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Original Research

Salivary Cortisol Analysis in Collegiate Female Lacrosse Athletes

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ABSTRACT

International Journal of Exercise Science 16(6): 242-251, 2023. Cortisol is a hormone that corresponds to physiological and emotional stress. The purpose of this study was to 1) evaluate the changes in cortisol in female Division I collegiate lacrosse players (n = 15) throughout the competitive season, and 2) evaluate the correlation between cortisol and athlete wellness and workload. Salivary cortisol samples were collected weekly in the morning throughout the entirety of the 2021 competitive season (12 weeks). Subjective athlete total wellness scores and sub-scores (muscle soreness, sleep quality, fatigue, and stress) were taken on the same days. Objective total weekly Athlete Load (AL, an amalgam workload metric) were tabulated from the previous training week. A significant effect of time was found on wellness ($p < 0.001$) and AL ($p < 0.001$) over the twelve weeks with weekly differences, such as weeks with more than one game, weeks with no games, weeks with students in quarantine (not competing), or weeks with academic stressors such as final exams. There were no weekly differences in cortisol ($p = 0.058$). Cortisol had negligible correlations with wellness ($r = -0.010$, $p = 0.889$) and AL ($r = 0.083$, $p = 0.272$) during the competitive season. These findings suggest that cortisol changed little for athletes throughout the season although training volume and wellness did. Thus, assessing acute responses of cortisol may prove to be more beneficial to evaluating athletes' stress.

KEY WORDS: Team sports; workload; stress; athlete

INTRODUCTION

Cortisol functions in metabolic activities including cardiovascular performance (e.g., regulation of blood pressure), immune system function (e.g., anti-inflammatory), and control of metabolic substrate selection (e.g., regulation of insulin) (25). Concentrations fluctuate with physiological and emotional stress over the course of the day with diurnal slopes. Typically, cortisol secretions are highest during the earliest part of the day, after the completion of the sleep cycle (25).

Previous research in cortisol and athletes have been conducted in elite male athletes (20, 21, 27), with new research providing some concepts of a stress response in female athletes. For female collegiate and youth athletes, salivary cortisol has been correlated with measures of athlete readiness and recovery, such as the countermovement jump and ratings of perceived exertion (3, 14, 18, 23). These concentrations have been shown to change throughout the competitive season, performance, and training (2, 13, 18, 20, 23). However, if the fluctuation in cortisol concentrations changes too drastically, it can harm the athlete's physical performance and mental health (15). Studies in soccer and volleyball female collegiate athletes indicated an elevated acute response to practice and games (14, 19). Cortisol concentrations throughout a competitive season have been shown to increase in elite competitive female swimmers (24), and decrease or show no change in elite female volleyball athletes (21) and collegiate female lacrosse players (7, 15). Fields and colleagues also showed weak inverse correlations between cortisol and heart rate variability (r = -0.232) and recovery (r = -0.185) (15). Carter and colleagues conducted a pilot study in female collegiate lacrosse that showed a low correlation between cortisol and training volume during the competitive season of female collegiate lacrosse athletes, but this study was limited by the presence of the COVID-19 pandemic (7). The present study will be similar to this, but will include a weekly assessment of cortisol rather than bi-weekly and will span the length of the competitive season. Collectively, these studies showed varied cortisol responses in collegiate female athletes in conjunction with training volume, recovery, and by sport, but with only five total studies evaluating cortisol in this population—two acute response and three chronic response—more research should be done.

Within the United States, lacrosse is a sport that is increasing in popularity (5). Previous literature has examined the collegiate game and training profiles (4, 11, 18, 23, 28), wellness scores (9), drill intensity analysis (1), and assessed the correlation between subjective and objective markers of athlete fatigue (16). These studies include internal and external load variables to evaluate training and game volume and intensity. External load metrics are typically measured through global positioning system (GPS) units and evaluate the mechanical load endured by the athlete. Examples of external load variables include total distance, sprints, accelerations, and decelerations. Internal load variables evaluate the physiological load of an athlete and include measures of heart rate and heart rate zones (4).

To date, the available literature has not provided a consistent understanding of the changes in physiological stress throughout the season. The purpose of this study was to 1) evaluate the weekly changes in cortisol in female Division I collegiate lacrosse players during the competitive season and 2) evaluate the correlation between cortisol and athlete wellness and Athlete Load (AL, an external load metric). We hypothesized that cortisol would be higher later in the season than early in the season, and that cortisol concentrations would moderately correlate with both AL and wellness. It was suspected that stress would increase as the season progressed towards the playoffs and as the athletes entered higher academic loads with final exams.

METHODS

Study design and ethical approval: This was a prospective observational study and was approved by the institutional review board. All participants completed a written informed consent and had the opportunity to ask questions before participating. This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science* (22).

Data were collected over 12 weeks during the competitive lacrosse season. The data collection period included 15 games and 54 practice sessions, averaging 1.25 ± 0.62 games/week and 4.5 ± 0.8 practices per week. Training sessions were typically two hours in length with some variation. Per National Collegiate Athletics Association (NCAA) guidelines, the team had one day off from practice and games each week.

Participants

Cortisol samples were taken from 15 female collegiate lacrosse players (attackers = 4, midfielders = 6, defenders = 5) who were recruited from a Division I lacrosse program. Participants were included in this study if they were 18 years or older, members of the varsity women's lacrosse team, and eligible to compete by academic standards. Participants were excluded if they were deemed ineligible for play by an athletic trainer or team physician and if they were not expected to play in at least 50% of the games during the season.

Measurements

Cortisol Analysis: Saliva samples were collected weekly on Friday mornings during the entirety of the competitive season, for a total of 12 samples per athlete. Research personnel gave participants the equipment for salivary collection on Thursdays. Participants provided the saliva sample first thing in the morning upon waking (between 6:30 a.m. and 6:50 a.m.) via passive drool. Participants refrained from eating, drinking, or activity prior to supplying the sample. The samples were collected by one person routinely on Friday mornings at 7:00 AM and were stored at -80° C per collection and storage protocol until analysis.

Saliva samples (25 µl) were analyzed for cortisol using a salivary cortisol ELISA kit (Salimetrics, State College, PA). Samples were thawed, vortexed, and centrifuged at 1500 x g for 15 minutes before adding to the ELISA plate. Samples were evaluated in duplicate using 96-well plates provided by Salimetrics, with appropriate standards and controls in each plate. Cortisol concentrations were determined using the manufacturer's instructions. The absorbance of the wells was measured at 450 nm 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 µg/dL to 0.012 µg/dL (Salimetrics, State College, PA). Per Salimetrics, the coefficient of variation for the ELISA kit ranges from 3-7%.

Athlete Wellness: Subjective athlete total wellness scores and sub-scores (muscle soreness, sleep quality, fatigue, and stress) were taken each morning shortly after waking between 6:30 a.m. and 10:00 a.m. This was done using a smart device linked to the 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?

The four questions were each scored accordingly, and then the average of the scores was tabulated to provide a composite wellness score. The daily composite wellness score was used for analysis. High scores indicated a more positive affect. The survey took approximately 1-2 minutes to complete.

Athlete Load: Athletes wore VX Sport (Wellington, New Zealand) GPS (collecting at 10 Hz) units to track objective training volume for AL. Data collection for external workload aligned with previous sport science literature (2, 4, 6). GPS units were inspected to ensure the proper working order and satellite connection before each training session and games. 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 and games, all data were uploaded to the VX Sport Training Tool software. Data were trimmed to remove inactive periods and split to supply data specific to the training plan provided by the coaches. AL—a proprietary VX Sport metric—was used to determine external volume. AL is an all-in-one metric that includes duration of the activity, total distance, high-intensity distance, and sprints. Daily AL values were collected for each athlete for all training and competition days.

Statistical Analysis

To address changes over time in alignment with the weekly cortisol values, athletes' daily wellness scores were calculated to create a weekly average and daily AL scores were totaled each week to account for weekly external load. There were errors during two games—one in week 6 and one in week 7—where AL was not collected. The mean game AL from the other 13 games was calculated and used in the weekly total for weeks 6 and 7 to account for these lost data.

Analyses were conducted in SPSS (Version 27.0, IBM, Chicago, IL) using an alpha level of 0.05 to determine the level of significance. Data were analyzed using a Shapiro-Wilks test for normality and deemed normally distributed, thus parametric analyses were performed. Weekly cortisol and training volume changes were evaluated using a repeated measures analysis of variance (RM-ANOVA). Partial eta squared (η_p^2) effect sizes were calculated and interpreted as small (0.01), medium (0.06), and large (0.14) (8). Specific differences between weeks were tested through univariate analyses and pairwise comparisons.

The second purpose of this study was to understand the relationships between cortisol concentrations, wellness scores, and training load. This purpose was carried out using repeated measures correlation analyses (3). Weekly cortisol values were correlated with the total of each training metric from the previous week and the average weekly wellness scores. Correlation coefficients were interpreted as low (< 0.30), moderate (0.30-0.49), and high (≥ 0.50).

RESULTS

The RM-ANOVA indicated a main effect for time for cortisol, wellness, and AL over the 12 weeks (*Lambda*(33,448) = 3.336, $p < 0.001$, $\eta_p^2 = 0.194$). Univariate tests demonstrated a difference over time in cortisol ($F(10,139) = 1.964$, $p = 0.042$, $\eta_p^2 = 0.123$), wellness ($F(10,133) = 4.267$, $p < 0.001$, $\eta_p^2 = 0.234$) and AL ($F(2,28) = 4.261$, $p = 0.019$, $\eta_p^2 = 0.248$). All effect sizes are interpreted as large. Cortisol concentrations (Figure 1) were highest during the first two weeks of the season compared to weeks 4-6 and 9-11 (p = 0.010 - 0.039). These results disagree with our hypothesis that cortisol would be highest later in the competitive season. Figure 2 shows that athletes had greater wellness scores during week 12 compared to weeks 3-11 ($p < 0.001$), and higher values during the first two weeks of the season compared to middle portions of the season (p = 0.004 - 0.034). AL was lowest during weeks 10 (p = 0.000 - 0.047) and 12 (p = 0.000 - 0.022) as depicted in Figure 3. Weeks 1, 2, 3, and 6 showed the highest AL scores (p = 0.000 - 0.047). It is important to note that as cortisol and AL decreased over the competitive season, wellness increased. Unlike wellness, both cortisol and AL trend upwards. As the competitive season proceeded, physiological cortisol concentrations fluctuated with the physiological work performed by the athlete, or the AL. However, wellness increased as these stressors decreased.

Repeated measures correlation analyses indicated negligible relationships between cortisol with AL ($r = 0.083$, $p = 0.272$) and wellness ($r = -0.010$, $p = 0.889$). These results do not support our hypothesis that cortisol would be moderately correlated with wellness and AL.

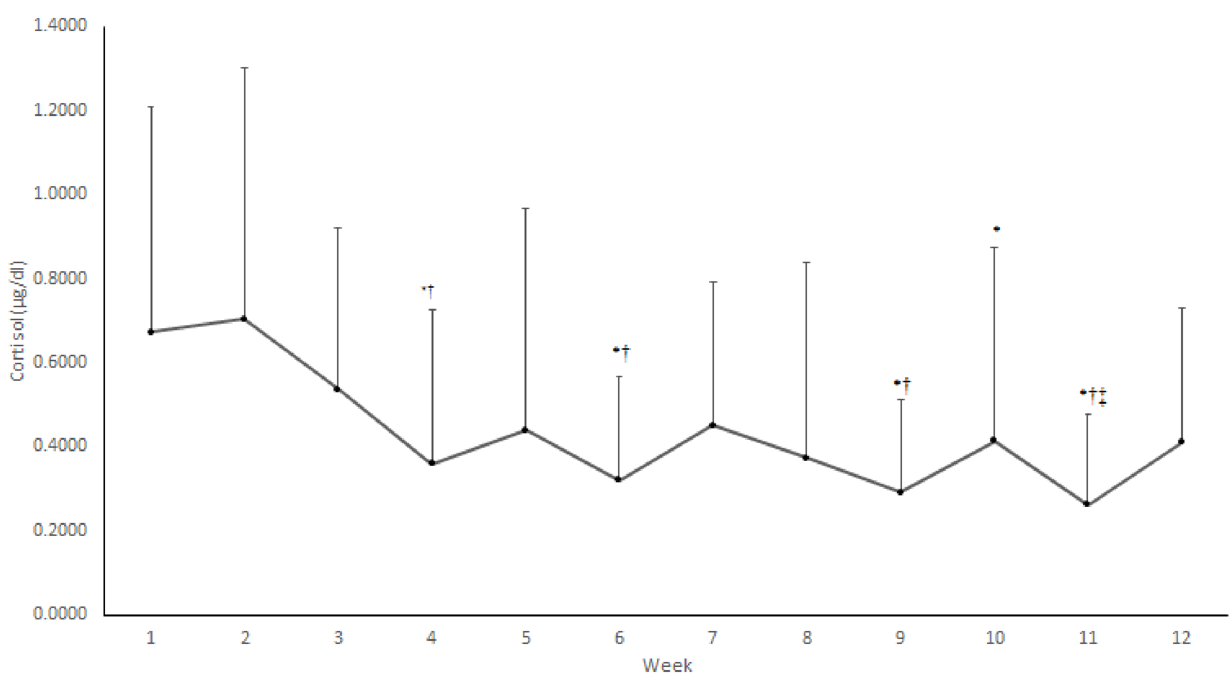

Figure 1. Weekly means and standard deviations for cortisol. * indicates a difference from week 1, † indicates a difference from week 2, and ‡ indicates a difference from week 3 ($p < 0.05$).

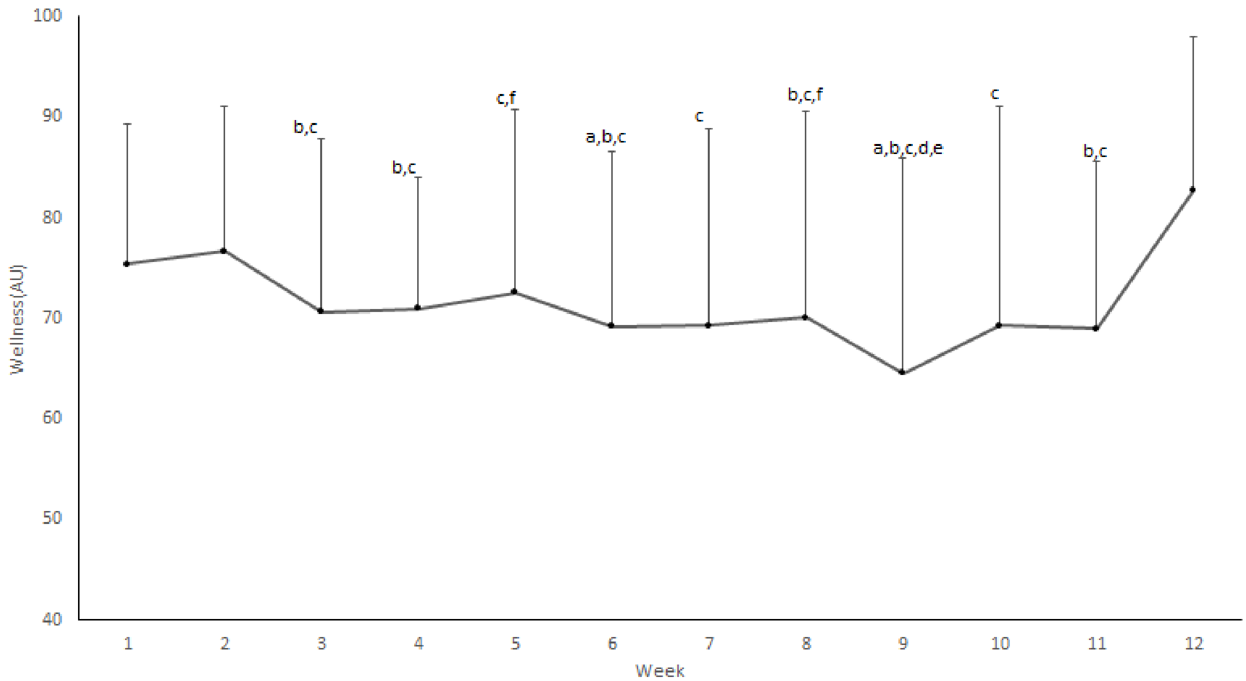

Figure 2. Weekly means and standard deviations for athlete wellness. a indicates different from week 1, b indicates different from week 2, c indicates different from week 12, d indicates different from week 5, e indicates different from week 8, f indicates different from week 9 ($p < 0.05$).

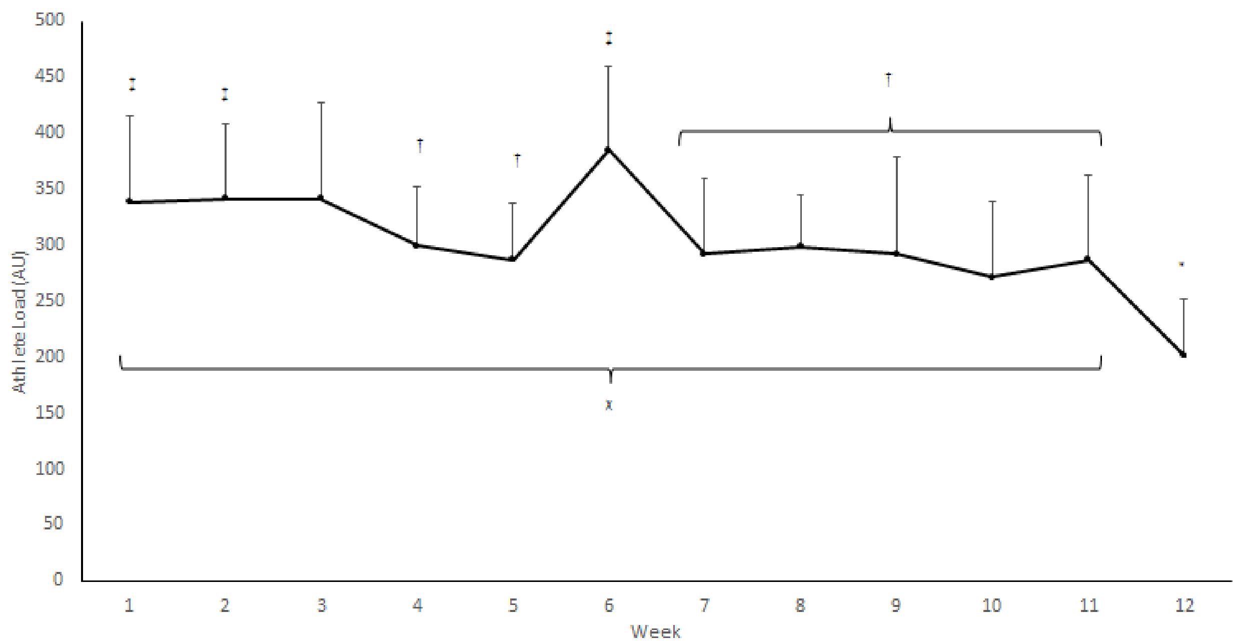

Figure 3. Weekly means and SDs for Athlete Load. * Indicates a difference from weeks 1-11, † indicates a difference from weeks 1, 2, and 6, ‡ indicates a difference from week 4, 5, 9-11, χ a difference from week 12 ($p < 0.05$).

DISCUSSION

The primary purpose of this study was to evaluate the changes in cortisol in female Division I collegiate lacrosse players. Cortisol decreased over the 12-week competitive season, with the highest values at the beginning of the season (the first two weeks) and slowly decreased over time, which was the opposite of our study hypothesis. Both cortisol and AL had downward trends over the 12 weeks while wellness remained even throughout the season, with a sharp increase in the final week. A second purpose of this study was to evaluate the relationship of cortisol with wellness and AL. Analyses indicated that cortisol had negligible correlations with AL and wellness, which does not support our study hypothesis that there would be a moderate correlation with these variables.

Cortisol was expected to be highest during the last weeks of the competitive season due to the multitude of stressors present as the team competed to keep their season going (21). In addition to academic stress of final exams, as well as other semester-end events (graduation or moving home), the athletes in lacrosse enter conference playoffs and perhaps NCAA playoffs. Managing final exams while playing to continue the season, seems stressful intuitively. While cortisol and AL showed a negative trend over the competitive season, overall wellness increased as the athletes approached conference championships and the end of the academic semester. The small weekly fluctuations in cortisol may be a result of varying numbers of games and practices throughout the week. Athletes participated in lacrosse six days/week throughout this observed period, but the number of games each week fluctuated. Weeks 9, 10, and 12 each had two games, but significant differences in cortisol and wellness were only noteworthy for week 9. This week displayed low cortisol and wellness, indicating low physiological stress, but high emotional stress or feelings of fatigue.

The cortisol concentrations measured in the present study were between 0.268 and 0.720 µg/dl and previous literature in a similar population showed higher season cortisol ranges of 0.611 to 0.767 µg/dl (7). The previous study ended early due to the global pandemic, thus the higher values seen near the end of the observed period may have been related to stress created from a pending pandemic because there was no correlation with cortisol and training load. The present study showed higher cortisol values early in the season followed by a steady decline. The greater physiological stress early may be attributed to the athletes adjusting to a higher training volume as the team transitioned from 8-hour training weeks, that were not included in this study, to 20-hour training weeks that were included. The present study began at the onset of the 20-hour training weeks, so we can only speculate about less physiological stress during the 8-hour training weeks. As the study observation period progressed, the athletes could have settled into a routine in these more intense training weeks, resulting in a decrease in physiological stress. In addition, the stress athletes experienced was perhaps influenced by nervousness associated with the beginning of a new competitive season, academic demands, personal demands (10), or related to the COVID-19 pandemic. COVID-19 has changed the way that activities of daily living occur such as social distancing, prolonged quarantine, and minimal social activities. This has been proven to have negative effects on mental health and overall success of college students

(26). During the present study, athletes were regularly screened for COVID-19 symptoms and only allowed to be in close contact with living partners. The athletes could also have experienced less pandemic-related stress as the new procedures became commonplace with the progression of the competitive season (12).

The data from the present study coincide with previous research from similar populations (7, 15). Fields and colleagues determined that there was no correlation between cortisol and internal load measured by heart rate variability (15), and Carter and colleagues also showed that cortisol concentrations were not correlated with external load measures such as total distance, high-intensity distance, sprints, accelerations, and decelerations (7). The first three of these external measures are used in the AL calculation from the present study. Notably, cortisol did not correlate with any of these external load measures. Carter and colleagues also indicated that cortisol did not correlate with wellness scores or sub-scores in muscle soreness, fatigue, sleep quality, or stress (7). These two studies indicate that, for collegiate female athletes, physiological stress does not align with subjective assessments of wellness. Conversely, Fields and colleagues showed cortisol concentrations greatly fluctuated across the competitive season and correlated with objective measures fatigue and recovery (15). It may therefore be possible that measures of fatigue and wellness do not follow the same time course. While wellness has been shown to be responsive with game load (17), it may be that subjective wellness has a delayed response to physiological stress. An acute assessment of cortisol concentration and athlete wellness may help to answer this question.

Limitations of this study include the small sample size and the lingering effects of the COVID-19 pandemic on overall morale and operations as compared to an athletic season where pandemic-related precautions did not have to be set in place. Another limitation is the game data that were missing from weeks 6 and 7. To overcome this loss, the mean game AL from 13 games was added to each athlete's AL for those weeks. This lack of game data was a result of technical and human error and may have impacted the correlation analyses. The use of the proprietary AL metric does limit comparison to other studies or equipment that do not have this type of metric. Lastly, we did not collect information to include the athletes' menstrual cycle or status of birth control. This may provide insight into weekly changes in performance, wellness, and stress response. A strength present in this study was the rigor surrounding the protocols for the saliva samples. The athletes were aware of and understood how to utilize the saliva kits and the exact times of when to produce a sample.

In conclusion, cortisol had negligible correlations with wellness and AL across the competitive lacrosse season. We speculate that athletes from this population experienced physiological stress due to factors not addressed in the wellness surveys and training. Because the athletes were students who are living away from family members, it is likely that their physiological stress may primarily be from academic or personal stress. Cortisol and AL tended to decline with each week of the season, with some exceptions. The results of this study are useful for coaches, athletic trainers, and athletes to understand the importance of routine monitoring and that physiological stress was not different in the beginning of the season compared to the end.

Routine monitoring is used to avoid overtraining, reduce the risk of injury, and optimize the strength and training regimens put in place by professionals (13). Coaches working with this population may also want to consider evaluating academic load and stress. The results do not support the correlation between cortisol and external load or wellness, indicating further analysis of physiological stress may be warranted. However, to note that the athletes did not have increases in cortisol throughout the season, rather a slight decline, indicates that the athletes' stress was reduced throughout the season. These data do suggest further inquiry into common stressors that collegiate female athletes experience outside of their sport. Some concepts of interest include assessing acute responses to training and games, rather than a chronic response. Additionally, evaluating correlations between cortisol and training intensity, instead of volume, may also be of interest.

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