maximal heart rate may have led to exercise prescriptions that were too low in intensity to elicit changes in executive function and stimulate a stress hormone response in some participants. Seventh, while measuring prefrontal cortex activity and oxygenation levels would have allowed us to compare our findings with those of pre-existing literature, we did not measure these variables. Lastly, the average BMI percentile rank of our population categorized them in the 40th percentile for their age and sex, which indicates a healthy weight. Therefore, these findings are not generalizable to populations of overweight or obese children. It is also possible that the healthy participants observed in the study demonstrated a ceiling effect with respect to their performance on the executive function tests.

In conclusion, due to methodological limitations of this study, we cannot definitively determine if stress hormone responses to exercise are related to improvements in executive function in a preadolescent population. However, with regard to attention/inhibition, a greater increase in alpha-amylase levels following exercise was marginally related to decreased RT, suggesting that future work should continue to investigate whether such changes in hormone levels underlie exercise-induced improvements in executive function. Future studies seeking to characterize the stress response to exercise in preadolescent children should consider utilizing more precise measures of exercise intensity and perhaps prescribe exercise intensity at a higher relative percentage of maximal capacity to ensure a statistically significant increase in stress hormone levels due to exercise. Additionally, these studies should seek to study these effects in populations that are sufficiently powered to detect differences in both executive function and stress hormones, as well as examine the impact of gender and weight status. Finally, studies looking to further examine potential mechanisms underlying acute improvements in executive function should consider limiting the number of administrations of such tests in order to avoid a creating a learning effect. Investigating the relationship between stress hormones and executive function in response to exercise has important implications for physical health, mental health, and public policy. A more mechanistic understanding of the relationship between exercise and executive function could further solidify the importance of physical activity and exercise as a part of the school day.

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