The Effect of Exercise on Serum and Salivary Cortisol in Male Children

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ABSTRACT

The purpose of this study is to examine serum and salivary cortisol responses to cycling exercise in male children, 10.6 ± 0.2 yr (mean ± SE). Each child performed a graded exercise test on a cycle ergometer to determine VO_2 max. On a separate day a 30 minute bout of exercise at 70% of VO_2 max was performed. Blood, obtained from a venous catheter, and saliva samples were collected at rest, at 15 and 30 minutes of exercise, and 15 minutes post exercise. The mean serum cortisol level at 15 minutes (7.94 ± 1.43ìg.dl⁻¹) and 30 minutes (8.72 ± 1.77ìg.dl⁻¹) of exercise and at 15 minutes post exercise (8.21 ± 1.59ìg.dl⁻¹) were significantly greater than rest (5.54 ± 0.86ìg.dl⁻¹). The increase in salivary cortisol levels over time approached (P = 0.08), but did not reach significance. However, effect size analysis indicates that increase in salivary cortisol at 30 minutes of exercise (0.64) and 15 minutes post exercise (0.62) was similar to the exchange in serum cortisol at these same two time points (0.72 and 0.66, respectively). Serum and salivary cortisol were correlated (P < 0.05) at 15 minutes of exercise (r = 0.77) 30 minutes of exercise (r = 0.90) and 15 minutes post exercise (r = 0.84), but not at rest (r = 0.46). In conclusion, 30 minutes of sub-maximal exercise at 70% of VO_2 max significantly increased serum cortisol level; and salivary and serum cortisol are correlated during and after exercise.
Keywords: Adrenocortical function, Boys, Glucocorticoid, Saliva, sub-maximal exercise.

INTRODUCTION

Cortisol is the primary glucocorticoid secreted by the adrenal cortex and an important regulatory hormone for blood glucose homeostasis (De Feo *et al*, 1989; Tharp, 1975). In adults the activation of the adrenocortical system by adrenocorticotropic hormone results in an increase in serum cortisol levels during exercise (Farrel, Garthwaite, and Gustafson, 1983). During sub-maximal steady-state exercise performed for at least 60 minutes at an intensity greater than 60% of maximal oxygen uptake (VO_2 max) blood cortisol levels have been reported to increase (Davies and Few, 1973). At higher intensities (70-100% of VO_2 max) and shorter durations (12-30 minutes), increases in plasma cortisol levels have also been reported (Farrel, Garthwaite, and Gustafson, 1983; O'Connor and Corrigan 1987). Salivary glucocorticoid concentration was first reported to be closely related to blood glycocorticoid levels in the late 1950s (Shannon, Prigmore, Brooks and Peller 1959). More recently, salivary cortisol has been confirmed to be a valid, reliable, and noninvasive indicator of the biological active free fraction of serum cortisol levels. Correlations ranging from r = 0.70 – 0.99 between salivary and serum cortisol have been reported in adults at

rest (McCraken and Poland 1989; Walker, Riad-Fahmy and Read, 1978) as well as during sub-maximal steady-state exercise (Bing and Junuen 1991; O'Connor and Corrigan 1987). There are several advantages of assessing salivary rate than serum cortisol. First, salivary cortisol reflects the unbound and biologically active form of circulating cortisol (Vining, McGinley, Maksvyntis and Ho, 1983). Second, salivary cortisol is not affected by changes in cortisol binding globulin (Vining, McGinley, Maksvyntis and Ho, 1983). Finally, saliva is easy to collect, less risky to handle than blood, and due to its noninvasive nature, it may be more appealing to the subject than the collection of blood.

Whereas the effect of exercise on serum and salivary cortisol levels has been established in adults, there are conflicting data on the effect of acute exercise on cortisol levels in healthy children (Garlaschi *et al.*, 1975; Sills and Cerny, 1983; Winter, 1974). Although healthy and diseased children were used in all of these investigations, the data from healthy children can be gleaned from each study. Two of these studies (Garlaschi *et al.*, 1975; Sills and Cerny 1983) report no statistically significant changes in serum cortisol levels as a result of exercise although the data of Garlaschi *et al.*, (1975) indicate a 56% increase in serum cortisol level following exercise. In contrast Winter (1974) reports a significant increase (30%) in plasma cortisol levels in 13 to 17 years old children following exhaustive exercise. Although the exact reason for the discrepancy between these studies is uncertain, there are several factors that are known to influence the response of serum cortisol to exercise. These factors would include the intensity and duration of exercise, the time of day, pre-exercise cortisol level, and whether or not food was ingested prior to exercise (Brandenberger and Follenius, 1975; Davies and Few, 1973; Few, Cashmore and Turton, 1980). More importantly though, these studies raise a question as to whether young children demonstrate a hormonal response to exercise in a manner comparable to adults. Although salivary and serum cortisol have been reported to be correlated at rest in children (Bober *et al.*, 1988; Burke *et al.*, 1985), there are apparently no reports relating serum and salivary cortisol in children during exercise. Since more and more children are participating in endurance sports activity, research examining hormonal responses during this type of activity is warranted. Therefore, the purpose of this study is to examine the effect of 30 minutes of exercise on a cycle ergometer at 70% of VO_2 max on serum and salivary cortisol concentrations; and , to determine the relationship between salivary and serum cortisol in children before, during and after exercise.

PARTICIPANTS AND PROCEDURE

Ten healthy male children were recruited to participate in this study. Their age, height, and weight were (mean $\pm$ SE) 10.6 ± 0.2 yr, 144.2 ± 2.0xcm and 36.4 ± 1.8kg respectively. Parental permission and written informed consent were obtained in compliance with the guidelines established by institutional review board. This study was conducted under the following restrictions: all testing took place within a 3-week period for each subject, no strenuous exercise was allowed 24 hours prior to testing, all testing took place between 1500 and 1800 hours and food and beverage (except water) ingestion was restricted 3 hours prior to testing. On their first visit to the laboratory, a through explanation was given

to the child and parent regarding the nature of the study. Although all children had previously participated in studies in this laboratory, each child was familiarized with the testing apparatus and procedures in an attempt to assess pubertal status, the height and handgrip strength method developed by Backous *et al.*, (1990) was employed. This procedure separates pubertal children (Tanner stages I, II, and III) from pubertal children (Tanner stages IV and V). Right and left hand grip strength was assessed using a hand grip dynamometer (Takei, Japan). Three trials were allotted for each hand and the overall average of the six trials was calculated. The cut off point for pubertal status is 165cm in height and 25kg of grip strength. Nine children met both criteria for the prepubertal category. One child slightly exceeded the cut off point for the grip strength (25.8kg); however, he did not meet the height criteria (158.4cm) for prepuberty.

Graded Exercise Test: On the second day of testing, a graded exercise test was conducted on a cycle ergometer to assess VO_2 max. Depending on the body weight of the subject one of two graded exercise protocols was employed. For those subjects weighing less than 35kg the protocol began at 30W for the first 2 minutes. Thereafter, the work was increased 15W every 2 minutes until maximal effort. Subjects weighing 35kg and above performed the same protocol; however, the starting work rate was set at 50W and increased 15W every 2 minutes. The primary criteria for terminating the graded exercise test was when VO_2 failed to increase (<2.1 ml. $kg^{-1}.min^{-1}$) despite an increase in work rate. In the absence of a plateau in VO_2, additional criteria used to aid in the determination of VO_2 max were the achievement of a $RER > 1.00$, and/or a $HR \pm 10b.min^{-1}$ of the age predicted (220-age) maximal HR. All subjects achieved at least two of these criteria.

VO_2 was assessed continuously during this test using open circuit spirometry. The subjects breathed through a two-way, non breathing valve (Hans Rudolt, model 2600, 49-ml dead space) while wearing a nose clip. Expired air samples were analysed from a mixing chamber using an Applied Electrochemistry SA-3 oxygen analyser (Sunnyvale, CA) and a sensormedics LB-2, Carbon dioxide Analyzer (Yorba Linda, CA). Pulmonary ventilation was measured during inspiration using a Parkinson Cowan dry gas meter. An integrated computer system reported respiratory gas exchange data every 30 seconds. Heart rate was monitored using physio-control Lifepeak – 9 electrocardiograph (Redmond, WA). Ratings of perceived exertion (RPE) were obtained throughout the exercise tests using Borg's 6-20 scale (Borg, 1982). All subjects read a standardized set of instructions recommended for children on the use of the RPE scale (Bar-Or, 1983).

Submaximal Exercise: On the third day of testing the subject assumed a supine position and a registered nurse inserted a 22-gauge Teflon catheter into a forearm or dorsal hand vein for the purpose of obtaining blood samples. The catheter was kept patent with a continuous drip of 0.9% sodium chloride. Following the insertion of the catheter, the subjects rested in a seat in a quiet room for a period of 30 minutes. After the 30 minutes rest period, a 3.0-ml blood and 1.0ml saliva sample were collected simultaneously for the purpose of establishing resting cortisol levels. Immediately after these samples were collected, the subject performed a sub-maximal trial on the cycle erogometer. The first 5 minutes served

as a warm-up period whereby the work rate was gradually brought up to the subject's target work rate (70% of VO$_2$ max). The subject then exercised for 30 minutes at this work rate. VO$_2$ was measured during the warm-up period, the first 3 minutes of steady – state exercise and from 20-23 minutes of exercise RPE and HR were recorded at 3 and 23 minutes of exercise. Blood and saliva samples were obtained at 15 and 30 minutes of steady-state exercise as well as 15 minutes after the exercise was completed following the 30 minutes blood sample, the subject got off the cycle ergometer and sat quietly until the final sample was obtained.

Blood Collection and Storage: To clear the dead space of the catheter and tubing a 2.0ml waste sample was collected and discarded immediately prior to each of the blood samples. Once obtained, blood samples were immediately transferred to test tubes and allowed to clot at room temperature. Prior to clotting approximately 50-70µl of blood were removed using a microhematocrit tube for the purpose of assessing the subject's hematocrit. Once the blood samples were clotted, they were centrifuged at 2500rpm for 20 minutes. Following centrifugation the serum was extracted and stored in a polystyrene tube at -20^{0}C until assayed for cortisol. Serum cortisol (25µl was analysed by radioimmunoassay using a coat-A-count cortisol assay kit with a sensitivity of 0.2µg.dl^{-1}) (Diagnostic products corporation, los Angeles, CA). The samples were measured in duplicate and averaged. The inter-assay coefficient of variation for serum cortisol was 7.9%. The averaged value was then adjusted for changes in plasma volume that occurred over time using the method of Van Beaumont, Greenleaf and Juhos (1972).

Saliva Collection and Storage: The procedure to collect saliva required the subjects to swallow the existing saliva in the mouth and to begin accumulating saliva on the floor of the mouth. A 2 x 1 inch band of parafilm was given after the subject had finished swallowing to stimulate new saliva production (Luisi and Franchi, 1984). After a 2 – minute period of saliva accumulation, the subject expectorated gently into a clean 25ml beaker, the saliva was then transferred to a polystyrene tube and stored at -20^{0}C. To prevent dilution of the samples, fluid intake was restricted 10 min prior to saliva collection. Prior to analysis, the salivary cortisol samples were thawed and centrifuged for 15min at 2500rpm for the purpose of removing mucins and any cellular debris. Salivary cortisol (200µl) was determined by radioimmunoassay using a coat – A – count assay for free cortisol in saliva with a sensitivity of 0.02µg.dl^{-1} (Diagnostic products corporation, los Angeles, CA). Each sample was measured in duplicate and averaged. The inter-assay coefficient of variation for salivary cortisol was 9.2%.

Statistical Analysis: All results are preserved as means ± SE. Two separate one-way repeated measures ANOVA were used to examine alterations in serum and salivary cortisol concentrations over time. Post-hoc analyses were performed using a Newman-Keuls test. Effect size analyses were also performed to examine the magnitude of the change in serum and salivary cortisol levels from rest over time (Cohen, 1988; Thomas, Salazar and Landers, 1991). Pearson product moment correlations were used to examine the relationship of serum cortisol to salivary cortisol at each time point. Statistical significance was set at P $\leq$ 0.05 for all comparisons.

RESULTS AND DISCUSSION

At maximal exercise VO_2 averaged 1.80 ± 0.09 l. min^{-1} or 49.5 ± 1.1 ml. $kg^{-1} min^{-1}$. The mean values for HR, RER, and RPE at this level of exercise were 198.5 ± 2.3 b. min^{-1}, 1.11 ± 0.01 and 15.3 ± 0.7, respectively. During the sub-maximal exercise trial, the percentage of VO_2 max was $69.5 \pm 0.9\%$ at the 3rd and $70.5 \pm 0.9\%$ at the 23rd minute of exercise. VO_2 averaged 34.3 ± 0.6 and 34.8 ± 0.7 ml. $kg^{-1}.min^{-1}$, while HR was 165.0 ± 3.0 and 169.1 ± 3.5 b. min^{-1} at these same time points. Mean RPE was 8.8 ± 0.6 at the 3rd minute and 10.9 ± 0.8 at the 23rd minute. Figure 1 shows the response of serum cortisol levels during sub-maximal steady state exercise. The statistical analysis indicated that exercise significantly elevated serum cortisol levels at 15 minutes (7.94 ± 1.43 µg $.dl^{-1}$ and $30µg.dl^{-1}$), as well as at 15 minutes post – exercise ($8.21 \pm 1.59µg.dl^{-1}$) compared with resting values (5.54 ± 0.86 µg . dl^{-1}). The effect size analysis comparing exercise and post exercise serum samples to the resting sample were 0.64, 0.72, and 0.66 for 15 minutes and 30 minutes of exercise, and 15 minutes post-exercise, respectively.

The results for salivary cortisol response to sub-maximal exercise are presented in figure 2. As a function of time, salivary cortisol tended to increase and approached, but did not achieve statistical significance ($P = 0.08$). Salivary cortisol levels were 0.079 ± 0.013 µg $.dl^{-1}$ at rest, 0.099 ± 0.022 µg. dl^{-1} at the 15th minute of exercise, 0.133 ± 0.035 $µg.dl^{-1}$ at the 30th minute of exercise, and 0.143 ± 0.044 µg . dl^{-1} at the 15th minutes of post exercise. The effect size analyses comparing exercise and post-exercise saliva samples with the resting sample were 0.35, 0.64, and 0.62 for 15 minutes and 30 minutes of exercise, and 15 minutes post-exercise, respectively. Significant correlations were found between salivary and serum cortisol during and after exercise, but not at rest. The correlation at rest was $r = 0.46$; at 15 and 30 minutes of exercise the correlation increased to $r = 0.77$ and $r = 0.90$, respectively. At 15 minutes post exercise the correlation was $r = 0.84$.

The results of the present investigation show that serum cortisol concentration increased by 43% and 57% above resting level at 15 and 30 minutes of exercise, respectively. At 15 minutes post-exercise, serum cortisol was still 48% higher than resting level. In comparison with previous research, the results of the present study are in agreement with those of Winter (1974), who also reports a significant increase (30%) in serum cortisol levels in a group of healthy adolescent children who exercised to exhaustion (15-30 minutes) on a cycle ergometer at a Heart Rate intensity equal to $170 – 180$ b. min^{-1}. Garcaschi *et al.*, (1975) report a large (56%), but no significant increase in serum cortisol level in a group of prepubertal children following 10 minutes cycle ergometry at 50% of VO_2 max. In contrast, Sills and Cerny (1983) report that continuous exercise performed at 50% of VO_2 max for 30 minutes and intermittent exercise performed at 100% of VO_2 max at $1 –$ minute intervals for 30 minutes resulted in no change in serum cortisol levels during or after exercise. The failure of exercise to augment serum cortisol level may be due to several factors including food ingestion prior to exercise (Brandenberger and Follenius, 1975), elevated pre-exercise values (Few, Cashmore and Turton, 1980) and an inadequate exercise stimulus with respect to intensity and duration (Davies and Few, 1973). This study attempts

to control for these factors. Food intake was restricted in the 3 hours preceding the test, and the pre exercise cortisol level was consistent with the circadian rhythm exhibited by cortisol (Kerrigan *et al.*, 1993). This would suggest that cortisol levels were not elevated prior to exercise. In addition, the exercise stimulus was of sufficient intensity and duration to increase in serum cortisol level. It is important to note that the measurement of serum cortisol concentration provides no direct information regarding adrenocortical function. This is to say, it cannot be ascertained whether the increase in serum cortisol levels was due to an increase in cortisol secretion, a decrease in the rate of cortisol removal from circulation, or a combination of the two. However, Davies and Few (1973) examine the rate of cortisol secretion and removal in adults performing exercise at different intensities. Their results indicate that when exercise intensities exceeded 60% of VO_2 max increases in serum cortisol concentration were more related to increases in the rate of cortisol secretion and not to decrease in the rate of removal. If this same information can be applied to children, then the increase in serum cortisol concentration in our study would be primarily due to an increase in cortisol secretion and less likely due to decrease in removal rate.

The mechanism that activates the hypothalamic – pituitary – adrenal axis during exercise is not completely understood. Although cortisol is an important hormone in the maintenance of blood glucose levels, other physiological factors may also stimulate adrenocortical activity during exercise. One of such factors may reside in metabolic disturbances, such as the accumulation of lactate that is sensed by muscle chemoreceptors in the contracting skeletal muscle (Few, Cashmore and Turton, 190). We did not measure blood lactate levels in this study. However, the exercise intensity used in this study was probably at or slightly above anaerobic threshold (AT) for most of the children and as a result, blood lactate levels were probably elevated at least to some extent. Kindermann *et al.* (1982) report that the exercise intensity corresponding to the subjects *Anaerobic Threshold* is associated with increased cortisol levels in adults. The use of exercise intensities relative to VO_2 max rather than AT may also explain the wide variation in serum cortisol responses we observed in our data. Perhaps the subjects who demonstrated little or no change in cortisol level were exercising below their AT while the subjects demonstrating increases in cortisol levels may have been exercising at an intensity above AT.

An alternative to the assessment of serum cortisol is the measurement of salivary cortisol (Bing and Junwen, 1991; McCraken and Poland, 1989; O'Connor and Corrigan, 1987; Port, 1991; Riad-Fahmy, Read and Walker 1983; Vining, McGinley, Maksvyntis and Ho 1983; Vining and McGinley, 1987; and Walker, Riad-Fahmy, and Read 1978). Although salivary cortisol concentration increased by as much as 81% above resting level, statistical significance was not achieved (P = 0.08). The failure to achieve statistical significance could be attributed to the small sample size or the large amount of variability in the salivary data. However, effect size analyses indicates that the increase in salivary cortisol measured at 30 minutes of exercise and at 15 minutes post exercise was similar to the effect sizes observed in the serum data at these same two time points. Significant correlations between the saliva and serum measurements, ranging from r = 0.77 to r = .90, were also noted during and after exercise. These correlations compare favorably with data obtained

from adult-subjects during and after exercise of a similar model, intensity and duration (O'Conor and Corrigan, 1987). These results suggest that the assessment of salivary cortisol may be an acceptable alternative to the measurement of serum cortisol in children during and after exercise. However, the correlation between resting serum and salivary measurements ($r = 0.46$), and the small increase in salivary cortisol at 15 minutes of exercise, suggests that some caution must be used when assessing cortisol in the saliva. The reasons for these findings are not clear, but may be partially attributed to the fact that salivary cortisol is an indicator of free serum cortisol, whereas the serum cortisol measurement was based on total serum cortisol (Vining, McGinley, Maksvyntis and Ho, 1983). In addition, the sensitivity of the salivary assay may have also contributed to the poor correlation at test and the small increase at 15 minutes of exercise.
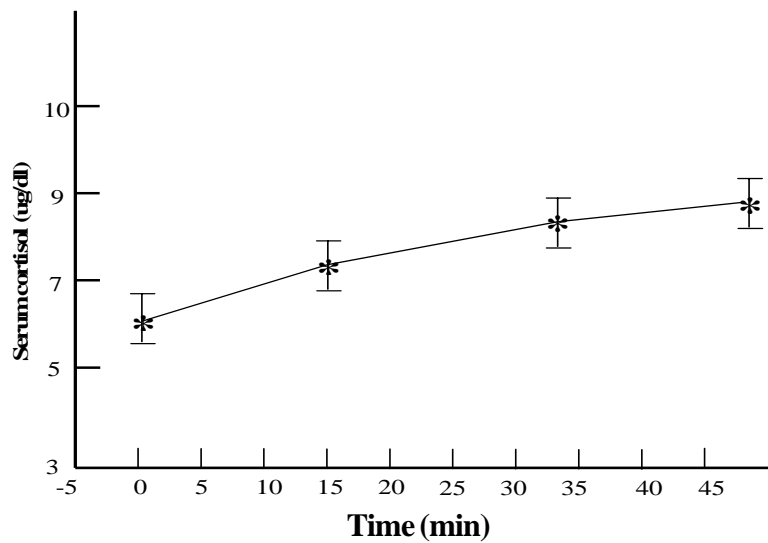

Figure 1: Mean ± Se serum cortisol concentration before, during, and after exercise (*P < 0.05 compared with resting cortisol levels).
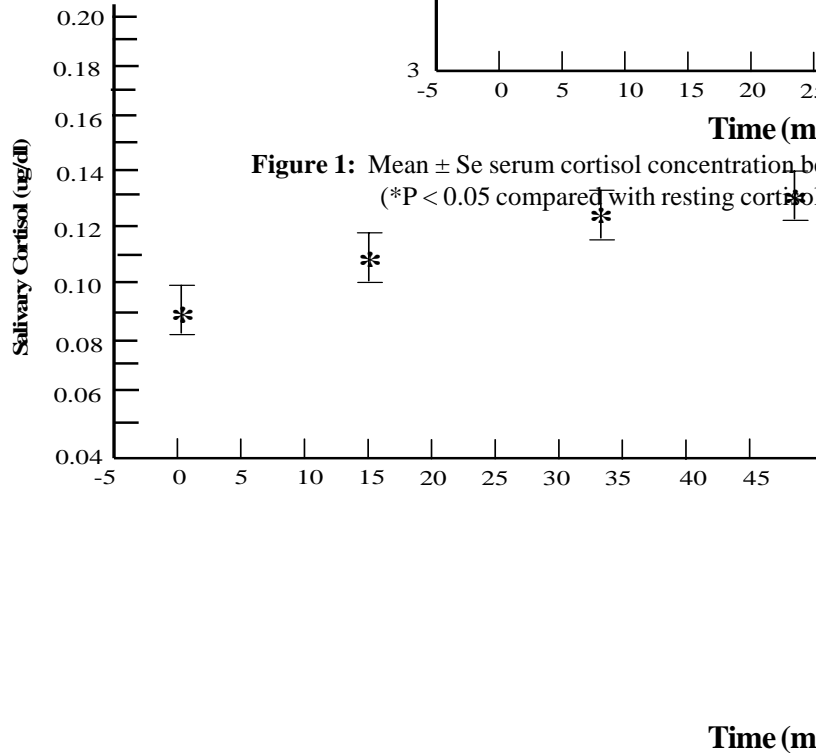
Time (min)

Figure 2: Mean ± SE salivary cortisol before, during and after exercise

CONCLUSION

The purpose of this study was to examine serum and salivary cortisol responses to cycling exercise in male children. Each child performed a graded exercise test on a cycle ergometer to determine VO_2 max. Thirty (30) minutes of exercise at 70% of VO_2 max significantly elevated serum cortisol concentrations in male children during and after exercise. Salivary cortisol was also elevated, but the increase was not statistically significant. Serum and salivary cortisol were significantly correlated during and after exercise, but not at rest. Although the assessment of salivary cortisol may be a more appealing procedure in this age group since it is noninvasive, more research is needed to understand the extent to which salivary cortisol can be used as an alternative means of predicting serum cortisol responses to exercise. Future studies should also address the effects of maturity, gender, and different exercise intensities and durations on serum and salivary cortisol responses in children.

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