

## Do weekly training loads alter hormone concentrations in athletes from the Brazilian under-19 men's volleyball team?

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**Abstract - Background:** We sought to verify the standard of weekly training loads (TL) of the Brazilian under-19 men's volleyball team, the salivary cortisol and testosterone concentrations and perceived recovery status (PRS), to understand how these variables respond after one day of recovery, and to observe the correlations between PRS and salivary cortisol and testosterone concentrations. **Methods:** PRS, salivary collections for the analysis of testosterone and cortisol concentrations were performed in 17 athletes ( $18.3 \pm 0.30$  years,  $87.39 \pm 9.02$  kg,  $198.34 \pm 8.13$  cm) at the beginning of the first training session (M1), at the end of the last training session (M2) and one day after the last training session of the week, at the beginning of the session (M3). The session rating of perceived exertion method (sRPE) monitored TL. **Results:** The monotony was 2.1 A.U., and weekly TL was  $5712 \pm 149$  (A.U.). Salivary cortisol concentrations showed significant differences between the moments evaluated ( $p = 0.001$ ). There was an increase between M1 and M2 ( $p = 0.001$ ; ES 1.18) and between M1 and M3 ( $p = 0.001$ ; ES 1.27). No significant differences were found between M2 and M3 ( $p = 0.387$ ; ES 0.30). Salivary testosterone concentrations remained similar between the three moments ( $p = 0.342$ ), as well as the PRS ( $p = 0.298$ ), with effect size insignificant and small between the moments. The PRS showed a positive correlation with testosterone and did not show correlations with cortisol. **Conclusions:** We conclude that the training loads generated significant increases in salivary cortisol concentrations, which remained after one day of recovery, without alterations in testosterone concentrations and PRS.

**Keywords:** recovery, collective sports, hormones.

### Introduction

High-performance sports teams require significant training volumes, with the preparatory period focusing on achieving the performance levels needed to meet competitive demands. During the training process, athletes may experience performance declines caused by prolonged excessive training, associated stressors, and inadequate recovery time<sup>1</sup>. Consequently, monitoring training loads becomes essential for understanding their impact on physiological variables, such as hormonal concentrations (testosterone and cortisol), as well as psychophysiological factors like perceived recovery status (PRS) and rating of perceived exertion (RPE). This approach facilitates the analysis of changes stemming from the applied training loads. The combination of objective and subjective tools is particularly

effective for monitoring fatigue and recovery, allowing for a comprehensive evaluation of responses<sup>2</sup>.

Among the methods for monitoring internal training loads, the session rating of perceived exertion (sRPE) method has been extensively used in team sports<sup>2-3</sup>. In volleyball, internal training load assessments based on sRPE have proven to be a reliable and easily applicable approach. Because volleyball emphasizes strength and power in practice, tools that measure effort intensity, such as the sRPE method, are crucial for analyzing training loads<sup>4-6</sup>.

About psychophysiological parameters, PRS evaluates individual recovery status, making it crucial for identifying performance declines associated with athletes reporting a feeling of not being recovered. It is also a simple method that can be applied daily<sup>7</sup>. The authors highlight the importance of PRS in identifying

performance declines when athletes report feeling unrecovered compared to those who report being recovered or moderately recovered. These practical and straightforward analyses can be associated with the concentrations of testosterone and cortisol hormones, which are also used as possible predictors of stress and recovery<sup>8-9</sup>. They can be evaluated through salivary concentration, providing insights into physiological changes related to the mental and physical demands on athletes during preparatory and competitive periods<sup>10</sup>.

Studies in different populations show that acute exercise sessions and training periods can lead to increased testosterone concentrations<sup>11-12</sup> and cortisol concentrations<sup>12-14</sup>, which seem to be associated with the imposed loads. However, other studies indicate no difference in acute<sup>11</sup> or chronic<sup>12,15,16</sup> cortisol concentrations despite the training loads. Evaluating volleyball athletes, some studies found no differences in testosterone and cortisol concentrations pre- and post-competitions<sup>17-20</sup>. However, De Freitas et al.<sup>21</sup>, assessing training periods, found no changes in cortisol concentrations in volleyball athletes over one week of training. Mazon et al.<sup>22</sup> observed increased testosterone concentrations after 12 weeks of training. Thus, the behavior of testosterone and cortisol concentrations during training periods and varying recovery times in volleyball athletes remains unclear.

Thus, the aim of the present work was to verify the behavior of TL during one week of athletes from the Brazilian under-19 men's volleyball team and their repercussions on salivary testosterone and cortisol concentrations and PRS, in addition, to understand how these variables behave after one day of recovery in which the athletes remain in a concentration regime at the training site.

Our hypothesis was that the salivary cortisol and testosterone concentrations would be altered with the loads imposed on athletes during the week, and with one day of recovery, the concentrations would decrease in relation to the end of the training week.

## Materials and methods

### Population

Seventeen athletes from the Brazilian under-19 volleyball team participated in the sample, with a mean age of  $18.3 \pm 0.3$  years, body mass  $87.39 \pm 9.02$  kg and height  $198.34 \pm 8.13$  cm. All athletes of the sample were selected by the Brazilian Volleyball Confederation to participate in the under-19 team in 2019.

Previously to the participation in the study, athletes read and signed the free and informed consent

form, which contained all information relevant to the study. The research project was approved by the Research Ethics Committee of the University of Rio Grande do Sul (number: 4,677,011). This study is in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), published in the British Medical Journal (July 18<sup>th</sup>, 1964).

### Procedures

The assessments were performed during the third week of the preparatory phase of training of the Brazilian under-19 men's volleyball team for the World Championship. In this phase, training routines of athletes consisted of two daily training sessions, with technical and strength training in the morning and tactical training in the afternoon.

In the recovery day, the athletes had free routines, but they had to stay at the Volleyball Development Center (CDV), place where the training camp was held.

At the time of the athletes' presentation for the first training session (on Monday), the saliva collection was performed for the analysis of testosterone and cortisol concentrations (M1), as well as at the end of the last training session of the week (Saturday) (M2) and at the moment of re-presentation of athletes for the following week of training, in the morning (Monday), after one day of recovery (M3).

PRS was collected before the start of the training sessions at the three moments evaluated. RPE was collected after the morning and afternoon training sessions to evaluate the training loads through the session RPE method.

### Training loads

The RPE assessment was performed through the CR-10 Scale<sup>23</sup> which was used to evaluate the training loads through the session RPE method proposed by Foster et al.<sup>24</sup>. At the end of training<sup>25-26</sup>, athletes were asked to answer the following question: what is your perception of effort for today's game or training? They should point their response on the scale. The scale indexes vary from 0 to 10, in which 0 is equivalent to no effort and 10 is equivalent to maximal effort. The athletes were previously familiarized with the use of scales for at least two years.

TL were monitored by the session RPE (sRPE) method, which is given by the product of the values observed by the RPE scale and the training time in minutes of each session, which corresponds to the session TL. For the values of daily TL, TL of morning and afternoon sessions were summed. The result is presented in arbitrary units (A.U.)<sup>24</sup>.

### Monotony

To obtain the monotony, mean daily training loads were calculated, including the recovery days, to compose the mean weekly training loads and the respective standard deviation. The calculation of monotony was given by the ratio of mean weekly training loads by its standard deviation. Values above 2 A.U. were considered a reflection of little oscillation of training loads<sup>24</sup>.

### Salivary cortisol and testosterone concentrations

Salivary cortisol and testosterone concentrations were evaluated through saliva samples. The procedures were the same in all collections. The athletes were asked to rinse their mouth with water to remove any residual food and asked to refrain from drinking caffeine and carbonated soda until 1 h before collection. Then, 1 mL of unstimulated saliva was passively collected in a 5 mL collector tube. The saliva samples were stored at  $-20^{\circ}\text{C}$  until further analysis and determined using enzyme immunoassay kits for the quantitative determination of free testosterone in human saliva (ELISA Salivary Testosterone, LDN, Germany) and enzyme immunoassay for determination of free cortisol in human saliva (ELISA Salivary Cortisol, LDN, Germany)<sup>11</sup>. Analyses were performed at the Laboratory of Exercise Research (LAPEX) at the Physical Education, Physiotherapy and Dance School of the Federal University of Rio Grande do Sul, by an experienced researcher in analyses, where the assay procedures were conducted according to the instruction manual of the kit used.

### Perceived recovery status

Through the PRS scale, validated by Laurent et al.<sup>7</sup>, the perception of recovery of athletes was assessed. The athletes were asked to answer the following question: What is your perception of recovery at the moment? They should point their response on the scale. The scale has scores ranging from 0 to 10, in which 0 is equivalent to no recovery and 10 is equivalent to total recovery.

### Statistical analysis

Data are presented by mean, standard deviation (SD) and 95% confidence interval (CI). Normality and homogeneity of data were verified through Shapiro-Wilk and Levene tests. For the comparison of mean daily TL, one-way repeated measures ANOVA was used. For the comparison of the different moments, the analysis of generalized estimation equations (GEE) was used, with a complementary LSD test. Significant differences were considered when  $p \leq 0.05$ . Statistical

tests were performed using SPSS software version 22.0 (IBM, Chicago, USA).

For effect size (ES) analysis, Cohen's d coefficient was used, in which an effect size of  $< 0.19$  was considered insignificant, 0.20-0.49 small, 0.50-0.79 medium, 0.80-1, 29 large and  $> 1.30$  very large<sup>27</sup>.

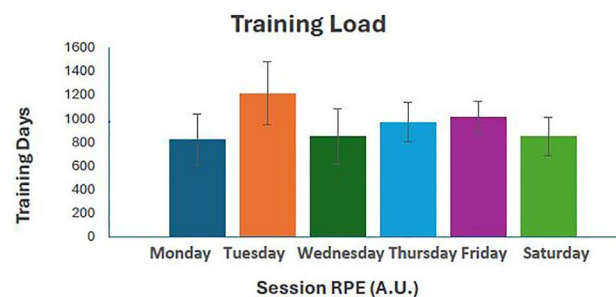
## Results

The monotony was 2.1 A.U., reflecting a low oscillation in TL throughout the week, despite the training loads showing significant differences between days. The TL was higher on day two (Tuesday) and lower on day one (Monday), day 3 (Wednesday) and day 6 (Saturday), as shown in Figure 1. The TL of the week was  $5712.1 \pm 149.8$  (A.U.).

Salivary cortisol concentrations presented significant differences between the moments evaluated ( $p = 0.001$ ). There were differences between M1 and M2 ( $p = 0.001$ ; ES 1.18) and between M1 and M3 ( $p = 0.001$ ; ES 1.27). On the other hand, there were no significant differences between M2 and M3 ( $p = 0.387$ ; ES 0.30). Testosterone concentrations remained similar between the three moments evaluated ( $p = 0.342$ ), as well as PRS ( $p = 0.298$ ) with effect size insignificant and small between the moments (Table 1).

## Discussion

The present study aimed to verify the behavior of training loads during one week of the Brazilian under-19 men's volleyball team athletes and their repercussions on salivary testosterone and cortisol concentrations and PRS, in addition, to understand how these variables behave after one day of recovery in which the athletes remained at the Volleyball Development Center (training camp). The monotony was 2.1 A.U., reflecting a low oscillation in TL throughout the week<sup>24</sup>, producing an increase in cortisol concentrations from the beginning to the end of the training



**Figure 1** - Mean values of the training loads of the week evaluated using the session RPE method (A.U. - arbitrary units). Letters of the same type indicate no significant differences, and different letters indicate significant differences between the training loads of the days of the week.

**Table 1** - Mean values, standard deviation, 95% CI of salivary cortisol concentrations (Sal-C) (pg/mL) and salivary testosterone concentrations (Sal-T) (ng/mL) evaluated in the three moments. Number of athletes: Sal-C (17), Sal-T (10), PRS (17).

|                                                     | M1                           |                  | M2                           |                  | M3                           |                  |
|-----------------------------------------------------|------------------------------|------------------|------------------------------|------------------|------------------------------|------------------|
|                                                     | Mean $\pm$ SD                | 95% CI           | Mean $\pm$ SD                | 95% CI           | Mean $\pm$ SD                | 95% CI           |
| Salivary cortisol concentration (Sal-C) (ng/mL)     | 3.36 $\pm$ 1.68 <sup>a</sup> | (2.55; 4.16)     | 7.53 $\pm$ 4.57 <sup>b</sup> | (5.34; 9.71)     | 6.37 $\pm$ 2.80 <sup>b</sup> | (5.03; 7.71)     |
| Salivary testosterone concentration (Sal-T) (pg/mL) | 140.29 $\pm$ 48.25           | (110.36; 170.22) | 163.18 $\pm$ 39.72           | (138.53; 187.82) | 144.71 $\pm$ 50.43           | (113.43; 176.00) |
| PRS                                                 | 7.64 $\pm$ 1.98              | (6.60; 8.68)     | 7.43 $\pm$ 0.97              | (6.92; 7.94)     | 8.00 $\pm$ 1.49              | (7.21; 8.79)     |

Letters of the same type indicate no significant differences, and different letters indicate significant differences in the three moments evaluated. M1 = morning before the start of weekly training sessions, M2 = after the end of the last training session of the week, M3 = morning of re-presentation to training after one day of recovery. Perceived recovery status (PRS), standard deviation (SD).

week and maintenance of these values after one day of recovery, but without differences in testosterone concentrations and PRS between the three moments evaluated.

The preparatory period for the U-19 team consists of 2 weeks. The first week consists of 6 days of training and 1 day of recovery and the second week consists of 4 days of training and 3 days of recovery. The organization of the 2-week micro-cycle can partly explain the low variation of the loads, as the Technical Committee organizes the LT in relation to the micro-cycle and not only in the first week. loads, as the technical committee organizes the LT related to the micro-cycle, and not just the first week.

Our findings show high weekly training loads in the week evaluated, and corroborate with Aoki et al.<sup>4</sup> who showed that under-19 athletes have training loads very similar to the demands of adult volleyball teams, and the high loads found in our study are compared to moments of intensification of training loads, which is probably related to the preparatory period stage evaluated.

An increase in salivary cortisol concentrations was expected after the training week, due to the imposed training loads, and a decrease in these levels after a recovery day compared to the values found after the end of the training week. Partially agreeing with our hypothesis, significant differences were found in the concentrations between M1 and M2 ( $p = 0.006$ ), but no difference between M2 and M3 ( $p = 0.100$ ). Cortisol has a catabolic effect, mobilizing energy stores and contributing to acute exercise responses. Increased cortisol concentrations are natural and expected after intensified training loads, as it aids in making metabolic substrates available and maintaining vascularization, leading to enhanced tissue sensitivity to glucocorticoids. Glucocorticoids neutralize muscle inflammation, cytokine synthesis, and muscle damage, aiming for tissue repair. However, when high concentrations are maintained over

prolonged periods, they can lead to overtraining syndrome<sup>28-29</sup>.

Differences were demonstrated from M1 (beginning of the week) to M2 (end of the week) ( $p = 0.006$ ), showing that there was an increase in cortisol concentrations from the beginning of the week to the end of the last training session of the week. Studies corroborate our findings<sup>17,20</sup> showing increases in cortisol concentrations after training periods or after competitions, and seem to be related to the training loads imposed. De Freitas et al.<sup>21</sup>, showed that the weekly training load of  $2354 \pm 492$  (A.U.) in professional volleyball athletes, evaluated by the session RPE<sup>24</sup>, did not generate changes in cortisol concentrations. The authors explain the lack of effects found in the study could be the adaptation of the players, related to the effect of repeated bouts, acquired during years of high level volleyball training. We emphasize that the values of the weekly training loads evaluated in the present study reached  $5712.1 \pm 149.8$  (A.U.), a higher value than the weekly training loads in the study by De Freitas et al.<sup>21</sup>, and the population of the present study is not professional athletes, may explain these differences.

Cortisol levels were expected to decrease after the recovery day proposed by the technical committee (from M2 to M3,  $p = 0.100$ ), however, there were no significant differences, showing that a recovery day was not a sufficient period to reduce the cortisol concentrations in these athletes. Furthermore, significant differences were found between M1 and M3 moments ( $p = 0.016$ ) with higher values in cortisol concentrations after a recovery day, when compared to the beginning of the training week. It is noteworthy that in the following week of training, only four days of training were performed, followed by three days of recovery. Therefore, this proposal of greater load increments may be a strategy of the team to apply a short period of functional overreaching, aiming at greater adaptations of the athletes in the preparatory period.

As well as cortisol concentrations, an increase in testosterone concentrations was expected during the training week, with a reduction after the recovery period. But testosterone levels did not show significant differences between the three moments evaluated ( $p = 0.925$ ). Testosterone release during physical exercise appears to be directly related to intensity and promotes anabolic effects with multiple physiological functions, particularly in the growth and maintenance of muscle and bone mass<sup>29</sup>.

Assessing testosterone concentrations, Filaire et al.<sup>17</sup> showed reductions in testosterone levels related to the increase in TL. On the other hand, Horta et al.<sup>5</sup> found no changes in testosterone concentrations after six weeks of training. Contrary to the findings of the present study, Mazon et al.<sup>22</sup> showed increased testosterone concentrations after a 12-week training period in volleyball athletes. The magnitude of the responses of testosterone concentration could be affected by many factors including the nutritional intake and training experience<sup>30</sup>. And conflicting results demonstrate the complexity of hormonal responses and the likelihood several factors are contributing to the response<sup>29</sup>.

PRS also remained similar in the three moments evaluated ( $p = 0.171$ ). PRS was assessed using the scale proposed by Laurent et al.<sup>7</sup> which has scores ranging from 0 (no recovery) and 10 (maximum recovery). Our findings show that despite the high TL, the athletes showed very good recovery at the time of presentation for training, despite the increased cortisol concentrations observed after the training week and after the recovery period. Volleyball athletes, when evaluated using the Total Quality Recovery scale (TQR) with scores ranging from 6 (minimum recovery) to 20 (maximum recovery)<sup>31</sup> showed that the mean scores of the preparatory period were between good and very good recovery, while Timoteo et al.<sup>32</sup> showed that volleyball athletes present scores close to very good recovery. These results are similar to those presented in the study.

The authors also point out that, although speculative, the psychophysiological dimensions and emotional state indices can distinguish the athletes' ability to deal with training stress, which may partially justify the very good recovery (PRS) despite higher values in cortisol concentrations. The findings by Berriel et al.<sup>33</sup> corroborate ours, showing an increase in creatine kinase (CK) concentrations in the preparatory phase, although the perception of stress and recovery remained stable (assessed by RESTQ-Sport). Therefore, it is so important to monitor psychophysiological and physiological markers to understand the demands of training loads. PRS scales proved to be sensitive when used in volleyball teams. Moreover, a proper inter-

pretation of these indexes along with the other tools can help to adjust the training loads more accurately and the optimal recovery period.

Possible limitations include the short evaluation period (one week of training), and only one recovery period proposed. A strength of our work is the fact that we showed that only one week of training can significantly change cortisol concentrations, despite having no changes in testosterone concentrations and PRS. This is an important information for practical application, showing the importance of monitoring loads and establishing training and recovery strategies for athletes to improve their sports performance, without taking them to possible situations of chronic stress.

## Conclusion

We conclude that the training loads imposed on athletes during a week showed low a low oscillation in TL throughout the week, can generate significant increases in salivary cortisol concentrations, being able to maintain these changes after one day of recovery, without changes in testosterone concentrations. These findings show the importance of monitoring the training loads of athletes, as well as the importance of uniting physiological and psychophysiological assessments for decision making.

## AI use statement

No AI was used in this research.

## Author contributions (CRediT)

**Ananda S Cardoso:** conceptualization, formal analysis, investigation: methodology, data curation, writing - original draft, writing - review and editing, visualization. **Guilherme P Berriel:** conceptualization, formal analysis, investigation: methodology, data curation, writing - original draft, writing - review and editing, visualization. **Natália S Ferreira:** methodology, data curation, writing - original draft, visualization. **Cleidir L Gerlach:** methodology, data curation, writing - original draft, visualization. **Francesco P Boeno:** formal analysis, data curation, writing - review and editing, visualization. **Leonardo A Peyré-Tartaruga:** investigation: methodology, data curation, writing - review and editing, visualization. **Luiz FM Kruel:** investigation: methodology, data curation, writing - review and editing, visualization.

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### Data availability statement

Research data is available in the body of the document.

### Conflict of interest

The authors declare that they have no conflicts of interest relevant to the content of this article. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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