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SALIVARY CORTISOL AND TESTOSTERONE RESPONSES TO RESISTANCE AND PLYOMETRIC EXERCISE IN 12-14 YEAR OLD BOYS

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Running Head: Hormonal responses to exercise in children

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Abstract

This study examined changes in salivary testosterone and cortisol following a resistance and a plyometric exercise protocol in active boys. Using a cross-over experimental design, 26 peri-pubertal (12-14 years) soccer players performed two exercise trials in random order, on separated evenings, one week apart. Each trial included a 30 min control session followed by 30 min of either a resistance or plyometric exercise. Saliva was collected at baseline, post-control (i.e. pre-exercise), 5 and 30 min post-exercise. There were no significant differences in the baseline hormonal concentrations between trials or between weeks ($p>0.05$). A significant effect for time was found for testosterone ($p=0.02$, $\eta^2_p=0.14$), which increased from pre- to 5 min post-exercise in both the resistance ($27\pm5\%$) and plyometric ($12\pm6\%$) protocols. Cortisol decreased to a similar extent in both trials ($p=0.009$, $\eta^2_p=0.19$) from baseline to post-control and then to 5 min post-exercise, following its typical circadian decrease in the evening hours. However, a significant protocol-by-time interaction was observed for cortisol, which increased 30 min following the plyometrics ($+31\pm12\%$) while continued to decrease following the resistance protocol ($-21\pm5\%$). Our results suggest that in young male athletes, multiple modes of exercise can lead to a transient anabolic state, thus maximizing the beneficial effects on growth and development, when is performed in the evening hours.

Key words: Resistance exercise, plyometrics, children, anabolic response, catabolic response

Introduction

Exercise can induce acute changes in anabolic hormones, such as testosterone, and catabolic hormones such as cortisol (Eliakim et al. 2009). In adults, both testosterone and cortisol have been found to increase as a response to resistance exercise (Borer 2003; Crewther et al. 2006, Kraemer and Ratamess 2005; Smilios et al. 2014). Specifically, resistance exercise protocols have been shown to result in both testosterone and cortisol increases measured 5, 15 and 30 min post-exercise (Kraemer et al. 1990, 1993). On the other hand, Beaven et al. (2008) reported small or no changes in salivary cortisol along with elevated or stable testosterone levels following resistance exercise. Others also reported an attenuated cortisol response to resistance exercise as compared to endurance exercise, which has been shown to elicit substantial cortisol increases 30 min post-exercise in adults (Smilios et al. 2003).

The acute testosterone and cortisol responses to exercise in children are less consistent. In a series of studies in adolescent boys Pullinen et al. (1998, 2002, 2011) reported no change or a very small increase in serum testosterone following resistance exercise. Likewise, Pilz-Burstein et al. (2010) reported a decrease in testosterone following a simulated fighting day (4 consecutive fights) among 12–17 years old male Taekwondo fighters, while serum cortisol significantly increased following the same simulation (Pilz-Burstein et al. 2010). On the other hand, serum cortisol has been shown to significantly decrease following a 3-hour gymnastics training session in the evening hours in 10–11 years old gymnasts and controls. The authors suggested that in children, cortisol follows its typical circadian pattern unaltered by the exercise intervention (Rich et al. 1992).

The literature is scarce regarding hormonal responses to plyometric exercise in general, and even more so in children. In adults, Beaven et al. (2008) found small increases in both

salivary testosterone and cortisol in semi-professional rugby players immediately after four bouts of jump squat and box squat exercises while Cadore et al. (2013) reported significant increases in serum testosterone and cortisol following 100, 200, and 300 jumps in young adult rugby players.

Late childhood and adolescence are characterized by wide hormonal fluctuations. Little is known about the hormonal response to different exercise protocols during this period of rapid growth. Yet, perhaps because of the rapid changes that take place during this period, it has been suggested to be a sensitive period during which exercise may have important positive effects (Cameron et al. 2002). For example, resistance and plyometric training are both recommended for youth due to their osteogenic effect (Zouch et al. 2014) without clear evidence on their functional benefits. In addition, testosterone has been linked to gains in muscle strength in adults. Beaven et al. (2008) recently suggested that strength gains following resistance exercise may be enhanced when prescribed according to salivary testosterone response. Thus, understanding the hormonal response to exercise and the resultant hormonal milieu may be important in enhancing the outcomes of training in children. Therefore, the purpose of this study was to examine the acute changes in salivary testosterone and cortisol following a resistance and a plyometric exercise protocol in active, peri-pubertal boys.

Materials and Methods

Participants

This study and all related procedures received ethical clearance from the University Research Ethics Board. Twenty-six peri-pubertal (12-14 years of age) soccer players participated in this study. Participants were recruited from a local soccer club. The researchers met with potential participants and their parents and provided them with a detailed description of the study. A

consent form was then completed and signed by the parents of those boys who agreed to participate in the study. An assent form was signed by the boys.

Experimental Design

A cross-over experimental design was used over a three week period in January 2015. During the three weeks of this study, participants were invited to one familiarization and two experimental trials, separated by one week. The familiarization session was used to ensure all participants can safely perform plyometric and resistance exercise as per the National Strength & Conditioning Association guidelines (Faigenbaum et al. 2014; Lloyd et al. 2011). During the experimental trials, each participant first completed a 30 min resting (control) session, which did not include any exercise. Participants were then randomly assigned to perform either a resistance or a plyometric exercise protocol, followed by a 30 min recovery session. Participants who performed the resistance protocol during the first experimental trial performed the plyometric training during the second trial, which was separated by one week, and vice versa. During the 30 min rest pre- and post-exercise recovery sessions the participants underwent anthropometric assessment and were asked to complete questionnaires and/or do quiet activities.

To ensure consistency and account for diurnal fluctuation in hormones, all trials and, thus, saliva collection were scheduled between 18:30 and 21:00 hours. Salivary cortisol and testosterone are characterized by similar diurnal fluctuations with peak concentrations in the morning and decreased levels in the evening in both children and adults (Dabbs 1990; Gröschl et al. 2003; Hayes et al. 2010; Rosmalen et al. 2005; Törnå 2002). While many studies examining the effects of exercise on hormonal levels are performed in the morning hours, during peak concentrations, the present study was planned for the evening hours for two reasons: a) It was expected that, if exercise increases hormonal concentrations, as previously demonstrated in

adults, that effect would be more apparent (and possibly of greater physiological significance) during low basal levels; and b) Most of pediatric training takes place in the afternoon/evening hours. Thus, conducting the study in the evening hours increases its construct validity. All participants were instructed to restrict eating at least 2 hours prior to each trial.

Exercise Protocols

Each exercise trial began with a standardized 15 min dynamic warm-up for both conditions. The plyometric protocol consisted of a 30 min session that included jumps, hops, and lunges (Table 1). The resistance protocol consisted of a 30 min session that included open kinetic chain exercises such as lunges, squats, and multi-directional movements using external loads (Table 1). Each exercise session was conducted and monitored by exercise professionals within the research team to ensure proper form and safety during the performance of each exercise. Ratings of perceived exertion (RPE) were used to monitor participants' subjective effort to indirectly control for differences in the training load between protocols. Specifically, boys were instructed to work at an intensity eliciting at an 8 out of 10 on a modified RPE scale that has been validated for children (Eston et al. 1994, 2009). Ratings of perceived exertion were recorded at the end of each set in the circuit and averaged to a final sessional score for each participant.

Measurements

Body mass (kg) was assessed using a weight scale, height (cm) and seated height were measured using a stadiometer. Skinfold thickness was measured using constant pressure skinfold calipers (Harpenden Skinfold Caliper, British Indicators, Body Care, UK). The sum of two (triceps and subscapularis) skinfold thickness measurements were then used to calculate the percent body fat, based on the equations by Slaughter et al. (1988). Somatic maturity was determined by calculating years from age of peak height velocity (aPHV) from height, sitting height, leg length

(height minus sitting height), and body mass, using sex-specific regression equations determined by Mirwald et al. (2002). The same investigator completed all anthropometric assessments.

Saliva collection and analysis

Each participant provided two pre-exercise saliva samples, one at baseline (i.e. pre-control) and one at post-control (i.e. immediately pre-exercise), and two post-exercise saliva samples, at 5 and 30 min, respectively. One milliliter of unstimulated whole mixed saliva was collected from each participant using salivette swabs (SARSTEDT Inc., Quebec, Canada). Participants moisten/chew lightly on the swab for one minute. After sampling, the swabs were placed directly into plastic tubes. All saliva samples were transported in a cooled box and stored at -20 °C until assayed.

Saliva samples were centrifuged at 3000xg for 10 min and only the supernatant was assayed. All enzyme immunoassays were carried out on NUNC Maxisorb plates. Cortisol (R4866) and testosterone (R156/7) antibodies and corresponding horseradish peroxidase conjugates were obtained from C. Munro of the Clinical Endocrinology Laboratory, University of California, Davis. Steroid standards were obtained from Steraloids, Inc. Newport, Rhode Island. Plates were first coated with 50 µl of antibody stock diluted at 1:10,000 in a coating buffer (50 mmol/L bicarbonate buffer pH 9.6) for the testosterone assay while cortisol antiserum was diluted at 1:8500 for the cortisol assay. Plates were stored for 12–14 h at 4 °C. 50 µl wash solution (0.15 mol/L NaCl solution containing 0.5 ml/L of Tween 20) were added to each well to rinse away any unbound antibody, then 50 µl phosphate buffer (pH 7.0) per well was added. The plates were incubated at room temperature for 30 min for testosterone, and 2 hours for cortisol before adding controls or samples.

For each hormone, two quality control samples at 30% and 70% binding (the low and high ends of the sensitive range of the standard curve) were prepared. For all assays, 50 µl

testosterone, or cortisol horseradish peroxidase conjugate were added to each well, with 50 µl of standard, sample, or control for testosterone or cortisol. Testosterone plates remained incubated for 2 h at room temperature while cortisol plates remained incubated for 1 h. Next, the plates were washed with 50 µl wash solution and 100 µl of a substrate solution of citrate buffer, H₂O₂ and 2,2'-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid] was added to each well and the plates were covered and incubated while shaking at room temperature for 30–60 min. Plates were then read with a single filter at 405nm on the microplate reader (Titertek multiskan MCC/340). Blank absorbances were obtained, standard curves generated, a regression line was fit to the sensitive range of the standard curve (typically 40 – 60 % binding) and samples were interpolated into the equation to give values in pg or ng per well. All samples were assayed in duplicate and ran in the same batch. The testosterone assay has been previously validated (Carré et al. 1996). The intra- and inter-assay CVs were 6.5% and 6.8% for salivary testosterone and 7.8% and 6.5% for salivary cortisol.

Statistical Analysis

Descriptive statistics were calculated for relevant study variables. Inspection of statistical outliers and examination of statistical assumptions was conducted. A series of paired t-tests were used to examine differences in baseline hormone concentrations taken prior to participation in each of the exercise protocols. A paired t-test was also used to examine whether there was a difference in the RPE between exercise protocols. Mixed-model ANOVAs were then used to examine differences in salivary concentrations of testosterone and cortisol between exercise protocols (protocol effect) and the within exercise protocols (time effect). The effect size (partial eta squared), as well as power estimates were also calculated. As per Cohen's (1973) suggestion, 0.01 is considered a 'small' effect size, 0.03 represents a 'medium' effect size and 0.14 a 'large'

effect size. An alpha level of <0.05 was used as the criterion for significance for all statistical analyses, which were conducted using SPSS version 19 for Windows (SPSS Inc., USA).

Results

Participants' descriptive characteristics are presented in Table 2. There was no significant difference in the RPE between exercise protocols (8.0 ± 0.6 and 7.8 ± 0.6 for resistance and plyometric, respectively). Likewise, there were no significant differences in the baseline salivary concentrations of testosterone and cortisol taken prior to participation in each of the exercise protocols.

There was a significant time effect for testosterone ($p=0.02$; power=0.79) but no exercise protocol effect. Moreover, interpretation of the effect size estimates ($\eta^2_p=0.14$) suggests a large effect size. Specifically, testosterone increased from pre- to 5 min post-exercise in both the resistance ($27 \pm 5\%$) and plyometric ($12 \pm 6\%$) protocols, with no difference between protocols and no protocol-by-time interaction (Figure 1). Testosterone levels returned to near baseline values 30 min post-exercise.

A significant ($p=0.009$; power=0.85) time effect was also found for cortisol, with the interpretation of the effect size estimates ($\eta^2_p=0.19$) suggesting a large effect size. As shown in Figure 2, cortisol decreased to a similar extent in both trials from baseline to post-control ($-14 \pm 7\%$ vs $-16 \pm 8\%$ for resistance and plyometric, respectively), and from post-control to 5 min post-exercise ($-12 \pm 8\%$ vs $-21 \pm 5\%$ for resistance and plyometric, respectively), following its typical circadian decrease in the evening hours. Most importantly, a significant protocol-by-time interaction was observed for cortisol, which increased 30 min following the plyometric ($+31 \pm 12\%$) but continued to decrease following the resistance protocol ($-21 \pm 5\%$).

Discussion

To our knowledge, this is the first study to examine the acute exercise-induced changes in testosterone and cortisol following two exercise protocols, resistance and plyometric, in young, active boys. Our findings suggest that despite the diurnal decrease in cortisol, plyometric exercise in the evening hours can result in elevated salivary cortisol. Testosterone transiently increased significantly from pre- to 5 min post-exercise as a response to both the resistance exercise protocol and the plyometric protocol, indicating a potential anabolic effect.

The post-exercise immediate increase of testosterone following the resistance protocol agrees with previous studies in adults (Crewther et al. 2006; Kraemer et al. 2005; Smilios et al. 2014). On the other hand, the response to our resistance protocol was of a higher magnitude than what was previously found in adolescent boys by Pullinen et al. (1998, 2002, 2011), who reported either no change or a very small increase in testosterone following resistance exercise. This difference in magnitude could be attributed to the dynamic multi-segmental exercises using multiple muscle groups in our protocol, as opposed to the isolated and movement-assisted devices used in previous studies (Pullinen et al. 1998, 2002, 2011)). The 5 min post-exercise increase in testosterone following our plyometric protocol also agrees with a study in young adult rugby players, which reported increases in serum testosterone in response to jumping exercises (Cadore et al. 2013). To the authors' knowledge, there are no studies on the hormonal responses to plyometric exercise in children or adolescents. Therefore, our results demonstrating increased testosterone levels immediately following exercise agree with previous studies on the effects of resistance training in adults and adolescents and extend these previous findings to pre- and early-pubertal boys. Furthermore, our study extends previously described effects of

resistance exercise to plyometric exercise. That is, testosterone transiently increases in boys following both, resistance and plyometric exercise.

Contrary to the typical adult response (Cadore et al. 2013; Kraemer et al. 1993, 2005), cortisol levels gradually decreased from baseline to 5 min post-exercise to a similar extent in both trials. The first part of this response, i.e. the decrease in salivary cortisol during the resting control period may simply reflect the typical diurnal decrease of cortisol in the evening. However, contrary to our hypothesis, there was an unexpected further decrease in cortisol 5 min post-exercise. It is possible that the time of the experiment strongly influenced these results. That is, despite the physical exertion, which was expected to result in increased cortisol levels, the latter continued to decrease, in line with the typical diurnal changes. It is also possible that the continued decrease in cortisol reflects a maturity-related insensitivity to stress for this age group, as previously suggested in children (Rich et al. 1992). The latter found cortisol to decrease after 3 hours of training in the evening hours in a sample of male gymnasts and controls, similar in age to our sample. According to these authors, gymnastics training, which combines resistance with plyometrics, was not sufficient to stimulate higher cortisol secretion in this age group, specifically during the evening hours (Rich et al. 1992).

Furthermore, in view of the consistent decrease in cortisol during the control period and immediately following both exercise protocols, the significant interaction showing a latent increase in cortisol 30 min following the plyometric exercise is surprising. Previous studies in adults have shown that following short-duration high intensity exercise, plasma cortisol does not increase immediately, but rather, following 15-20 min (Buono et al. 1986; Kindermann et al. 1982). In the present study, the exercise session duration was 45 min (15 min warm-up + 30 min plyometric training) and consisted of short, high intensity plyometric exercises. Thus, it is

possible that the delayed cortisol increase observed in the present study following the plyometric exercise reflects an adrenocortical response to intense, short-duration bouts of exercise, in line with the previous studies in adults (Buono et al. 1986; Kindermann et al. 1982). Unfortunately, we did not obtain saliva samples beyond 30 min post exercise and therefore cannot comment further on the pattern of the cortisol response during recovery.

The present study has strengths and limitations. The cross-over design allowed all participants to complete both exercise protocols thus reducing inter-individual variability. In addition, the soccer players who participated in the study were similar in age, somatic maturity, physical activity, training and anthropometric characteristics, which resulted in a very homogeneous sample. However, in this study we did not measure other metabolic or endocrine markers such as androgen receptor, so a mechanistic interpretation of the findings cannot be provided. Finally, since experiments were carried out in the evening, while both testosterone and cortisol levels are low, the results may not be generalized to the morning hours, when these hormonal levels are high.

In summary, our findings indicate that both the resistance and plyometric exercise protocols resulted in salivary testosterone transient increases from pre- to post-exercise. Despite the diurnal decrease in cortisol, plyometric exercise in the evening hours resulted in elevated cortisol. This suggests that in young male soccer players, exercise may facilitate an acute state of anabolism by changing the anabolic to catabolic balance, especially when performed later in the day, when cortisol's secretion is at its nadir. However, the duration of this state, especially given the increase in cortisol after plyometrics, is not clear. This can be encouraging for coaches to consider scheduling exercise training for this population later in the day (i.e. after school). Finally, these results provide preliminary evidence that in children both resistance and

plyometric exercise can lead to a transient state of anabolism, which may have potential long-term functional benefits. However, the long-term implications of these changes on muscle anabolic processes in children require further research.

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Table 1. Exercises included in each exercise protocol.

Resistance protocol		Plyometric protocol	
Exercise	sets x repetitions	Exercise	sets x repetitions
One legged squat	3 x 12	Drop jump	3 x 12
Lunges	3 x 12	Power Step Up	3 x 12
Sumo Squat	3 x 12	Repeated Long Jump	3 x 3 x 4
Step Ups	3 x 12	Jump Lunge	3 x 12

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Table 2. Descriptive characteristics of the participants (mean $\pm$ standard deviation).

Variables	Participants (N=26)
Age (years)	12.3 $\pm$ 0.8
years from age of Peak Height Velocity	-1.06 $\pm$ 0.7
Height (cm)	158.4 $\pm$ 8.0
Body Mass (kg)	47.0 $\pm$ 9.1
Body Mass Index	18.5 $\pm$ 2.4
Percent Body Fat (%BF)	17.7 $\pm$ 5.6
Weekly energy expenditure (METs)	82.5 $\pm$ 28
Training experience (years)	8 $\pm$ 1
Training volume (hours/week)	6 $\pm$ 1

Figure Legends

Figure 1. Salivary testosterone responses to both exercise protocols in young boys .

Note: *indicates significant ($p < 0.05$) increase from post-control (i.e. immediately pre-exercise) to 5 min post-exercise.

Figure 2. Salivary cortisol responses to both exercise protocols in young boys.

Note: * indicates significant ($p < 0.05$) decrease from previous time point; # indicates significant ($p < 0.05$) exercise protocol by time interaction, reflecting the increase in cortisol following plyometric exercise but not resistance exercise.

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