EVALUATION OF SALIVARY CORTISOL LEVELS DURING A LONG CROSSFIT® CHAMPIONSHIP

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Abstract

Objective: This study measured the cortisol levels of professional athletes during a CrossFit® championship. Then, it evaluated the physiological response caused by cortisol. Increased cortisol levels may interfere with the athletes' performance and muscle recovery. Materials and Methods: A prospective observational cross-sectional study was performed with a sample of eight female professional athletes, aged between 20 and 34 years, enrolled in the 2017 Brazil CrossFit Championship (TCB), held in Valinhos, state of Sao Paulo, Brazil. The salivary samples were obtained during the three days of the championship in two different moments: in the morning, before athletes' warm-up, and 15 minutes after the last workout of each day. Results: Before warming up, the mean values of salivary cortisol were 15.9 ng/mL, 13.2 ng/mL, and 13.1 ng/mL, respectively. After the last workout, the mean values for cortisol were 6.4 ng/mL, 9.6 ng/mL, and 7.1 ng/mL, respectively. A statistically significant difference was found on the first day of competition. The results presented the expected diurnal cycle of this hormone. Conclusion: CrossFit® is a sport that alternates medium- to high-intensity exercises, including gymnastics, metabolic conditioning, and weight lifting. This competitive sport presents a variability of stressors, which may increase cortisol production and secretion according to different studies. This study found that the physiological stress of a CrossFit® championship affects the production of cortisol and increases the stimulation of the hypothalamic axis. However, the normal cortisol secretion response suggests a physiological adaptation or alteration in the cortisol receptor in the athletes studied. Further studies carried out with a larger sample are necessary to assess these neuroendocrine changes.

Keywords: CrossFit®. Cortisol. Physical activity. Women.

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INTRODUCTION

CrossFit® (CrossFit®, Inc., Washington, DC, USA) is a relatively new but extremely popular non-Olympic sport with mixed workouts that encompass many types of functional movement patterns in a single session, performed at a medium- to high-intensity level[1]. Since its creation in the 1990s, it has become a competitive sport with about 11,000 gym affiliates and over 200,000 athletes worldwide in 2014[2].

Physical exercises during training sessions and competitions are constantly varied. Three sports are the basis of CrossFit® exercises: gymnastics (G), metabolic conditioning (M), and weight lifting (W)[3]. The literature states that CrossFit® training elicits a high acute cardiovascular response as well as large increases in aerobic and anaerobic performance[4].

CrossFit® is a competitive sport with varied stressors and external and internal pressures not controlled by athletes, which can physically and mentally destabilize athletes before and during a competition. External pressures include, among other factors, athletes' performance evaluation by their coach and other participants, coaches' expectations about athletes' performance, and cheering behavior. Internal pressures include the achievement of personal goals, expectations of success or failure, and athletes' own perceptions of wins and losses[5].

Considering hormonal responses, cortisol is the main glucocorticoid released by the adrenal cortex in a stressful situation. It is considered the stress hormone, since its production and secretion increase during and after exposure to some stressors[6]. In this context, the presence of cortisol in competitive situations is an indicator of stress. Cortisol levels can be assessed by blood plasma, urine or saliva samples. Salivary cortisol, specifically, is an important, effective, affordable, fast, and non-invasive measurement variable. Salivary cortisol can be collected in any competitive and extracompetitive situation without causing reactivity, or practical or ethical embarrassments[6].

As mentioned above, physical exercises affect cortisol levels. The hormonal response to exercises depends on several factors, such as exercise intensity, duration, mode and level of training. It is important to assess cortisol levels during high-performance competition in professional athletes because the increase in physiological cortisol levels may result in a decrease in testosterone levels, loss of strength and impaired muscle recovery. High cortisol levels increase loss of muscle mass (cortisol catabolic effects) and contribute to the occurrence of overtraining, as it causes chronic fatigue, loss of appetite, decreased performance, infections, sleep disorders, mood swings, and disinterest in training.

Therefore, this study aims to analyze the effects of three consecutive CrossFit® training sessions on salivary cortisol. The initial hypothesis is that three consecutive CrossFit® training sessions increase cortisol responses, harming physiological stress.

METHODS

This is a prospective observational cross-sectional study performed with convenience sampling: volunteer women. They signed a free and informed consent form approved by the Research Ethics Committee of the University Center of Brasilia (Centro Universitário de Brasília), whose process number was 1.791779.

The inclusion criteria comprised Brazilian women enrolled in the 2017 Brazil CrossFit® Championship (TCB) who succeeded in the first selection phase of the championship.

The championship selected 48 female athletes in the main category. The volunteers in this study were high-performance female athletes, CrossFit® practitioners for over a year, aged 20 to 34 years, not using any oral contraceptive. Among them, only eight female athletes accepted to participate in this research and met the inclusion criteria, which corresponded to a sample of 16.6%.

Table 1: Sample characteristics presented by the mean and standard deviation.

Age years)	31.2 (±2.9)
Body mass (kg)	67.52 (±7.0)
Height (cm)	164.0 (±0.1)
Body mass index (kg/m^2)	25.13 (±1.0)
Experience (years)	5.0 (±1.8)

The exclusion criteria were women who used glucocorticoids, missed one day of the championship or reported to be very anxious about the competition during the interview. The second phase of the competition was held in Valinhos, state of São Paulo, Brazil, on August 25, 26, and 27, 2017. The saliva samples were collected during the three days of the championship.

DATA COLLECTION AND ANALYSIS

Saliva samples were obtained with salivette® (Sardstedt, Germany) at two different times using the procedure indicated by the manufacturer. The first collection was before athletes' warm-up (D1A, D2A, and D3A), where participants could not be fasting and they were instructed to make a quick mouthwash with clean water. Sample D1A was collected considerably before the warm-up to be characterized as basal cortisol. The other sample was collected about 15 minutes after the last workout (D1P, D2P, and D3P), when participants were instructed to repeat the same procedure of the first collection. Control data collection was not performed before the championship, because we did not have access to all participants before it and due to collection logistics, as the participants came from different states of Brazil.

Saliva was collected using a sterile dental cotton roll on the participant's tongue, where it remained until it was saturated with saliva; then it was removed and placed inside a plastic tube provided by the manufacturer. Samples were then kept in a refrigerator (4 °C) until they were sent to the laboratory, where they were centrifuged and frozen at -20 ºC until the salivary cortisol concentration analysis.

Between the first and the last collection, there was an average twelve-hour interval. Participants had, on average, ten hours of rest between the days of competition. After performing the ANOVA statistical treatment and Tukey's range test, this study considered the significance level of 5% (p[?]0.05).

TYPES OF PHYSICAL EXERCISES PERFORMED

The competition comprised exercises based on metabolic conditioning (M), weight lifting (W) and gymnastics movements (G), as shown in Table 2. Exercises are performed individually. Each athlete performs all exercises under the supervision of a judge.

Table 2: Characteristics of the tests and exercises performed in the TCB 2017 competition.

FRIDAY
TEST 1
50 Air Bike Calories 6 Series de: - 5 Behind-Neck Pull-Up - 10 Bench Press (Supino) (40kg) - 15 Dumbbell Squat (15kg) 50
SATURDAY
TEST 3
HANG CLEAN LADDER ** 30s attempt / 15s transition Female: 75-130kg
SUNDAY
TEST 5
TIMECAP: 12'
3 Series: - 20m Sledge Prowler - 10 Heavy Ball Clean (40/70kg) - 20m Sledge Prowler - 25 Toes To Bar - 30 WallBall (20/3

In the competition, athletes had the right and access to medical care, if necessary, physiotherapists and osteopaths to assist them in their recovery. Four athletes of the sample (50%) reported to have used venous rehydration with saline solution and electrolytes to recover from the workouts. They also used pneumatic compression devices to reduce the level of lactic acid in their lower limbs. No significant difference was found in the analyses of the athletes who adhered to these practices.

Subjective stress levels were not assessed by existing questionnaires (perception of overtraining levels, French Society of Sports Medicine). As mentioned above, CrossFit® is a new sport with high, acute cardiovascular response, which increases aerobic and anaerobic performance[4], and requires different techniques for a good performance. For this reason, it is necessary to develop and validate a Scale for Psychological Stress Analysis in CrossFit® to investigate particular conditions (internal and external pressure) that may provoke a reaction in athletes and a subjective self-assessment of efficiency of the behavior adopted.

Some potential biases found in this study were the collection of the first sample before athletes used any stimulant supplements; the collection of saliva after the last workout of the day, due to athletes' difficulty to produce saliva; and the risk of sample loss due to the little material collected during the study.

RESULTS AND DISCUSSION

This study evaluated the behavior of salivary cortisol levels during a long CrossFit® championship. The initial hypothesis, found in literature review, is that salivary cortisol levels increase after workouts. However, this was not demonstrated in this study. Athletes' saliva samples were collected in the morning, before the competition (D1A – 15.9 ± 10.3 ng/mL, D2A – 13.2 ± 8.0 ng/mL, and D3A – 13.1 ± 9.9 ng/mL), and immediately after the last workout (D1P – 6.4 ± 6.9 ng/mL, D2P – 9.6 ± 7.9 ng/mL, and D3P – 7.1 ± 6 , 7 ng/ml). A statistically significant difference (Figure 1) was found only in D1P compared to D1A, although data showed a trend of fall in cortisol levels throughout the day, indicating that the circadian rhythm of cortisol seemed to react as expected[7].

In general, the literature has emphasized that the behavior of cortisol varies according to the intensity of physical exercise; thus, the higher the intensity, the greater the need to mobilize amino acids of tissues. Concurrently, the transportation of cortisol from the extracellular zone to the liver cell increases, which increases their availability for further conversion to glucose. In addition, the increased peripheral destruction of cortisol, the decreased hepatic metabolism rate and the increased ACTH secretion, mostly derived from the influence of stress-related mechanisms, also contribute to increased cortisol secretion during physical exercises[7].

Authors, such as Nunes et al.[8], demonstrated increases in cortisol concentrations during endurance, power and hypertrophy exercise protocols. This increase is related to the intensity of the physical effort performed; the high metabolic demand of higher efforts tend to induce higher concentrations of cortisol. The idea that CrossFit® is considered an extreme exercise program[9]supports this fact. However, this study's data showed that the physiological condition of cortisol behavior was maintained.

Other authors have stated that increased cortisol levels play an important role in athletes' performance, ultimately meeting their physical demands during a long-term competition[10,11,12]. Some authors have corroborated that cortisol increases during recovery, indicating a delay in post-training cortisol secretion in women[13,14].

Georgopoulos et al.[15] found that female Olympic gymnasts showed higher stress response, and their salivary cortisol showed an inverse relation to fat percentage. However, this study was conducted with adolescent gymnasts, who were thinner than the Average female gymnasts at their age, had delayed skeletal maturation, suffered from amenorrhea, and had a significant reduction in fat mass. This phenotype probably results from multifactorial events; that is why this behavior cannot be applied to this study.

Our findings, however, contradict the research studies presented above. In our study, Figure 1 shows the mean values and confidence intervals for each concentration of salivary cortisol (ng/mL) at each time on evaluation days, in the pre and post phase. All athletes presented a physiological performance contrary to the one expected concerning the cycle of cortisol after a competition based on salivary cortisol. This finding may partially result from the behavior of cortisol secretion/metabolism.

Figure 2 reveals that there was a statistical difference ($p>0.05$) in the concentration of salivary cortisol (ng/mL) between each group of evaluation days.

Figure 1. Mean values and confidence intervals for each concentration of salivary cortisol (ng/mL) at each time on evaluation days.

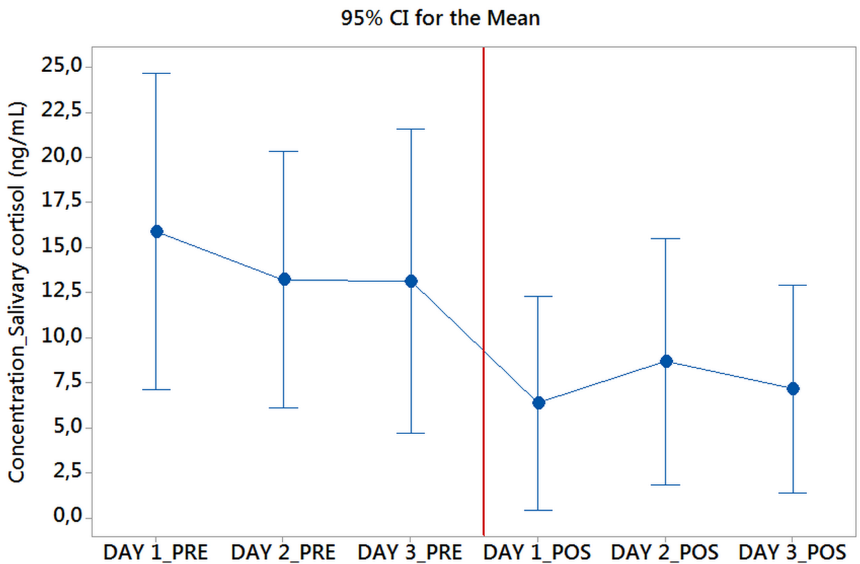
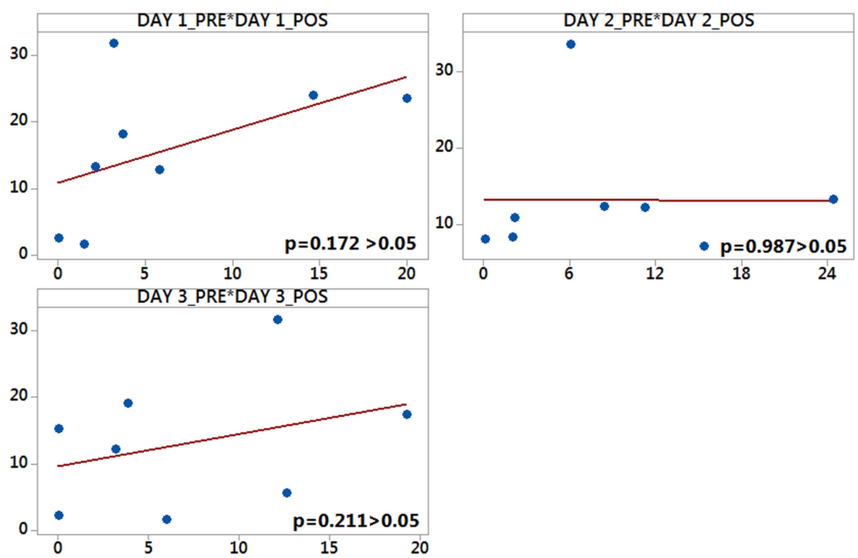


Figure 2. Statistical difference in the concentration of salivary cortisol (ng/mL) between each group of evaluation days.



The classical negative feedback loop for cortisol secretion is associated with inhibition and production of CRH and ACTH to suppress cortisol release and concentration.

On one hand, high salivary cortisol concentrations since the beginning of the day may be associated with female gene, specifically the production of cortisol intensified by estrogen in the hypothalamus-pituitary-adrenal axis, a higher mean value of cortisol, and a greater predisposition to its absorption[15].

On the other hand, the high salivary cortisol concentration found may also be associated with a suppression of glucocorticoid receptors (GR, glucocorticoid receptor – NR3C1). This fact is related to the effect of adaptation to training and to a protective effect regarding overtraining[16, 17].

GRs are a family of receptors consisting of three main domains. The first one is the N-terminal domain (NTD), responsible for transcriptional activation function (AF1), which interacts with co-regulators, representing the main site for post-translational modifications (PTMs). The second one is the DNA-binding domain (DBD), which is the most preserved domain in the entire receptor. It has two zinc finger regions, which recognize and associate with the target DNA sequence, called glucocorticoid-responsive elements (GRE), and it is responsible for the transcriptional activation function (AF1) in association with co-regulators. The last one is the C-terminal region of the ligand-binding domain (LBD), comprising 12 beta propellers and four beta sheets. It constitutes a hydrophobic region for the ligand substance (e.g. cortisol) and contains an activation function (AF2) that depends on cortisol to interact with co-regulators[18, 19, 20].

Barrientos et al.[21] demonstrated a decrease in the levels of expression of GR in the hippocampus in elderly animals submitted to exercises. These findings corroborate previous studies and suggest that prolonged basal increases in cortisol levels in the hippocampus are associated with potential inflammatory responses, as recently demonstrated by other authors[22, 23]. This seems to support the idea that a decreased expression of GR may be associated with a protective effect.

Previous findings have indicated that high cortisol levels increase insulin resistance and metabolic syndrome. Paradoxically, evidence indicates that aerobic exercises attenuate the development of metabolic syndrome, although they stimulate acute increases in circulating cortisol levels[24, 25].

This action seems to be associated with the lipolytic nature of cortisol. The literature has shown that increased glucocorticoids in insulin-sensitive tissues is related to lipid accumulation and metabolic complications, independently of plasma concentrations. The action of intracellular cortisol is determined by both 11beta-hydroxysteroid dehydrogenase type 1 (11-βHSD1) and GR. Research studies have proved that training can increase the concentration of 11βHSD1 as well as GR in the adipose tissue, resulting in increased lipolysis[26, 27].

Another important aspect to be considered is the so-called anticipatory salivary cortisol response before competition, which is well described in the literature. It does not depend on the type and nature of the sport, not even on the existence or not of an opponent and/or physical contact[28]. The anticipatory salivary cortisol response has been strongly related to precompetitive anxiety[29,30].

Cevada et al. [31] reviewed the literature and showed that most studies have evaluated male athletes, while three have evaluated female athletes, and one study have included both genders. Among these studies, only those involving women showed statistically significant differences, showing that female athletes seem to be more vulnerable to physical stress than men.[32]

Changes in both cortisol and testosterone concentrations have been associated with motivational factors and aggression; in this context, a positive correlation between dominance and fighting status is a central aspect of this change[31]. These aspects have been well described in a recent meta-analysis that compared the effects of this association on individuals of both genders, demonstrating a clear increase in cortisol levels in women, which was associated with competitive events (e.g. games and fights).

STUDY LIMITATIONS

During a sports championship, many hormonal factors can affect the performance of athletes. Measuring salivary cortisol levels during a long CrossFit® competition was important to understand the impact of this sport on the body of athletes and to discover whether their performance can be improved.

With increasing numbers of CrossFit® practitioners and more competitions throughout the year, understanding the action of cortisol is critical, either to identify overtraining or a differential diagnosis of post-competition burnout syndrome.

The main limitation of this study was the impossibility to control the rest time of athletes and to confirm if athletes did not use any stimulant substance.

CONCLUSION

Based on the data collected in this study, physiological stress in a long CrossFit® championship plays a significant role in the production of cortisol and in the increased stimulation of the hypothalamic axis. However, the normal cortisol secretion response suggests a physiological adaptation or alteration in the cortisol receptor in the athletes studied, which prevents salivary cortisol from increasing in a long physical exercise session, as found in the research studies presented below.

Further studies should repeat the salivary cortisol analysis in a larger group during a long CrossFit® championship and include a group of men in the sample. Also, they could measure salivary testosterone levels in order to analyze the relation between cortisol/testosterone and sports performance.

Conflict of interest

The authors declare no conflict of interest.

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