ACUTE ENDOCRINE RESPONSES TO PLYOMETRICS VERSUS RESISTANCE EXERCISE IN CHILDREN

by

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Abstract

The purpose of this study was to examine the acute hormonal responses to a bout of resistance versus plyometric exercise in young male athletes. Specifically, changes in salivary cortisol, testosterone and testosterone-to-cortisol ratio from pre- to post-exercise between the two different exercise protocols were examined. Twenty-six peri-pubertal active boys participated in this cross-over study, completing two exercise sessions. During each session, participants first completed a 30 min control period, which did not include any exercise, and then was randomly assigned to perform a 45 min of either a resistance exercise or a plyometric exercise protocol. All participants crossed over to perform the other exercise protocol during their second exercise session, a week later. Four saliva samples during each protocol were taken at: baseline, pre-exercise, 5 min post-exercise and 30 min post-exercise. Significant increases in testosterone values were reported 5 min post-exercise following the resistance protocol, but not the plyometric protocol. Both exercise protocols resulted in significant cortisol decreases overtime, as well as significant testosterone-to-cortisol ratio increases. The post-exercise increases in salivary testosterone and testosterone-to-cortisol ratio followed the typical exercise induced anabolic response seen in adults. However, the post-exercise decrease in salivary cortisol was different than the typical adult response indicating an insufficient stimulus for this age group maybe due to their stage of the biological development. Thus, in the adolescent boys, exercise appears to change the anabolic to catabolic balance in favor of anabolism.
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CHAPTER 1 – Introduction

1.1 Rationale

Resistance training refers to a specialized method of conditioning whereby an individual is working against a wide range of resistive loads, such as body weight, weight machines, free weights, elastic bands and medicine balls, to enhance health, fitness and performance (Lloyd et al. 2014). Over the past 10 years, resistance training has had increased exposure and popularity, which combined with a recent concern for decreasing youth fitness levels, has led to a closer examination of the efficacy of resistance training in youth (Lloyd et al. 2014). Plyometrics on the other hand, are exercises that involve muscles exerting maximum force in short intervals of time, with the goal of increasing both speed and power (Donald, 1998). This type of exercise training focuses on learning to move from a muscle extension to a contraction in a rapid or "explosive" manner (Donald 1998). Due to its high impact, plyometrics is a highly osteogenic exercise, thus it is important to use in youth (Zouch et al. 2014).

Resistance training can induce acute changes in numerous growth-related hormones, mainly those in the hypothalamic-pituitary-gonadal (HPG) axes. Although exercise is known to stimulate anabolic components of testosterone, it also leads to an increase in catabolic hormones such as cortisol (Eliakim et al. 2009). Testosterone is believed to be the major promoter of muscle growth and subsequent increase in muscle strength in response to resistance training in men (Vingren et al. 2010). Cortisol, on the other hand, increases protein degradation and decreases protein synthesis in skeletal muscle (Viru & Viru 2006). However, increased cortisol levels may also have a functional influence on anabolic processes, thus the balance between exercise-induced
hormonal changes may provide new insights into quantification of different types of exercise training (Pilz-Burstein et al. 2010).

The acute cortisol response to exercise generally reflects a stress response and is related to the intensity of exercise (Inder et al. 2006). In adults, cortisol has been observed to increase as a response to a session of resistance exercise (Crewther et al. 2006; Kraemer et al. 2006). On the other hand, the observed cortisol changes in a study by Beaven et al. (2008) consisted of uniform decreases, whereas testosterone of the matching salivary sample either increased slightly or stayed constant. In youth, significant increases in testosterone and cortisol have been reported in junior weightlifters following a training session (Fry et al. 1993; & Kraemer et al. 1992). Likewise, resistance exercise has been shown to increase testosterone in moderately trained youth (Eliakim & Nemet 2008). In contrast, a series of studies by Pullinen et al. (2011, 2002) on the acute testosterone response to resistance exercise demonstrated either no change or a very small post-exercise increase in testosterone. In an earlier study, Pullinen et al. (1998) found testosterone to increase in adolescent boys and young men following a session of resistance exercise. However, the boys’ pre- and post-exercise testosterone levels were lower than those observed in men and the increase in the boys was relatively small. In addition to that, Pullinen et al. (2011, 2002) demonstrated an increase in testosterone concentrations following resistance exercise to exhaustion in men, but not in adolescent boys.

The results in regards to plyometric training are limited and are only in adults. In a study by Beaven et al., small increases in salivary testosterone and cortisol were observed in response to the same volume of different jump exercises (Beaven et al. 2011).
2011). As well, post-exercise increases were observed in testosterone after 4 sets of squat exercise with 90 seconds of rest between sets for both adult groups (Kraemer 1998).

It terms of timing, hormone concentration has been measured pre-, post-, and 30 minutes post-exercise. According to Kraemer et al. (2005), anabolic hormones such as testosterone have been shown to be elevated during 15–30 minutes post-resistance exercise providing an adequate stimulus is present. High volume exercise protocols of moderate to high intensity tend to produce the greatest acute hormonal elevations. Moreover, strength protocols have failed to elicit a significant cortisol response whereas endurance protocols elicited more substantial acute elevations through 30 minutes post-exercise (Smilios et al. 2003). Kraemer et al. (1990, 1993) have shown that high-intensity strength exercise sessions that engage large muscle groups with short rests lead to testosterone increases, but this is not the case with exercise of moderate volume and long rest periods between sets (Kraemer et al. 1990, 1993). The same was shown for serum cortisol, with increases only after high volume exercise protocol at 0, 5, and 15 min post-exercise. However, all the above studies are in adult populations. In children, serum cortisol decreased 30 min following a 3-hour gymnastics training session of moderate intensity indicating that cortisol followed its typical circadian pattern unaltered by this type of training, which is a combination of resistance and plyometric exercise (Rich et al. 1992).

The testosterone-to-cortisol (T/C) ratio has been suggested as an indicator of the anabolic/catabolic status of skeletal muscle during resistance training (Hakkinen et al. 1989). Either an increase in testosterone, a decrease in cortisol, or both would indicate a potential state of anabolism. In adults, periodised, higher-volume programs have been
shown to produce a significantly greater increase in T/C ratio than a low-volume, single-set program (Marx et al. 2001). However, the use of post-exercise T/C ratio remains questionable (Kraemer & Ratamess 2005) while there are limited data in children and adolescents. Rich et al. (1992) found that the T/C ratio significantly increased 30 min after a 3-hour gymnastics training session of moderate intensity in 10-11 year old gymnasts and controls despite the significant drop in serum cortisol.

To sum up, the inconsistent testosterone and cortisol post-exercise responses in children and adolescents may reflect the wide hormonal fluctuations, which are characteristic of this period of rapid growth (Pullinen, 1998, 2002, 2011). In addition, there are limited results in regards to the hormonal changes after resistance exercise, and even more limited in regards to plyometric exercise in youth. The purpose of this study is to examine the differences in the acute hormonal responses to a single bout of resistance versus plyometric exercise in young active boys at pre- and post-control periods as well as immediately post- and 30 minutes post-exercise.
CHAPTER 2 – Review of Literature

Resistance training refers to a specialized method of conditioning whereby an individual is working against a wide range of resistive loads to enhance health, fitness and performance (Lloyd et al. 2014). Five forms of resistance training include the use of body weight, weight machines, free weights (barbells and dumbbells), elastic bands and medicine balls (Lloyd et al. 2014). Plyometric, also known as "jump training" or "plyos", are exercises based on having muscles exert maximum force in short intervals of time, with the goal of increasing both speed and power. This training focuses on learning to move from a muscle extension to a contraction in a rapid or "explosive" manner, for example with specialized repeated jumping. Plyometric exercises are primarily used by athletes, especially martial artists and high jumpers, to improve performance, and are used in the fitness field to a much lesser degree (Donald 1998, Behm et al. 2008).

Traditionally, interest in the hormonal adaptations to resistance training has focused on the anabolic hormones that are involved in tissue growth or repair (testosterone), the catabolic stress response (cortisol), or the anabolic-catabolic relationship (testosterone-to-cortisol ratio). Although exercise is known to stimulate anabolic components of testosterone, it also leads to an increase in catabolic hormones, such as cortisol (Eliakim et al. 2009). Thus, it was suggested that the balance between exercise-induced hormonal changes may provide new insights into the quantification of training and competition loads, and contributes in the development of a sport-specific conditioning program (Pilz-Burstein et al. 2010). This is really important, taking into account the benefits of resistance and plyometric exercise for children and adolescents,
including improved muscular endurance, as well as increased bone mineral density, maturation growth, decrease in the severity of sport injuries, rest blood pressure, improved body composition and psychosocial skills (Behm et al.2008).

2.1 Testosterone response to exercise

Testosterone is the most important androgen in humans that regulates several bodily functions including libido, metabolism, immunity, muscle development and bone health. In males, Leydig cells in the testes synthesize testosterone. In females, the ovaries and adrenal glands synthesize a much smaller amount of testosterone. Testosterone secretion is controlled by GNRH (gonadotropin-releasing hormone), which is released by the hypothalamus in pulses. These pulses stimulate the pituitary gland to secrete LH (luteinizing hormone). LH causes the enzymatic conversion of cholesterol into testosterone in the Leydig cells (Beers et al. 1999, Borer 2003).

Resistance training can induce acute changes in numerous growth-related hormones, mainly those in the hypothalamic-pituitary-gonadal (HPG) axes such as testosterone (Vingren et al.2010). The initiation of the HPG axis, which ultimately leads to increased testosterone release, is caused either by direct nervous stimulation of the hypothalamus by the Central Nervous System (CNS) or by reduced feedback inhibition on the hypothalamus by testosterone (Vingren et al. 2010). In muscle, testosterone stimulates protein synthesis (anabolic effect) and inhibits protein degradation (antiacatabolic effect). Combined, these effects account for the promotion of muscle hypertrophy by testosterone. In fact, testosterone is believed to be the major promoter of
muscle growth and subsequent increase in muscle strength in response to resistance training in men (Vingren et al. 2010).

Anabolic hormones such as testosterone have been shown to be elevated 15–30 minutes following resistance exercise providing an adequate stimulus is present. Protocols high in volume (sets and repetitions during a single session), moderate to high in intensity, using short rest intervals, and stressing a large muscle mass tend to produce the greatest acute hormonal elevations of testosterone compared with low-volume, high-intensity protocols using long rest intervals (Kraemer et al. 2005). Thus, the acute testosterone response to resistance exercise in adults varies depending on the intensity or volume of training (Crewther et al. 2006; Kraemer et al. 2005).

Generally, resistance exercise has been shown to acutely increase total testosterone concentrations in most studies in men (Smilios et al. 2013). According to Smilios et al. (2013), the acute response to traditional strength training has been shown to be volume dependent because greater responses are observed as the number of sets increases. Beaven et al. (2011) indicated that exercise designed to improve maximal strength via morphological adaptation (i.e., hypertrophy inducing bouts of moderate load, high volume and short rest periods), generally produces larger relative increases in testosterone than those designed to enhance strength through neural adaptation (maximal strength type bouts of heavy load, low volume and long rest periods). Training that combines both power and strength (power defined as the ability of a muscle to exert force to overcome the most resistance in one effort and strength as the amount of work performed per unit of time) stimulus has been reported to be superior to more
conventional weight training bouts in actualizing strength and power gains (Beaven et al. 2011).

Pullinen et al. (2011, 2002) has shown an increase in testosterone concentrations following maximum resistance exercise in men. Moreover, regarding whether changes are attenuated in older subjects who remain physically active, few data are available on the responses of an entire ensemble of anabolic and catabolic hormones to a single heavy resistance exercise (HRE) stimulus in younger and older physically active men (Bunt et al. 1986). Kraemer et al. (1997) examined the acute responses of total and free testosterone to a single bout of heavy resistance exercise in younger versus older men and found that testosterone increased after four sets of 10 Repetition Maximum (RM, defined as the most weight someone can lift for a defined number of exercise movements.) squat exercise with 90s rest between sets for both 62 and 30 year old men. Beaven et al. (2008) identified large individual differences in testosterone response to four distinct resistance exercise protocols pre, post, and 30 min post exercise in elite adult male rugby players. The results of that study indicated a trend towards a post-exercise increase in testosterone, although that increase was small and not significant. The observed testosterone of the matching salivary sample either increased slightly or stayed constant.

Free testosterone has also been shown to increase by 25% in young women following acute resistance exercise (6 sets of 10 RM squats with 2-min rest intervals), however no changes have been observed following resistance exercise in middle-aged and elderly women (Hakkinen et al. 2000).

In summary, in adults, especially in men, testosterone acutely increases following a session of resistance exercise. However, there are few data regarding the hormonal
response to plyometric exercise. In a study by Beaven et al. (2011), small increases in salivary testosterone were observed in response to the same volume of different jump exercises in 19-21 year old male rugby players over a 4-week period (8 training bouts). These authors reported hormonal responses to 4 exercise bouts indicating that acute testosterone responses are enhanced after a complex training bout. A small, clear increase in testosterone was observed in the strength-power bout relative to the power–power bout immediately after exercise (Beaven et al. 2011).

Changes in the testosterone concentrations after single sessions of endurance and strength training were measured in well trained men, as evident from blood samples for testosterone measurements before, immediately after, and 2 hours after the training sessions. The mean testosterone concentration increased during both the strength and endurance training session, returning to the pre-training level 2 hours later (Jensen et al. 1991). That longer recovery period suggests that exercise-induced increases in testosterone might occur in a rebound fashion later into recovery (90 or 120 min) after certain heavy resistance exercise protocols (Kraemer et al. 1990). Thus, when examining hormonal responses to exercise, the timing of sampling may be important. However, most studies rarely examine post-exercise hormone concentrations at more than one time point. In Kraemer’s study (1999), blood was obtained before, immediately after, and 5, 15, and 30 min after exercise at rest before and after training for analysis of total and free testosterone (Kraemer et al. 1999). Raastad et al. (2000) also collected blood samples before, and every 15 min for the first hour after exercise, examining the acute hormonal responses in male power-lifters. As expected, the testosterone concentration was higher during and for 1 hour after the 100% protocol as compared to the 70% protocol.
All the above studies and results refer to adult groups, both males and females. Over the past 10 years, resistance exercise training has had increased exposure and has become popular; which, in turn, has led to its acceptance among adults as a method to improve certain aspects of physical fitness. That, combined with a recent concern for youth fitness, has led to a closer examination of the efficacy of resistance training for youth (Pullinen et al. 1998, 2002, 2011). Pullinen et al. (1998) examined the post-exercise testosterone increases in young and adult male athletes following single sessions of 4 different half-squatting exercise protocols. The post-exercise serum concentrations of testosterone were significantly lower in the young than the adult participants in every exercise unit. This may suggest a reduced sympathetic nervous activity in the younger subjects compared to the adults in response to exhausting resistance exercise (Pullinen et al. 1998). In two later studies, Pullinen et al. (2011, 2002) demonstrated an increase in testosterone concentrations following resistance exercise to exhaustion in men, but not in adolescent boys. The lack of response, or lower response in boys compared with men may be explained by less synchronized regulation of the HPG axis in the boys (Crewther et al. 2006) although in at least one of Pullinen et al. (2002) studies, resting testosterone levels were similar to those of men. These findings are consistent with an early study, which reported that a resistance exercise session resulted in an increase in testosterone in moderately trained young adults (Fahey et al. 1976), but interestingly, no such increase was detected in untrained adolescents.

Most of the above studies found either no change or a very small testosterone increase following resistance exercise in adolescent boys. However, following a session of simulated Taekwondo fighting, testosterone levels (blood samples were taken) seemed
to decrease in male adolescent fighters, whereas in females, no change was observed in testosterone (Pilz-Burstein et al. 2010). Additionally, Kraemer et al. (1992) demonstrated that in 14–18-year-old weightlifters, testosterone concentrations increased following a single weight-lifting session. In fact, the testosterone response was higher in athletes with more than two years of experience, compared with those with less experience, although there was no age difference between the two groups. Kraemer et al. (1992) suggested that in children, testosterone may affect neural pathways which, in turn, affect children’s trainability and performance following resistance training. In view of the general lack of hypertrophic effects of resistance training in children, this purported role of testosterone should be considered. Nevertheless, there is no evidence that testosterone affects the neural adaptations to resistance training in youth nor in adults (Viru & Viru 2005).

In summary, in adolescents, the post-exercise testosterone response is less consistent than what has been observed in adults. Several studies have demonstrated an increase in testosterone concentrations following a session of resistance exercise in adolescent males, although this increase is lower than observed in men and may simply reflect changes in plasma volume (reductions) as well as maturation effect.

2.2 Cortisol response to exercise

Cortisol is a hormone that belongs to a family of steroid hormones known as glucocorticoids. It’s secreted by the adrenal cortex, which is located in the adrenal glands that sit bilaterally atop the kidneys. Cortisol is the main glucocorticoid in humans that affects every cell in the body. In particular, glucocorticoids released in the body send feedback to the brain and influence the release of corticotropin-releasing hormone (CRH)
and adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal glands to secrete cortisol. The rise in cortisol secretion follows ACTH release with a 15 to 30 min delay (Thibodeau et al. 1999, Borer 2003, Beachle & Earle 2000). Cortisol is a stress hormone having both metabolic and anti-inflammatory functions. It stimulates gluconeogenesis and lipolysis, while it also inhibits the production of numerous inflammatory factors. In relation to resistance exercise, its catabolic role is of interest. Cortisol increases protein degradation and decreases protein synthesis in skeletal muscle. It can play a role in tissue remodeling in that the catabolic effect provides free amino acids increasing the amino acid pool available for renewal of protein structure (Viru & Viru. 2005). Therefore, increased cortisol levels may have functional importance for anabolic processes.

The acute cortisol response to exercise generally reflects a stress response and is related to the intensity of exercise (Inder et al. 2006). In adults, most studies report an increase in cortisol following a session of resistance exercise, with similar increases in men (Crewther et al. 2006, Kraemer et al. 2006). In adolescents, increased cortisol levels have been observed following resistance exercise, but relative to adults, it is not clear whether the increase is comparable. Kraemer et al. (1997) examined the acute responses of cortisol to a single bout of heavy resistance exercise in younger versus older men. No differences between young and old men were observed for cortisol concentrations at any time point and both groups responded with a similar pattern of cortisol increase. In contrast, Beaven et al. (2008) observed a consistent cortisol decrease after four distinct resistance exercise protocols from pre- to post-, and 30 min post-exercise in rugby players, aged 22-26 years old.
Resistance exercise has been shown to acutely increase cortisol concentrations in most studies in men. In fact, some strength protocols have failed to elicit a significant cortisol response whereas hypertrophy and endurance protocols performed by the same group of participants elicited more substantial acute elevations up to 30 minutes post-exercise (Smilios et al. 2013). However, there are few data regarding the hormonal response to plyometric exercise. In a study by Beaven et al. (2011), small increases in salivary cortisol were observed in response to the same volume of different jump exercises.

Cortisol has been observed to respond to high-volume strength exercise (10-RM sets) with short rest periods between sets, but not to strength exercise of moderate-volume (5-RM sets) and long rest periods between sets (Kraemer et al. 1993). Only the high total work-exercise protocol demonstrated significant increases in serum cortisol at 0, 5, and 15 min post-exercise, indicating that the duration of the force production and the length of the rest periods between sets are key exercise variables influencing increases in serum cortisol.

A higher increase in serum cortisol level in boys observed by Pullinen et al. (2002) may reflect a trend for a stronger stress reaction in the boys than in men. Two studies of highly trained 17-yr-old boys demonstrated acute increases in cortisol following sessions of intense resistance exercise (Fry et al. 1993, Kraemer et al. 1992). An attenuated increase was observed in less experienced boys, but no comparison was made with an adult group. Whether the greater cortisol increase in the more experienced boys reflects the ability for greater effort is not clear. Likewise, Pilz-Burstein et al. (2010) reported that among young male Taekwondo fighters (12-17 years old), a simulated
fighting day (4 consecutive fights) resulted in an increase in cortisol for both, male and female adolescent fighters (Pilz-Burstein et al. 2010).

In summary, in adolescents, the post-exercise cortisol response is less consistent than what has been observed in adults. The inconsistent cortisol response in adolescence may be a reflection of the wide hormonal fluctuations, which are characteristic of this period of rapid growth.

2.3 Testosterone-to-Cortisol ratio response to exercise

The testosterone-to-cortisol (T/C) ratio has been suggested to be indicator of the anabolic/catabolic status of skeletal muscle during resistance training (Hakkinen et al. 1989). Either an increase in testosterone, a decrease in cortisol, or both would indicate a potential state of anabolism (Kraemer et al. 2005). Periodised, higher-volume programs have been shown to produce a significantly greater increase in the T/C ratio than a low-volume, single-set program (Marx et al. 2001). However, this appears to be an oversimplification and an indirect measure of the anabolic/catabolic properties of skeletal muscle. Some studies have shown changes in the T/C ratio during strength and power training, and this ratio has been positively related to performance improvements (Alen et al. 1988).

In fact, the T/C ratio indicates the balance of the body’s anabolic to catabolic state and generally it is an exercise intensity index. Specifically, the T/C ratio is considered to reflect states of anabolism and tapering off when it is high and, inversely, states of catabolism and overreaching when it falls by 30% or more. Moreover, a single bout of exercise induces transient changes in the anabolic-catabolic balance, depending on the
intensity and duration of the exercise bouts. Repeated heavy endurance exercise without a sufficient period of recovery can cause a persistent disturbance in this balance (Maso et al. 2003). On the other hand, serum T/C ratio was found not significantly increased following one session of karate training in young elite karate athletes (Boostani et al. 2013).

Moradi et al. (2012) examined concurrent endurance- resistance versus resistance-endurance exercise on the T/C ratio in non-athletes. Blood samples were taken 5 min before and one hour after each exercise protocol. The post-exercise T/C ratio increase was greater in the resistance-endurance group compared to the endurance-resistance group, indicating a higher anabolic response after the endurance-resistance exercise (Deakin 2004). In another study, however, the endurance-resistance group had a lower T/C ratio compared to the resistance-endurance group (Moradi et al. 2012). Thus, the response of the T/C ratio to exercise remains questionable because its examination seems to be quite limited, especially in adolescents during resistance and even more during plyometric exercise.

2.4 Specific Objectives and Hypotheses

In this study, we specifically:

- examine the changes in salivary concentration of salivary cortisol, testosterone and T/C ratio from pre-exercise to 5 min and 30 min post-exercise in young active boys;
- investigate whether the exercise-induced changes of these hormones differ with the type of exercise; resistance versus plyometric.
Based on the above literature we hypothesize that: a) salivary testosterone would significantly increase immediately following both types of exercise protocols returning to baseline levels 30 min post-exercise; b) salivary cortisol would not change following both types of exercise; c) the T/C ratio would follow the same pattern as testosterone, thus increasing immediately after both types of exercise protocols and returning to baseline levels 30 min post-exercise.
CHAPTER 3 – Methodology

3.1 Participants

This study and all related procedures received ethical clearance from the Brock University Research Ethics Board (file #13-305). After receiving permission from parents and coaches, young active boys were recruited from a soccer club in Niagara Region, Ontario. Twenty-six 11 to 14 years old competitive soccer players participated in this study.

3.2 Study design

This crossover exercise study was conducted in early January. A small group of researchers from Brock University met with the young athletes and their parents prior to data collection. During this meeting, the parents and coaches were provided 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.

Over the three-week duration of this study, participants were invited to one familiarization and two exercise sessions a week apart in their soccer club. During these sessions, each participant first completed a 30 min control period, which did not include any exercise, and then was randomly assigned to perform a 45 min of either a resistance exercise or a plyometric exercise protocol. Participants who performed the resistance training session first performed the plyometric training during the second exercise session a week later while participants who performed the plyometric training session first
performed the resistance protocol a week later, during the second session. This design was chosen so that all participants performed both protocols acting as their own controls.

Figure 1 presents the testing protocol for both exercise sessions. 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 then completed the 45 min exercise protocol followed by two post-exercise saliva samples, at 5 min and 30 min, respectively. During the 30 min control session the participants underwent anthropometric assessment and were asked to complete questionnaires and/or do quite activities (Figure 1). Additionally, all participants’ last meal was consumed at least 2 hours before the first saliva collection.

**Figure 1.** Data collection timeline during exercise session 1 (top box) and exercise session 2 (bottom box).
3.3 Exercise Protocols

The plyometric exercise session consisted of 15 minutes of warm-up and 30 minutes of plyometric exercises. Exercises included jumps, hops and bounds of varying volumes and intensities. The plyometric training protocol was structured in a manner which allows participants to safely progress through the various exercises, minimizing stress placed on joints and allowing for optimal power-associated muscular adaptations to occur. The resistance exercise session consisted of 15 minutes of warm-up and 30 minutes of resistance exercises. Resistance exercises included lunges, squats and various resistance band/medicine ball exercises, at varying volumes and intensities. The resistance exercise protocol was structured in a manner, which allows participants to safely progress through the various exercises, minimizing stress placed on the joints, allowing for optimal strength associated muscular adaptations to occur.

Prior to each session, participants were shown how to safely conduct the various exercises in a controlled and safe manner by the research team. Below is a detailed description of both protocols (Table 1). Each of the protocols was repeated 3 times during the session. In order to check if the intensity of the protocols were comparable, we used the Compendium of Energy Expenditures for Youth (see Appendix A), to calculate each protocol’s metabolic equivalent (MET). The Compendium provides a coding scheme that links a five-digit code representing the specific activities performed in various settings with their MET. Modelled after the adult Compendium of Physical Activities (Ainsworth et al. 2000), the Compendium of Energy Expenditures for Youth contains a list of over 200 activities commonly performed by youth and their associated MET intensity levels (Ridley et al. 2008). Using the exercise tracking guide, we added the MET of the
exercises included in each protocol (Table 1). Both our protocols’ total expenditure was 8.0 MET.

**Table 1.** Exercises included in each exercise protocol.

<table>
<thead>
<tr>
<th>Resistance protocol</th>
<th>Plyometric protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of Perceived Exertion: 7.8/10</td>
<td>Rate of Perceived Exertion: 7.8/10</td>
</tr>
<tr>
<td>One legged squat x 10</td>
<td>Drop jump x 10</td>
</tr>
<tr>
<td>Lunges x 12</td>
<td>Power step ups x 10</td>
</tr>
<tr>
<td>Steps ups x 12</td>
<td>Repeated long jumps x 5 (3 sets)</td>
</tr>
<tr>
<td>Sumo squats x 12</td>
<td>Jump lunges x 10</td>
</tr>
</tbody>
</table>

### 3.4 Experimental Measurements

**Descriptive Measures**

Body mass (kg) was assessed using a weight scale, height (cm) and seated height were measured using a stadiometer. Body mass index (BMI) was calculated by dividing the subject’s weight (kg) by their height squared (m²). Relative body fat (%BF) was estimated from skinfold thickness with constant pressure skinfold calipers (Harpenden Skinfold Caliper, British Indicators, Body Care, England). The sum of two (triceps and subscapularis) skinfold thickness measurements were then used to calculate the percent body fat, using the Slaughter et al. (1988) equations. The same investigator completed all anthropometric testing in order to eliminate inter-observer variability.

Somatic maturity was determined by calculating years from age of 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).
All participants completed a physical activity questionnaire to determine current weekly physical activity energy expenditure. This was documented by using their weekly physical activity MET values estimated from the Godin-Shephard Leisure Time Exercise Questionnaire (Godin & Shephard 1985) (Appendix C). This questionnaire asks participants to indicate the number of times in a typical week that they engage in light, moderate and strenuous physical activity for at least 15 consecutive minutes. These frequency scores are further multiplied by known energy consumption values in order to obtain weekly MET scores. This questionnaire has demonstrated adequate validity and reliability in children (Scerpella et al. 2002; Sallis et al. 1993). An in-house developed training questionnaire was also used to record any additional sport activities the boys performed regularly (Appendix B).

**Saliva Collection**

One milliliter of unstimulated whole mixed saliva was collected from each participant using salivette swabs (SARSTEDT Inc., Quebec, Canada). Subjects moisten/chewed lightly on the swab for one minute. After sampling, the swabs were placed directly into plastic tubes.

To ensure consistency and account for diurnal fluctuation in hormones, all testing sessions and, thus, saliva collection were scheduled between 18:30 and 21:00 hours. Both salivary cortisol and testosterone are characterized by a standard fluctuation during the day. Cortisol exhibits peak levels in the morning while it drops in late afternoon (Gröschl et al. 2003, Horrock et al. 1990, Kiess et al. 1995, Rosmalen et al. 2005, Price et al. 1983, Pruessner et al. 1997). Salivary cortisol in children fluctuates in the morning from 3.3 to 26.6 nmol/L while it decreases in the evening (0 to 7.1 nmol/L) (Price et al. 1983,
Rosmalen et al. 2005, Törnhage 2002). One more study verifies the descending way that cortisol follows throughout the day in children. Gröschl et al. (2003), reported morning values of salivary cortisol are 24.7 nmol/L, SD (8.5) whereas at noon the circadian effects drop to 8.0 nmol/L, SD (4.0). In the evening exhibited the lowest values at 1.7 nmol/L, SD (1.4). On the other hand, continuous stress may alter HPA function caused of high levels of cortisol in saliva and serum throughout the day. As well, salivary cortisol peaks 30-45 minutes after awakening independently of the time of day or the total time slept (Rosmalen et al. 2005, Pruessner et al. 1997). Testosterone is also characterized by a diurnal rhythmicity with peak concentrations in the morning and decreased levels in the evening (Dabbs 1990, Hayes et al. 2010, Plymate et al. 1989, Riad-Fahmy et al. 1983). Early morning samples of testosterone being more than double compared with those of late evening (Riad-Fahmy et al. 1983).

Salivary Cortisol and Testosterone Analysis

All saliva samples were transported on portable fridge bag and stored at -20 °C until assayed. Saliva was centrifuged at 3000xg for 10 minutes 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 standards, samples, or controls.

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 (Carre 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.
3.5 Statistical Analysis

Univariate normality (i.e., means, standard deviation, skewness and kurtosis) of the data was inspected. Descriptive statistics were calculated for relevant study variables. Inspection of statistical outliers and examination of statistical assumptions was conducted. A series of t-tests were subsequently used to check for differences in the percent hormonal changes from each time point to the next between groups i.e., those who first performed the plyometric protocol versus those who started with the resistance protocol. A second series of paired t-tests were also used to examine the percentage changes between the resting periods of session 1, during which the participants completed the anthropometric evaluation and questionnaires, and those of session 2, during which they did quiet activities, such as playing card games, in order to stay inactive for a while. Since no differences were found between resting periods, the data of both sessions were pooled by type of exercise for further analysis.

Six separate one-way, repeated measures ANOVAs were used in order to examine the time effect in the salivary hormonal concentrations for each exercise protocol. In these analyses, the dependent variable was the salivary concentration of cortisol, testosterone or T/C ratio, and the independent variable was the time of saliva collection (pre- to post-control, 5 min post- and 30 min post-exercise). The effect size (partial eta squared) as well as power estimates were also calculated. As per Cohen’s suggestion, 0.1 is considered a 'small' effect size, 0.3 represents a 'medium' effect size and 0.14 a 'large' effect size. Percent changes were calculated as \[\frac{(T2 - T1)}{T2} \times 100\]. 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).
Chapter 4: Results

Participants’ baseline characteristics, including age, years from age of peak height velocity, height, body mass, BMI, %BF and activity data are presented in Table 2.

Table 2. Baseline characteristics of the participants (mean ± standard deviation).

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

The series of t-tests that were used to check for differences in the percent hormonal changes between the resting periods of each session indicated no significant differences between each pair of resting periods for the testosterone, cortisol or T/C ratio changes either from baseline to post-control or from 5 min to 30 min post-exercise. Thus, the data of both sessions were pooled for the repeated measure ANOVA analysis testing the time effect for each exercise protocol.
Figure 2 shows the results for salivary testosterone levels for both types of resistance and plyometric exercise protocols. The repeated measures ANOVA analysis demonstrated a statistically significant time effect for testosterone following the resistance exercise protocol ($F (3,75) =3.50, p=0.02$, power=0.76, $n^2_p=0.12$) but not the plyometric protocol ($F (3,66)=1.75, p=0.17$, power=0.44, $n^2_p=0.07$). Interpretation of effect size estimates suggests a medium to large effect size. Post-hoc, pairwise analysis showed a significant 27% increase in testosterone from the post-control to 5 min following resistance exercise, which was then decreased by 13% to near baseline levels at 30 post-exercise.

Figure 2. Salivary testosterone responses to both exercise protocols in young boys. Note: *indicates $p<0.05$ from post-control (i.e. immediately pre-exercise) to 5 min post-exercise for the resistance exercise.
Figure 3 shows the salivary cortisol changes across all time points from pre-control to 30 minutes post-exercise. The repeated measures ANOVA analysis demonstrated a statistically significant time effect with salivary cortisol significantly decreasing after for both the resistance \( F(3,75)=5.38, p=0.002, \text{power}=0.92, \rho^2=0.17 \) and the plyometric protocol \( F(3,69)=3.70, p=0.030, \text{power}=0.66, \rho^2=0.14 \). Interpretation of effect size estimates suggests a relatively medium effect size. An examination of the post-hoc analyses demonstrated that cortisol significantly decreased after resistance exercise, and this decrease was consistent across time (22% from baseline to post-control, 7% at 5 min post-exercise and by 24% at 30 min post-exercise). Likewise, cortisol decreased significantly when participants engaged in plyometric exercise with significant decreases noted from baseline to post-control (29%), and from post-control to 5 min post-exercise (20%). However, cortisol increased by 27%, thus returning to pre-exercise levels at 30 min post-exercise.
Figure 3. Salivary cortisol responses to both exercise protocols in young boys. Note: *indicates p<0.05 from post-control (i.e. immediately pre-exercise) to 5 min post-exercise for resistance exercise; **indicates p<0.05 from 5 min to 30 min post-exercise for resistance exercise; # indicates p<0.05 from baseline to post-control for plyometric exercise; ## indicates p<0.05 from post control (i.e. immediately pre-exercise) to 5 min post-exercise for plyometric exercise).

Figure 4 presents the T/C ratio changes during both exercise protocols. The repeated measures ANOVA analysis demonstrated a statistically significant main effect for T/C ratio for both the resistance (F (3,75)= 6.84, p=0.000, power=0.97, $n^2_p=0.21$) and the plyometric protocol (F (3,66)=5.60, p=0.007, power=0.82, $n^2_p=0.20$). Interpretation of effect size estimates suggests a relatively large effect size. Specifically, when engaged in resistance exercise, participants’ T/C ratio was significantly increased by 39% at 5 min post-exercise with a further 4% increase 30 min later. When engaged in plyometric training, the ratio was also increased significantly by 37% and then slightly decreased (7%) at 30 min post-exercise.
Figure 4. Salivary testosterone-to-cortisol (T/C) ratio changes over time for both exercise protocols in young boys. Note: *indicates p<0.05 from post control (i.e. pre-exercise) to 5 min post-exercise for resistance exercise; **indicates p<0.05 from post control (i.e. pre-exercise) to 30 min post-exercise for resistance exercise; #indicates p<0.05 from post control (i.e. pre-exercise) to 5 min post-exercise for plyometric exercise; ## indicates p<0.05 from post control (i.e. pre-exercise) to 30 min post-exercise for plyometric exercise.
Chapter 5. Discussion

The aim of this study was to determine whether there are any differences in the acute exercise-induced changes in cortisol, testosterone and their ratio between two different exercise protocols. To our knowledge, there is no study that examined the acute hormonal responses to both plyometric and resistance exercise in youth aged 11-14 years old. As per our hypothesis, testosterone significantly increased from pre- to post-exercise as a response to the resistance but not to the plyometric exercise protocol. Opposite to our hypothesis, and the typical for adult response (Beaven et al. 2008, 2011, Kraemer et al. 1993, 2006), cortisol showed a gradual decrease from baseline to 30 min post-exercise for both the resistance and plyometric protocols. Taking into account the typical diurnal decreases of cortisol in the evening as well as other training (i.e., inadequate exercise load) and developmental (i.e., attenuated stress response in prepubescent boys) factors, could give alternative interpretation to these cortisol decreases (20-29%). The T/C ratio, however, displayed a gradual, significant increase from pre- to up to 30 min post-exercise (37-39%) following both exercise protocols.

5.1 Salivary Testosterone

Exercise can stimulate a short-term testosterone release, which may amplify muscle growth (Beers et al. 1999, Borer 2003). Stimulation of beta-adrenergic receptors encourages testosterone synthesis and release in a dose dependent fashion — the more stimulation, the more synthesis. Thus, increases in plasma concentrations of testosterone are relative to the intensity of exercise. In other words, the harder someone exercises, at least with resistance exercise or metabolic conditioning, the more testosterone is released.
(Beers et al. 1999, Borer 2003). However the type of exercise also matters. For example, extended bouts of endurance exercise seem to suppress testosterone. Plasma testosterone increases occur in men after various forms of exercise, as long as that exercise is of high intensity. On the other hand, women respond to intense exercise with very small and/or delayed testosterone increases, or even no testosterone increases at all (Beers et al. 1999, Borer 2003). There are sparse and inconsistent data in children.

One of the main findings of this study is the elevation of testosterone following the resistance exercise bout while testosterone’s response to the plyometric protocol was non-significant. This testosterone response to the resistance protocol agrees with previous studies in adults and trained youth (Beaven et al. 2011, Eliakim & Nemet 2008, Smilios et al. 2013). On the other hand, Pullinen et al. (2011) found that a 40% RM protocol resulted in testosterone increases only in men, giving them an anabolic advantage compared to women and adolescent boys. This study demonstrated lower testosterone in boys compared to men, clearly showing a maturation effect on testosterone (Pullinen et al. 2011). Lower testosterone concentrations have been reported previously in adolescents (15 year old) versus adults (25 year old) after four different half-squatting exercises suggesting that adolescents may have reduced sympathetic nervous activity when compared to the adults in response to resistance exercise (Pullinen et al. 1998). Likewise, college male athletes had a post-exercise increase in testosterone after weight training while high school students did not. This difference could indicate a maturation effect although it is also possible that was caused by a higher training intensity by the college students group. Possible inexperience and lack of aggressiveness towards the exercise may have resulted in less vigorous weight training sessions in the high school students
group suggesting that in youth, training type and intensity may have a more prominent effect on testosterone than maturation (Fahey 1975). Therefore, our resistance protocol load was probably more vigorous for the young soccer players than the plyometric load resulting in a higher testosterone response. However, the estimated MET value of our protocols was the same, and the RPE values showed no difference in the perceived intensity between the resistance and the plyometric protocol. This is supported by a previous study, where the testosterone responses to heavy resistance protocols differ in magnitude and duration, even when the identical total work was performed (Pullinen et al. 2011). In our study, the same duration of resistance exercise resulted in a 27% increase in post-exercise testosterone while the plyometric protocol led to an attenuated (16%), non-significant increase in testosterone. Nevertheless, the post-exercise testosterone levels (resistance) decreased 30 minutes later to near baseline values.

5.2 Salivary Cortisol

The second finding of this study was that cortisol significantly decreases from pre- to post- exercise. This result is not in agreement with adult studies, which have consistently shown a post-exercise increase in cortisol (Kraemer et al. 1997, Pilz-Bulstein et al. 2010; Pullinen et al. 2002). Furthermore, previous pediatric studies have reported higher post-exercise cortisol increases in boys than in men, speculating that this could be reflective of a stronger stress reaction due to anxiety, a lesser adaptation to resistance exercise or prolonged diurnal increases throughout the day after fighting simulation (Pilz-Bulstein et al. 2010; Pullinen et al. 2002).
High intensity resistance exercise is associated with acute increases in plasma cortisol concentration. The most dramatic increases occur when rest periods are short and total volume is high. Cortisol responses to increased training volume are variable and depend on the specific training protocol and its diurnal variations (Beachle & Earle 2000, Borer 2003, Thibodeau et al. 1999). It is also important to distinguish between acute and chronic cortisol release; when muscle glycogen concentrations are low, cortisol is released and fuel use shifts toward protein or fat so that judicious use is made of the remaining glucose but during long-term, excessive exercise cortisol will encourage fat synthesis and storage, along with provoking appetite (Beachle & Earle 2000, Borer 2003, Thibodeau et al. 1999). Endurance trained individuals typically have a higher cortisol response, while resistance trained individuals have a higher testosterone response. Secretion of cortisol is elicited at exercise intensities between 80% and 90% of VO2max, which means that in this case, we are referring to endurance training (Beachle & Earle 2000, Borer 2003, Thibodeau et al. 1999).

On the other hand, decreased salivary cortisol levels were reported in adults by Beaven et al. (2008) following different strength protocols, as high as 33% post-exercise. Smilios et al. (2003) also found a 22% decrease in serum cortisol following some of their exercise protocols while other protocols resulted in approximately 27% increase in serum cortisol. A later study by Smilios et al. (2013) indicated that in hypertrophy-type resistance exercise of maximum velocity cortisol concentrations remained unchanged, while at protocols performed at sub-maximal velocity, cortisol decreased in accordance with its circadian rhythm. The differences in the results could be attributed to different exercise loads. As a stress hormone cortisol increases more after the execution of higher
intensity protocols combined with high metabolic stress as compared to lower intensity protocols (Smilios et al. 2013). The exercise protocols used in our study could therefore be characterized of low intensity, as both led to constant decreases of cortisol which could be interpreted as low or no stress inducing for athletic boys of this age group. In fact, individual cortisol values showed that those who are practicing hockey, baseball or basketball as second sport, did not reach high values of cortisol (lower than 1 ng/ml most times), so one could argue that they are coping with stress better than boys playing only one sport. However, based on the Compendium of Energy Expenditures for Youth (Ridley et al. 2008), the estimated expenditure value of the protocols was 8.0 MET, and the participants rated their perceived exertion as hard (7.8/10) for both the resistance and the plyometric protocol.

The other argument, of a maturity related insensitivity to stress, is supported by Rich et al. (1992), who found that cortisol decreased after 3 hours of training in pre-pubescent male gymnasts. According to the authors, the training was not intense enough to stimulate a higher cortisol secretion in this age group, which simply followed its typical circadian pattern. Thus, the total load of exercise in combination with the saliva collection time is an important determinant of the magnitude of the adrenocortical response in younger populations. This interpretation supports our results in that the total load of exercise was probably an inadequate stress stimulus for our participants and their training background. This argument can be further supported by Papadopoulos et al. (2013), who found no differences in salivary cortisol levels and no change in its variability between a typical, non-competitive week and a week leading to a significant competition in a group of young swimmers indicating a low (or immature) catabolic
response in this age group. Additionally, no differences in the post-exercise cortisol values lower than 1 ng/ml were observed by Beaven et al. (2011). According to these authors, the time of evening collection, i.e. at the nadir of cortisol’s circadian rhythm, has a potential effect on the cortisol response to exercise. Our samples were all collected within a 2-hour time frame between 1830 and 2030 hours. This time frame is similar to the Rich et al. (1992) study suggesting that cortisol secretion followed the typical adult circadian change, seemingly unaltered by exercise training.

5.3 Testosterone-to-cortisol ratio

The T/C ratio significantly increased from pre- to post- exercise following both the plyometric and the resistance exercise protocol, and this was because of a higher testosterone increase compared to the cortisol decrease. In line with these results, Maso et al. (2014) showed higher anabolic (testosterone) than catabolic (cortisol) variability in rugby players after a game. Others on the other hand, found no significant differences in the T/C ratio after both a resistance-endurance and an endurance-resistance exercise protocol in adults although the second protocol led to a higher anabolic response (Moradi et al. 2012).

Our results also agree with Rich et al. (1992), who found that the T/C ratio increased significantly from 1630 to 2000 Hours for both the young controls and gymnasts. Although they did not report changes in testosterone, the authors attributed this ratio increase to the circadian decrease of cortisol. Nevertheless, the increased T/C ratio indicates a favourable state of anabolism as a result of both exercise protocols in the
young boys, mainly due to the catabolic influences being reduced in the evening as seen by the decreased post-exercise evening cortisol.

5.4 Strengths and Limitations

The present study has several strengths. First, the cross-over design allowed all participants to complete both exercise protocols ensuring equal sample sizes. Second, the soccer players who participated in the study were matched for age, somatic maturity, physical activity, training and anthropometric characteristics, which resulted in a very homogeneous study group. The second strength was the consistent season and the time of sampling. All samples were collected in mid-January to avoid seasonal variations. However, it is not certain that these results would be similar had we run this experiment in the summer, spring or fall so they cannot be generalized. In terms of sample size, although relatively small it was adequate for a relatively high statistical power (0.80 – 0.97) for testosterone, cortisol and the T/C ratio. A limitation of the study was that not all of the participants had long-term soccer experience, which is a factor that could affect their stress and their familiarity with the exercise protocol. In addition, based on the rate of perceived exertion and the cortisol results the intensity for our protocols was probably inadequate to elicit a stress response.

5.5 Conclusion

Our findings indicate that resistance exercise led to a significant increase in salivary testosterone from pre- to post-exercise that was not seen with the plyometric exercise protocol. Salivary cortisol, on the other hand, showed a non-typical post-exercise
decrease in both the resistance and plyometric protocols. Finally, the testosterone-to-cortisol ratio followed the typical exercise induced anabolic response seen in adults resulting mainly from the decreased evening cortisol. This suggests that in adolescent boys, exercise can change the anabolic to catabolic balance leading to an acute state of anabolism as long as 30 minutes post-exercise, especially when performed later in the day when cortisol’s secretion is decreased.

5.6 Significance of the Study and Take Home Message

The relationship between stress and exercise in athletes is an area of interest, which is clouded by conflicting results. The present study tried to provide answers pertaining to this association. The findings of the present study show the acute hormonal responses to two different types of exercise protocols in young soccer players. Exercise-induced stress seems to be low in these pre-pubertal athletes leading to a favorable anabolic response during exercise.

5.7 Future recommendations

Further research is needed to examine the effects of a variety of exercise protocols on salivary markers of stress in young athletes. Lastly, a future study is needed to examine the relationship between stress and exercise in trained versus untrained adolescent athletes. This way we would be able to better understand the difference in the stress response among young athletes and how the adapt to different loads. Finally, using precise for the participants in terms of intensity protocols, was found to be major factor in their responses.
References


8) Boostani MH, Boostani MA, Fathollahzadeh E and Abbariki Z (2013). The acute response of testosterone, cortisol and the ratio of testosterone to cortisol serum in one


40) Papadopoulos E. Interaction between stress and immunity during a week leading to competition in young athletes. Brock University 2013


APPENDICES
APPENDIX A

The Compendium of Energy Expenditures for Youth
(energy cost values derived from child measures are in bold)

<table>
<thead>
<tr>
<th>Code</th>
<th>Activity</th>
<th>MET</th>
<th>Rationale for energy cost</th>
<th>Reference</th>
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<tbody>
<tr>
<td>342050</td>
<td>bobsled toboggan luge</td>
<td>5.3</td>
<td>0.75 × moderate value</td>
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<td>342051</td>
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<td>7.0</td>
<td>Ainsworth* 19180 - sledding, tobogganing,</td>
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<tr>
<td>342052</td>
<td>bobsled toboggan luge - moderate effort</td>
<td>8.8</td>
<td>1.25 × moderate value</td>
<td></td>
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<tr>
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<td>playground games measured in 1 child study, n = 37 [27]</td>
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<tr>
<td>342853</td>
<td>chasey/tag/tips/tiggy – hard effort</td>
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<td>dancing (general)</td>
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</table>

APPENDIX B

INFORMATION AND CONSENT TO PARTICIPATE IN RESEARCH

Additional session

Plyometric and Resistance Training in Children

You and your son are being invited to participate in a voluntary, additional study session being conducted by the investigators listed below. This testing session is part of the larger, Plyometric and Resistance Training Study; however, participation in this additional testing session is optional and will not impact your participation in the Plyometric and Resistance Training Study. Therefore, any participant wishing to participate in this study session is asked to complete this additional consent form. This study will be completed at BP Sports Park (CAT Soccer Field) in Welland, or at Brock University, in accordance with your convenience, on a day following the post-training testing. Please read this form to find out about the purpose and the tests of this study. This study is part of the Faculty of Applied Health Sciences of Brock University.

INVESTIGATORS: DEPARTMENT: CONTACT:
Dr. Bareket Falk FAHS, Brock University (905) 688-5550 ex. 4979
Brandon McKinlay FAHS, Brock University (905) 688-5550 ex. 5623
Raffy Dotan FAHS, Brock University (905) 688-5550 ex. 5272
Dr. Craig Tokuno FAHS, Brock University (905) 688-5550 ex. 4365
Dr. Nota Klentrou FAHS, Brock University (905) 688-5550 ex. 4358
Angeliki Giannopoulou FAHS, Brock University (905) 688-5550 ex. 5623

* FAHS = Faculty of Applied Health Sciences

PURPOSE:

The purpose of the study session is to investigate the changes in salivary concentration of cortisol, testosterone following plyometric or resistance training.

DESCRIPTION OF TESTING PROCEDURES:

If your child agrees to volunteer for this study, he will participate in either a plyometric or resistance exercise session, as well as one session which will not include any exercise. Before and after these sessions, we will ask your child for a saliva sample. We will also ask you child to complete a nutrition questionnaire (about 30 min).

Please note that participation in this study will not affect the status of your child within the Elite Soccer Development Academy.
Your child will undergo the measurements and procedures listed below; please note that in all questionnaires, your child may choose not to answer any question. Your child may also choose not to participate in any procedure listed below.

Because food consumption may affect the results, we will ask your child not to eat 2 hours prior to testing. For the training session, we will ask your child to wear athletic attire.

**A. Assessments (pre- and post-training):**

1. Your child will complete a nutritional questionnaire, specifically designed for children. It measures servings of fruit, vegetables, dairy, meat, legumes and whole grains, as well as sugars, and saturated fat. Your child may choose not to answer any question without penalty.

2. A saliva sample will be collected to determine the relationship between hormonal levels (cortisol and testosterone) and muscle function during exercise, as well as to determine the response of these hormones to one session of exercise. Therefore, a sample will be collected before the exercise session, immediately after, and 30 min after exercise. Likewise, for the session with no exercise, saliva samples will be collected at a similar time of day. These samples will be collected using specifically designed swabs which will be chewed lightly for a minute before sample collection into a plastic tube.

**B. Exercise Protocol:**

Your child will be assigned to take part in a plyometric or resistance exercise session, held within BP Sports Park (CAT Soccer Field) in Welland.

**Plyometric exercise:**
The session will consist of 5 minutes of warm-up, 15 minutes of plyometric training exercises, and 5 minutes of cool down. Exercises will include jumps, hops and bounds of varying volumes and intensities. The plyometric training protocol has been structured in a manner which allows your child to safely progress through the various exercises, minimizing stress placed on joints and allowing for optimal power-associated muscular adaptations to occur. Prior to the session, your child will be shown how to safely conduct the various exercises in a controlled and safe manner by the research team.

**Resistance exercise:**
The session will consist of 5 minutes of warm-up, 15 minutes of on field resistance training exercises, and 5 minutes of cool down. Resistance exercises will include lunges, squats and various resistance band/medicine ball techniques, at varying volumes and intensities. The resistance training protocol has been structured in a manner which allows your child to safely progress through the various exercises, minimizing stress placed on joints and allowing for optimal strength associated muscular adaptations to occur. Prior to the session, your child will be shown how to safely conduct various exercises in a controlled and safe manner by the research team.
CONFIDENTIALITY:
All data collected during this study will remain confidential and will be stored in offices and on secured computers to which only the principal and co-investigators have access. You should be aware that the results of this study will be made available to scientists, through publication in a scientific journal but your child’s name and any personal data will not appear in compiling or publishing these results. The name of the soccer organization may appear in the report. Data will be kept for 5 years after the date of publication, at which time all information will be destroyed. Additionally, you will have access to your child’s data, as well as group data when it becomes available and if you are interested. This can be provided to you by simply contacting the principal investigator.

PARTICIPATION AND WITHDRAWAL:
You and your child can choose whether to participate in this study or not and may withdraw or remove your child’s data from the study, by simply telling one of the investigators. In case you or your child chooses to withdraw from the study by telling the investigator, you will be asked whether his data can still be used for analysis. In case your child withdraws by not showing up, partial data will be used. Your child may also refuse to answer any questions posed to him during the study and still remain as a participant in the study. The investigators reserve the right to withdraw your child from the study if they believe that it is necessary.

Your child should not feel obligated to participate in the study and his decision to participate or not participate or withdraw from participation will in no way impact the standing of your child within the Elite Soccer Development Academy.

If your child choses to withdraw from the study, he will still be able to participate in the training.

RISKS AND BENEFITS:
Participation will allow you and your child to become exposed to a research protocol, potentially improve your child’s physical performance, contribute to the advancement of science, and gain personal and general knowledge about your child’s fitness.

The only foreseeable risks involved in participation include:

a) Possible muscle fatigue from plyometric or resistance exercises. This phenomenon is typical of most physical training programs and may last 24-72 hours.

b) With any participation in physical activity there is a risk of injury. Therefore, at each training session there will be one or more team members who have certified first aid training.

FEEDBACK and STUDY RESULTS:
Your child’s and group results will be provided to you upon request. If any results outside the norm appear during data collection, you and your child will be informed within one month. Additionally, you and your child will receive a comprehensive report of your child’s nutritional intake, along with recommendations (provided by NutritionQuest). This report will be provided to you and your child, whether your child completes the study or decides to withdraw from it.

RIGHTS OF RESEARCH PARTICIPANTS:
You will receive a signed copy of this consent form. The study has been reviewed and received ethics clearance though the REB (file #13-305). If you have any pertinent questions about your
rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca).

**INFORMATION:**
Please contact Dr. Bareket Falk at 905 688-5550 (ext. 4979), bfalk@brocku.ca, Dr. Nota Klentrou at 905 688-5550 (ext. 4358), or Brandon McKinlay at 905 688-5550 (ext. 5623), bm13tj@brocku.ca if you have any questions about the study.

I have read and understand the above explanation of the purpose and procedures of the project. I have also received a signed copy of the information and consent form. My questions have been answered to my satisfaction and I agree to participate in this study.

__________________________________________________________
SIGNATURE OF PARENT/GUARDIAN                                         DATE

__________________________________________________________
PRINTED NAME OF PARTICIPANT

In my judgment the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

__________________________________________________________
SIGNATURE OF INVESTIGATOR                                         DATE
APPENDIX C

ANTHROPOMETRIC MEASUREMENTS

ID NUMBER: ________

NAME: ______________________

TEST DATE (MM/DD/YYYY): ____________

AGE: ______

DATE OF BIRTH (MM/DD/YYYY): ____________

SUBJECT HEIGHT (cm): ________

SEATED HEIGHT (cm): ________

SUBJECT WEIGHT (kg): ________

BMI: ________

% BODY FAT: ________

SKINFOLD MEASUREMENT (mm):

<table>
<thead>
<tr>
<th>SITE</th>
<th>TRIAL 1</th>
<th>TRIAL 2</th>
<th>TRIAL 3</th>
<th>TRIAL 4 (&gt;1 mm diff)</th>
<th>MEDIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRICEP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUBSCAP.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TRAINING INFORMATION:

<table>
<thead>
<tr>
<th>SPORTS</th>
<th>YEARS OF TRAINING</th>
<th>HOURS/WEEK</th>
<th>MONTHS/YEAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
APPENDIX D

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a 7-day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free-time (write on each line the appropriate number)?

<table>
<thead>
<tr>
<th>Times Per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) STRENUOUS EXERCISE (HEART BEATS RAPIDLY)</td>
</tr>
<tr>
<td>(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)</td>
</tr>
<tr>
<td>(b) MODERATE EXERCISE (NOT EXHAUSTING)</td>
</tr>
<tr>
<td>(i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)</td>
</tr>
<tr>
<td>(c) MILD EXERCISE (MINIMAL EFFORT)</td>
</tr>
<tr>
<td>(i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)</td>
</tr>
</tbody>
</table>

2. Considering a 7-day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

   1. OFTEN  2. SOMETIMES  3. NEVER/RARELY

   □      □      □