

## **Analysis of cortisol response and load in collegiate female lacrosse athletes: A pilot study**

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### **ABSTRACT**

Currently, it is unknown how cortisol fluctuates across a season in female athletes and how it is related to other measures of training load. The purpose of this study was to 1) evaluate changes in salivary cortisol in Division I female collegiate lacrosse athletes ( $n = 26$ ) and 2) assess the relationship between cortisol and athlete wellness and training load. Saliva samples were collected biweekly on Monday mornings during the first six weeks of the competitive season. Subjective athlete total wellness scores and sub-scores (muscle soreness, sleep quality, fatigue, and stress) were collected on the same days. Objective total weekly workload for distance, high-intensity distance (HID), sprints, accelerations, and decelerations were tabulated from the previous week. A repeated measures analysis of variance assessed weekly changes in cortisol, wellness, and training load across the season. Pearson correlations were used to determine relationships among cortisol, wellness, and training load. There was an upward trend in cortisol (wk 0:  $0.637 \pm 0.296 \mu\text{g/dL}$ , wk 2:  $0.611 \pm 0.450 \mu\text{g/dL}$ , wk 4:  $0.767 \pm 0.495 \mu\text{g/dL}$ ), but no difference in time points. HID and sprints were lower in week 2 than weeks 0 and 4 ( $p < 0.05$ ). Although cortisol did not correlate with wellness scores or objective workload, the upward trend from week 0 to week 4 suggested that as the season progressed, the athletes had increasing levels of stress, possibly resulting from performance or game settings. These findings provide coaches with a better understanding of competition-related stress throughout the season and allow for the implementation of pre- and post-game strategies for stress management among their athletes.

### **1. Introduction**

Cortisol is a glucocorticoid hormone that fluctuates with sympathetic response to stress and is associated with neuromuscular performance (Balsalobre-Fernández et al., 2014a). This plays an important role in metabolic activities including blood pressure regulation, cardiovascular function, regulation of metabolic substrate selection, and immune system function (Sapolsky et al., 2000). Depending on the stressors present, cortisol levels can fluctuate throughout the day, but typically cortisol is secreted in the highest concentrations in the morning, also known as diurnal variation (Lippi et al., 2009). Cortisol is traditionally assessed using blood serum assay; however salivary cortisol tests have shown to be a simple and less invasive method

to achieve reliable results (Haneishi et al., 2007). In addition, salivary cortisol levels have high correlations with serum cortisol levels (Dorn et al., 2007).

In sport, cortisol levels have been shown to negatively correlate with several markers of athlete readiness and recovery (e.g., countermovement jump and subjective wellness), as well as fluctuate throughout a competitive season with performance and workload demands (Balsalobre-Fernández et al., 2014b; Drew & Finch, 2016; Mason et al., 2020; Skoluda et al., 2012). Training can be affected by the fluctuation of cortisol levels within the athletic body and can hinder physical and mental wellbeing (Duclos et al., 2007). Salivary cortisol can be used to determine the effects of high and low intensity exercises during immediate and prolonged periods of time after training. This is important

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because cortisol levels can show how the body is truly responding to stress and how training can be improved by adapting the protocol to the individual. Cortisol has been assessed in male collegiate and professional athletes across a variety of high-intensity and endurance sports. Results from these studies show an acute increase in cortisol with workload (Balsalobre-Fernández et al., 2014a, 2014b; Filaire et al., 2001; McGuigan et al., 2004); however, cortisol did not appear to impact athlete performance. Weekly assessments of cortisol had low correlations with workload ( $r = -.366-0.171$ ) but showed no differences between an athlete's best performance and their worst performance (Balsalobre-Fernández et al., 2014a). The measurement of cortisol can provide evidence to coaches in an effort to fine tune or alter their training schedule to better serve and improve the effectiveness of the current training regimen and reduce the risk of injury and overtraining.

Stressors from sport can be physical (illness, trauma, surgery, fever, physical exercise and extreme temperatures) or psychological (clinical depression, anxiety, strain, fear and pain) (Sapolsky et al., 2000). Self-reported wellness scores and ratings of perceived exertion (RPE) are subjective measurements that evaluate athlete stress levels in various aspects of training (Gallo et al., 2016; op de Beéck et al., 2019). Pre-training wellness scores have been shown to be correlated with external load output, which suggests that wellness scores may be used to help coaches determine training intensity (Gallo et al., 2016). While previous literature has addressed athlete stressors and cortisol response, few studies have examined this relationship in high level female athletes. The present study will only be the second to delve into cortisol responses in female lacrosse athletes (Fields et al., 2020). It is important to evaluate the cortisol response in various athlete populations because it cannot be assumed that all athletes respond the same to the physical and emotional stressors they undergo in-season.

Lacrosse is a sport quickly growing in popularity within the United States and across the globe (Burton & O'Reilly, 2010; Hayhurst, 2005). However, there is little research regarding optimal training load and recovery in female collegiate lacrosse athletes. Current literature provides concepts of game profiles by position (Devine et al., 2022), the relationship between wellness scores and external load output (Crouch et al., 2021), analysis of drill intensities (Alphin et al., 2020), evaluation of important training metrics by training mode (Bunn et al., 2021), and an assessment of the relationship between objective and subjective markers of athlete fatigue (Frick et al., 2021). The first study evaluating cortisol levels in female collegiate lacrosse athletes showed weak relationships between physiological, hormonal, and psychological markers of load and no change in cortisol across a competitive season (Fields et al., 2020). Because this was the first study to evaluate cortisol in this population, it is unknown if these results hold true outside of the sample athletes assessed.

Evaluating athlete wellness may serve as a surrogate of objectively measuring stress through salivary cortisol, but this has not yet been supported with data from collegiate female athletes (Haneishi et al., 2007; op de Beéck et al., 2019). The primary purpose of this study was to evaluate changes in salivary cortisol in Division I female collegiate lacrosse athletes during the

competitive season. A secondary purpose of this study was to assess the relationship between cortisol and athlete wellness and training load using RPE.

## 2. Methods

### 2.1. Study design and ethical approval

This was a non-experimental observational study design that took place during the first six weeks of the competitive season. On average, the team completed  $4.4 \pm 0.5$  practices per week and  $1.6 \pm 0.5$  games per week. During the week of baseline assessments and week 3, athletes participated in five practices and one game, and weeks 1, 2 and 4 each consisted of four practices and two games. Salivary cortisol was measured bi-weekly in the morning. Athlete wellness was assessed each morning prior to any practice or physical activity. Workload measures were obtained through microtechnology during each training and game day.

This study was approved by the institutional review board and conducted in accordance with the Declaration of Helsinki. All participants had the opportunity to ask questions prior to participation, and all participants completed a written informed consent. All elements of the study coincided with FERPA guidelines.

### 2.2. Participants

In this study, cortisol samples were collected from 26 female athletes (aged 18-22) who were recruited by association with the women's lacrosse team at Campbell University. Participants were included in this study if they were members of the varsity women's lacrosse team and eligible for play. Participants were excluded if they were deemed ineligible for play by an athletic trainer or team physician.

### 2.3. Measurements

*Salivary Cortisol Assessment.* Saliva samples were collected biweekly on Monday mornings during the first six weeks of the competitive season, for a total of three samples per athlete. Athletes provided saliva samples into the provided tubes via passive drool shortly after waking and prior to eating or drinking. Athletes provided the sample in compliance with previously validated collection methods provided by Salimetrics (Salimetrics, 2021a). Samples were stored in  $-80^{\circ}\text{C}$  per collection and storage protocol until analysis. Saliva samples were analyzed for cortisol using the Salivary Cortisol ELISA Kit (Salimetrics, State College, PA). These analyses have been shown to provide low coefficients of variation (3-5%) between samples and labs (Salimetrics, 2021b). Samples were thawed, vortexed and centrifuged at  $1500 \times g$  for 15 minutes before adding to the ELISA plate. Cortisol levels were determined using the manufacturer's instructions. The absorbance of the wells at 450 nm was measured using a BioTek plate reader (Winooski, VT). To calculate the concentration of cortisol, a standard curve was generated for the B/Bo from known standards provided in the Saliva ELISA kit ranging from 300  $\mu\text{g/dL}$  to 0.012  $\mu\text{g/dL}$  (Salimetrics, State College, PA).

**Athlete Wellness.** Subjective athlete total wellness scores and sub-scores (muscle soreness, sleep quality, fatigue, and stress) were taken each morning prior to training. This was done using a smart device linked to VX Sport Cloud (Wellington, New Zealand). A five-point Likert scale (0/25/50/75/100) was used with the following questions to determine athlete wellness:

1. How are your muscles feeling today?
2. How did you sleep last night?
3. How are your energy levels feeling for your training today?
4. How stressed are you?

**Athlete Training Load.** Athletes wore VX Sport (Wellington, New Zealand) units in order to track objective training load for distance, HID, sprints, accelerations, and decelerations. The units included a global positioning system (GPS; collecting at 10 Hz), 3-axis accelerometer (104 Hz per channel), 3-axis magnetometer (18 Hz), 3-axis gyroscope (18 Hz) and heart rate monitor (2.4 GHz) and have been shown to provide valid and reliable measures (Alphin et al., 2020; Malone et al., 2014). GPS units were inspected to ensure proper working order and satellite connection prior to each training session. Athletes used only their assigned unit in conjunction with their corresponding vest equipped by VX Sport. The unit was placed in the designated pocket on the vest located between the shoulder blades. After training each day, all data were uploaded to the VX Sport Training software. Data were trimmed to remove inactive time periods and split to supply data specific to the training plan provided by the coaches. Objective total weekly training load for distance, high-intensity distance (HID), sprints, accelerations, and decelerations were tabulated from the previous training week. HID represents the distance run at greater than 60% of maximum sprint speed (MSS). Sprints repetitions were counted when sprint efforts exceeded 80% of MSS. Accelerations and decelerations were determined by a change of  $\pm 3 \text{ m/s}^2$ . MSS was determined using the methods previously described by Alphin et al. (2020).

2.4. Statistical analysis

All analyses were conducted in SPSS (IBM, Chicago, IL), and an alpha level of  $p < 0.05$  was used to determine significance. Data were first analyzed for normality using a Shapiro-Wilks test. Data were determined to be normally distributed, so parametric analyses were utilized. Changes over time in cortisol, wellness, and training load were evaluated using a repeated measures analysis of variance (RM-ANOVA). Partial eta squared ( $\eta^2$ ) effect sizes were calculated in conjunction with the RM-ANOVA. Effect sizes were interpreted as small (.01), medium (.06), and large (.14). An LSD post-hoc analysis was used to determine specific differences if the RM-MANOVA indicated a main effect for time. Due to the ordinal nature of the wellness data, a Friedman test was used to analyze differences over time for these data. The second aim of this study—relationships between cortisol, wellness, and training load—was assessed using repeated measures Pearson correlation (Bland & Altman, 1995). This analysis incorporates the dependence of multiple measures taken

over time. Correlation values ( $r$ ) were interpreted as low (.29), moderate (.49), and large (.50), (Bland & Altman, 1995).

3. Results

The RM-ANOVA indicated a main effect for time (Lambda(12,14) = 8.212,  $p < 0.001$ , partial  $\eta^2 = .876$ , large). Univariate analyses showed no difference over time for cortisol ( $p = 0.241$ , partial  $\eta^2 = .056$ , medium; wk 0:  $0.574 \pm 0.297 \mu\text{g/dL}$ , wk 2:  $0.701 \pm 0.481 \mu\text{g/dL}$ , wk 4:  $0.772 \pm 0.603 \mu\text{g/dL}$ ) as shown in Figure 1. Workload metrics across the three time points are shown in Table 1. There was a difference in HID ( $p = 0.001$ , partial  $\eta^2 = .283$ , large) and sprints ( $p = 0.028$ , partial  $\eta^2 = .148$ , large). Post-hoc analyses showed that HID was lower in week 2 ( $2660.4 \pm 770.3 \text{ m}$ ) compared to baseline ( $3593.4 \pm 917.4 \text{ m}$ ,  $p < 0.001$ ) and week 4 ( $3238.7 \pm 1560.3 \text{ m}$ ,  $p = 0.008$ ). The same was shown in sprints with lower repetitions in week 2 ( $22.9 \pm 10.2$  efforts) compared to baseline ( $27.4 \pm 12.5$  efforts,  $p = 0.013$ ) and week 4 ( $29.5 \pm 18.3$  efforts,  $p = 0.006$ ). There was no difference over time for total distance ( $p = 0.836$ , partial  $\eta^2 = .006$ ), accelerations ( $p = 0.152$ , partial  $\eta^2 = .073$ , medium) or decelerations ( $p = 0.087$ , partial  $\eta^2 = .093$ , medium).

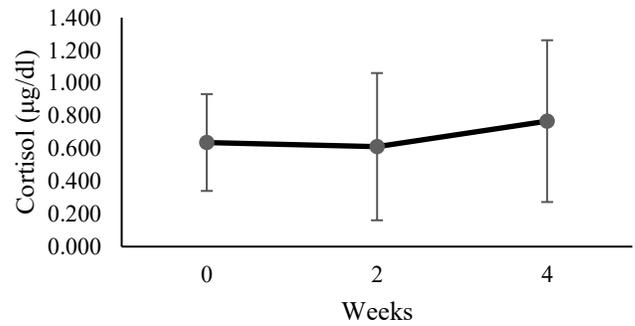


Figure 1: Mean ± standard deviation for changes in cortisol from baseline (week 0) to week 4 of the study.

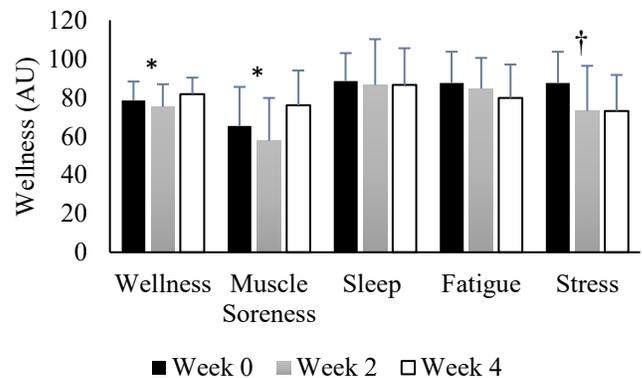


Figure 2: Subjective wellness scores across the assessment period. Note: \*Indicates a difference between week 4 and week 2 for wellness and muscle soreness,  $p < 0.05$ . †Indicates a difference between week 0 and weeks 2 and 4 for stress,  $p < 0.05$ .

Table 1: Training load values (mean  $\pm$  SD) over three time periods demonstrating the trends within each training load measure.

	Week 0	Week 2	Week 4
<b>Distance (m)</b>	27483.9 $\pm$ 4162.6	27402.5 $\pm$ 7593.1	26807.5 $\pm$ 6688.4
<b>HID (m)</b>	3593.4 $\pm$ 917.4	2660.4 $\pm$ 770.3*	3238.7 $\pm$ 1560.3
<b>Sprint (efforts)</b>	27.4 $\pm$ 12.5	22.9 $\pm$ 10.2*	29.5 $\pm$ 18.3
<b>Acceleration (efforts)</b>	542.2 $\pm$ 142.2	588.9 $\pm$ 203.6	536.5 $\pm$ 164.5
<b>Deceleration (efforts)</b>	151.6 $\pm$ 48.5	153.7 $\pm$ 61.8	134.9 $\pm$ 53.0

Note: HID, high-intensity distance. \*Indicates a significant difference from week 0 and week 4,  $p < 0.05$ .

The subjective wellness scores are shown in Figure 2. Higher values indicate better wellness, less soreness, improved sleep, less fatigue, and less stress. Analyses indicated time differences for wellness ( $p < 0.001$ ), muscle soreness ( $p = 0.001$ ), and stress ( $p < 0.001$ ). Pairwise comparisons showed week 4 scores were higher than week 2 for wellness ( $p = 0.001$ ) and muscle soreness ( $p = 0.011$ ). These data indicate that athletes felt better and had less muscle soreness in week 4. Athletes indicated lower feelings of stress at baseline compared to week 2 ( $p = 0.031$ ) and week 4 ( $p = 0.046$ ). There were no time differences for sleep quality ( $p = 0.917$ ) or fatigue ( $p = 0.109$ ).

Correlational analyses were all low and not statistically significant. For objective workload, cortisol had low correlations with HID ( $r = .158$ ,  $p = 0.168$ ) and sprints ( $r = .205$ ,  $p = 0.071$ ), and negligible correlations with distance ( $r = .043$ ,  $p = 0.708$ ), accelerations ( $r = .083$ ,  $p = 0.469$ ), and decelerations ( $r = .017$ ,  $p = 0.884$ ). For the subjective wellness measures, cortisol had low correlations with fatigue ( $r = .160$ ,  $p = 0.162$ ) and sleep ( $r = 1.53$ ,  $p = 0.181$ ) and correlations with wellness ( $r = .062$ ,  $p = 0.589$ ), muscle soreness ( $r = .801$ ,  $p = 0.485$ ), and stress ( $r = .100$ ,  $p = 0.385$ ).

#### 4. Discussion

The purposes of this study were to 1) evaluate changes in salivary cortisol in Division I female collegiate lacrosse athletes ( $n = 26$ ) and 2) assess the relationship between cortisol and athlete wellness and training load. There was a small non-significant upward trend in cortisol as the season progressed, and the athletes experienced increasing levels of stress within the body. This stress could be a result of stress induced from performance or game settings. However, cortisol did not correlate with wellness scores or objective load measures. The present study was conducted during the 2020 season which was halted early due to the COVID-19 pandemic. If carried out to completeness, this trend could be analyzed more thoroughly across the competitive season.

Previous literature showed no change in cortisol between in season and out of season training, but an upward trend was observed throughout the 13-week competitive season (Fields et al., 2020). A similar trend was also observed among collegiate female soccer players (Haneishi et al., 2007) and judo athletes (Filaire et

al., 2001). This trend is analogous to the slight trend present in our study. Previous literature in female swimmers and volleyball players showed a decline in cortisol throughout the competitive seasons (Roli et al., 2018; Santhiago et al., 2011). The conflicting evidence of cortisol response in female athletes provide evidence for more research and for a more consistent assessment in cortisol response. Studies are not consistent in the time parameters employed in measuring cortisol (e.g., weekly, bi-weekly, monthly), nor are they comparable in the timing of assessment as it relates to workload (e.g., beginning of the training week, end of the training week, before/after a recovery day). In the present study, salivary cortisol was collected biweekly at the beginning of the training week after a recovery day. The sample collection after a recovery day may have resulted in a reduced cortisol response. Moreover, the athletes represented in this study are also college students who engaged in a full academic workload. Stress from schoolwork was not evaluated in this study, or parsed out from the general stress question utilized in the daily wellness survey. Because college athletes also carry a role as a student, it may be useful to differentiate school-based stress versus physical stress.

According to Fields et al. (2020) weak relationships were established between physiological, hormonal, and psychological markers of load. Similarly, none of the workload variables evaluated within the current study correlated strongly with the cortisol levels. Additionally, no correlations were found between muscle soreness, sleep quality, or stress. Collectively, these studies indicate that cortisol levels do not follow the same trend as subjective ratings of stress. In a systematic review, Saw et al. (2016) concluded that subjective assessments of wellness were more sensitive to athletes' physical demands, recovery, and fatigue, than that of common objective measures. Utilizing daily and weekly subjective assessments are also a more cost-effective method to measure stress response and—with the right questions—may provide more specific data about the type of stress the athlete is experiencing (e.g., physical, personal, school-based). Similarly, data from subjective wellness surveys have been shown to predict the daily workload response in female lacrosse athletes, male American football players, male Australian football players, and elite male football athletes (Crouch et al., 2021; Gallo et al., 2017; Gastin et al., 2013; Govus et al., 2018; Malone et al., 2018). Cortisol assessment provides concept of

general physiological stress, but because it cannot be measured and evaluated at the same frequency and speed as subjective assessments, it may have less value for athlete workload management. Nevertheless, it is valuable to know the changes in physiological stress athletes experience throughout a competitive season, especially with some of the evidence suggesting that athletes' stress levels do return to baseline or near baseline levels with each training week (Fields et al., 2020; Roli et al., 2018; Santhiago et al., 2011).

A number of limitations may have affected the outcomes of our study. Due to the COVID-19 pandemic in the United States, the NCAA terminated the collegiate lacrosse competitive season after six weeks of competition. This provided this study with only three collection points worth of data. In addition, saliva samples were only collected on a biweekly basis. This could limit the validity of the trend by allowing too much time to pass between each collection time, therefore altering the results of this study. Other limitations that could have affected the outcome of this study was the small sample size and the reliance on the participants to strictly adhere to protocol.

## 5. Conclusion

This study sought to evaluate changes in salivary cortisol in Division I female collegiate lacrosse athletes and assess the relationship between cortisol and athlete wellness and training load. There was an upward trend for cortisol as the season progressed, but only small relationships with subjective assessments of workload and athlete wellness. There was also increased variation in cortisol in subsequent weeks from baseline, which warrants further analysis. These data agree with the one previously published finding indicating that cortisol did not have significant changes throughout the competitive season in collegiate female lacrosse athletes. Future research should examine these variables across a full-length competitive season with data collection occurring on a weekly basis. Analyses may also want to consider the occurrences on the student component for collegiate athletes and how that affects their cortisol and wellness. Coaches and support staff can then use this knowledge to help manage workload during practices in conjunction with their course load. These data may also be used to help educate and improve self-awareness of athletes and their stress.

## Conflict of Interest

The authors declare no conflict of interests.

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