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Weekly Salivary Biomarkers across a Season for Elite Men Collegiate Basketball Players

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ABSTRACT

International Journal of Exercise Science 11(6): 439-451, 2018. The purpose of this study was to monitor weekly the salivary free testosterone (FT), cortisol (C), and the FT/C ratio in elite men NCAA Division I basketball athletes across an entire season. Twelve athletes gave salivary samples for 30 consecutive weeks, beginning in the pre-season and ending one week after the end of post-season competition. Samples were assayed for FT and C. Additionally, a composite value (CBD) composed of Z-scores for weekly practice minutes, game minutes, resistance training repetitions, academic demands, and travel schedules was determined. One-way RM ANOVAs were used to determine which weekly values were different (α=.05) from the season mean. For FT, 10 weeks were different from the season average (5.1 nmol/L). For C, 11 weeks were different from the season mean (9.0 nmol/L). For FT/C, weeks 7 (p=.007), 17 (p=.007), and 25 (p=.005) were different from the season mean (FT/C=0.69). During Wk7, at the start of regular season play, FT/C was above, while CBD was below the season means. During Wk17, which was leading into a series of important conference games, FT/C was below, while CBD was not different from season means. During Wk25, which was one week before the conference tournament, FT/C was below, while CBD was above season means. The methods of this study can be used for monitoring athlete responses and adaptations to sport-specific training and competition; specifically, for evaluating responses to challenging pre-season training, recovery for regular season play, and how they physiologically cope with the competitive season.

KEY WORDS: Hormones, biomarkers, stress, performance

INTRODUCTION

In response to a single stressful training session, the hormone testosterone (T) will slightly increase or decrease (5). Conversly, the hormone cortisol (C), will typically experience a large increase, resulting in a decrease in their ratio (5). The free testosterone/cortisol ratio (FT/C) is the amount of T in its unbound or free form, divided by the amount of C. In some instances, the total amount of testosterone (TT) is examined instead, resulting in the ratio of TT/C. Numerous investigations suggest that strength, power, and athletic preparedness are positively correlated with T and these T/C ratios in male athletes, irrespective of the time during the season in which they are evaluated (1, 3, 6, 9, 10, 13-16, 24, 30, 31). That is, when measures of performance are favorable, T and T/C are typically elevated. As such, the ratios between these two hormones are often considered important endocrine markers for performance. However, this may be an oversimplification, as it should be noted that correlations do not imply causality (21). Regardless, it still may be a useful biomarker for stress management in athletes (5, 8-10, 14, 20, 23, 25, 28, 30, 31).

As training stress increases over time, the T/C ratios tends to decrease. With a taper, or reduced training stress, the ratio will usually return to baseline or increase above baseline due to super-compensation (5, 24). However, in instances of non-functional overreaching, an athlete's performance and hormonal markers will merely return to baseline after a taper (23), suggesting that T/C ratios may help indicate the effectiveness of a peak-taper cycle.

While many studies have addressed the relationship between T/C ratios and training and competition stress in strength and power athletes in the lifting sports (1, 8-10, 13-16, 21, 31), there have also been investigations observing changes in these hormones for athletes from sports with competing physiological demands (i.e., anaerobic power, muscular endurance, aerobic endurance) (20, 24, 28, 30). Significant correlations have been reported between the T/C ratios and the performances or training adaptations for collegiate soccer (20), track and field (24), rowing (28), and American football (30). Conversely, these hormonal ratios were not associated with winning or losing in wrestling (11), or with non-functional overreaching in American football (23).

Few studies have attempted to monitor T, C, and T/C ratios in elite male basketball players. One investigation observed changes in T, C, and T/C in elite professional basketball players during a 4-week training camp (18). While significant increases in C were found from the beginning of the camp to the end, there were no observable changes in T or T/C (18). Schelling et al. sampled serum T and C from elite professional basketball players at 8 time-points throughout a competitive season (27). Testosterone levels and T/C were significantly lower at the later collection dates, while C remained the same throughout the season, suggesting the stresses from the training and competition schedules were reflected in the hormonal ratios (27).

To date, the majority of studies attempting to use T/C ratios to monitor athletic performance have used serum or plasma to measure these hormones. However, saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (3, 19, 22, 25, 29, 32). It should be noted that most of the T measured in saliva is in the unbound or free state by the time it reaches this biocompartment, although it is still indicative of T in the blood (3). The use of saliva as a means of evaluating T and C has increased in recent years. This methodology allows researchers to collect data simultaneously from a group of athletes in a non-laboratory setting (12, 17, 30), minimizing interference in the athletes' training (12). It also facilitates frequent hormonal data collection from athletes (12, 26) , even allowing week-to-week monitoring across an entire competitive season (2).

As noted above, the literature has shown that T/C can relate to training stress in anaerobic athletes and team sport athletes with multiple physical demands, including professional basketball players. However, no studies have addressed additional aspects of training and off-court demands weekly throughout an entire basketball season. Therefore, the purpose of this study was to monitor weekly salivary biomarkers (i.e., free T, C, and their ratio) in elite men NCAA Division I basketball players throughout an entire season. Additionally, the salivary biomarkers were compared to other demands for collegiate basketball training and competition (i.e., practice minutes, game minutes, resistance training repetitions, travel schedules, and academic demands), to examine their relationships.

METHODS

Participants

This study was observational and time-series in nature. The athletes performed their normal training, competitions, and non-sport activities as assigned by the coaching staff. Additionally, each gave a weekly salivary sample immediately upon arriving for a regularly-scheduled afternoon practice across the 30 weeks of an entire training and competition season**.** Finally, data regarding weekly playing and practice time, resistance training volume, travel, and academic demands, were also collected.

Twelve men NCAA Division I American collegiate basketball student-athletes volunteered to serve as subjects for research purposes. The study was approved by the University Human Subjects Committee and was performed in cooperation with the basketball coaching staff. All subjects gave written informed consent to participate in this study as approved by the Institutional Review Board at the University.

Protocol

Student-athletes gave salivary samples immediately prior to an afternoon practice in the middle of the week for 27 different weeks within a 30-week season. Saliva was utilized instead of serum since it permitted simultaneous sample collection for all subjects, in the field, in a non-invasive manner. Sample collection began one week prior to the beginning of pre-season practice and concluded one week after the end of post-season competition. All salivary collections were conducted with the participants at rest, in the afternoon (1300-1500 hrs), in the middle of the week. The participants were instructed not to eat, brush their teeth, or consume any drinks other than water for one hour prior to giving salivary samples. When the student-athletes arrived for the mid-week afternoon practice, they rinsed their mouths out with water and sat quietly for 5 minutes. Participants were then given oral swabs (Salimetrics Oral Swab, Salimetrics, PA, USA) to place and hold in their mouths. After two minutes, the swabs were released directly into a centrifuge tube. These samples were immediately frozen and stored at -80° C for later analysis.

Salivary free T and C were analyzed using enzyme immunoassay kits using 96-well microtitre plates (Salimetrics, PA, USA). A separate assay was used for each student-athlete, with all samples, standards, and controls analyzed in duplicate. Resulting absorbances were measured with an automated plate reader and software (KC4, Biotek Instruments, Winooski, VT, USA). Salivary measures of FT and C have been shown to be highly correlated to values from plasma (3) or serum (19, 29), and have previously been suggested as valuable indicators for sport science (25).

In addition to the saliva samples, the following data was also recorded throughout the season: playing time (minutes), practice time (minutes), resistance-training volume (total repetitions), travel schedules (scale), and academic demands (scale). Resistance-training volume was calculated as total repetitions completed within a workout. Typical exercises included included barbell back squat, barbell bench press, barbell deadlift, barbell power snatch, barbell power clean, barbell power jerk, and dumbbell presses and rows. The travel scale was assigned by the team coaches such that a score of "1" was given for an overnight bus trip where they returned before midnight, a "2" was given for an overnight flight, and a "5" was given for a multiple day trip with flight. After consultation with the coaches, it was determined that this scale fairly represented how challenging the travel would be for the athletes. The academic demands scale was generalized so that a score of "0" was given when college classes were not held, a score of "2" was given during normal academic weeks, and a score of "4" was given during exam periods. Both travel and academic scores were averaged for each week. Additionally, a composite value composed of Z-scores for playing time, practice time, resistance-training volume, travel schedules, and academic demands was used in an attempt to quantify weekly cumulative demands so that an increase in the score indicated an increase in cumulative demands. All variables were converted to Z-scores to standardize each player's values, and a comprehensive Collegiate Basketball Demands score (CBD) was compiled from all of the various demands.

Statistical Analysis

Statistical tests were conducted using IBM SPSS Statistics 24 (SPSS Inc., Chicago, Illinois, USA). Of 324 total samples for the 30-week period, 20 individual samples were missing due to players being absent from practice. As has been previously suggested (4), missing values were replaced with the team average for that week. After verifying all assumptions had been met, one-way repeated-measures analyses of variance with LSD pairwise comparisons were used to determine which weekly values (FT, C, FT/C, and CBD) were different from the season mean. Pearson correlations were used to determine relationships between team FT, C, or FT/C and team CBD scores, and their individual components, throughout the season. Significance was determined *a priori* ($\alpha < .05$).

RESULTS

The overall ANOVA models were significant for FT ($F[27, 297] = 2.832$, $p < .001$, $n_p^2 = .205$), C ($F[27, 297] = 5.957$, $p < .001$, $n_p^2 = .351$), FT/C ($F[27, 297] = 2.660$, $p < .001$, $n_p^2 = .195$), and CBD ($F[27, 297] = 335.465$, $p < .001$, $n_p^2 = .968$). The observed power was 1.0. For salivary FT, 10 weeks were different from the season mean (0.51 nmol·L^{-1}), for salivary C, 11 weeks were different from the season mean (9.0 nmol·L^{-1}), and for FT/C, weeks 7 (p=.007), 17 (p=.007), and 25 (p=.005) were different from the season mean (FT/C=0.069). A significant negative correlation (r = -0.47; r_{crit} = 0.38; $p < .05$) was observed between FT/C and game minutes. No other correlations were statistically significant. Inter- and intra-assay variances (CV) were 4.2% and 3.7% respectively for FT, and 3.6% and 3.2% respectively for C. All hormone values fell within the normal physiological range for male college students.

During Week 7, at the start of regular season play, FT/C was more than 3 standard deviations (SD) above, and CBD was significantly below the season averages. During Week 17, which was at the beginning of a series of important games, FT/C was more than 2.5 standard deviations below the season average, while CBD was not different. During Week 25, which was one week before the conference tournament, FT/C was more than one standard deviation below, and CBD was significantly above the season averages. All data are reported in Tables 1 and 2 and Figures 1-5.

Table 1. Weekly hormonal concentrations (Mean ± Standard Deviation).

Week	1	2	3	4	5
FT(nmol/L)	.52±.06	.50±.12	.50±.09	.58±.24	.57±.23
C (nmol/L)	9.5±6.4	7.4±2.7*	7.2±4.4	9.6±4.8	9.2±5.2
FT/C	.073±.039	.076±.031	.085±.035	.071±.033	.074±.032
Week	6	7	8	9	10
FT(nmol/L)	.47±0.06	.51±.31	.51±.15	.51±.17	.50±.09
C (nmol/L)	6.9±2.7**	5.5±3.3**	8.0±3.9	8.5±6.8	6.9±2.6**
FT/C	.078±.031	.110±.050**	.073±.027	.077±.032	.077±.032
Week	11	12	13	14†	15
FT(nmol/L)	.52±.09	.51±.09	.58±.11*	n/a	.59±.10*
C (nmol/L)	9.0±4.6	8.9±4.1	11.6±5.1	n/a	13.1±5.8*
FT/C	.070±.031	.067±.027	.058±.025	n/a	.053±.026
Week	16	17	18	19	20
FT(nmol/L)	.37±.08**	.69±.25*	.49±.07	.50±.17	.50±.16
C (nmol/L)	6.9±3.1	20.3±8.9**	7.7±2.4	7.8±4.0	7.8±3.2
FT/C	.060±.20	.041±.25**	.070±.27	.073±.29	.073±.25
Week	21	22	23††	24	25
FT(nmol/L)	.40±.08**	.40±.08*	n/a	.39±.06**	.41±.05**
C (nmol/L)	7.6±3.0	6.9±2.8*	n/a	6.9±2.1**	8.1±3.0
FT/C	.058±.23	.062±.19	n/a	.061±.19	.056±.17**
Week	26	27	28	29††	30
FT(nmol/L)	.59±.42	.43±.07*	.62±.14*	n/a	0.49±.11
C (nmol/L)	10.5±6.1	6.4±2.2**	17.7±12.6*	n/a	7.1±3.1*
FT/C	.063±.36	.075±.25	.050±.34	n/a	.078±.30

Different from the season mean (*p<.05; **p<.01; † Holiday break; ††Travel)

Table 2. Correlations between Collegiate Basketball Demands (CBD) components and hormones.

	CBD Composite Score	Game Minutes	Practice Minutes	Resistance Training Volume (reps)	Academic Demands	Travel Demands
Free Testosterone	-.02	-.13	.13	.16	.02	-.31
Cortisol	.13	.18	.19	.21	-.16	-.07
FT/C Ratio	-.17	-.47*	-.06	-.13	.21	-.16

*p<.05; r_{crit}=.38

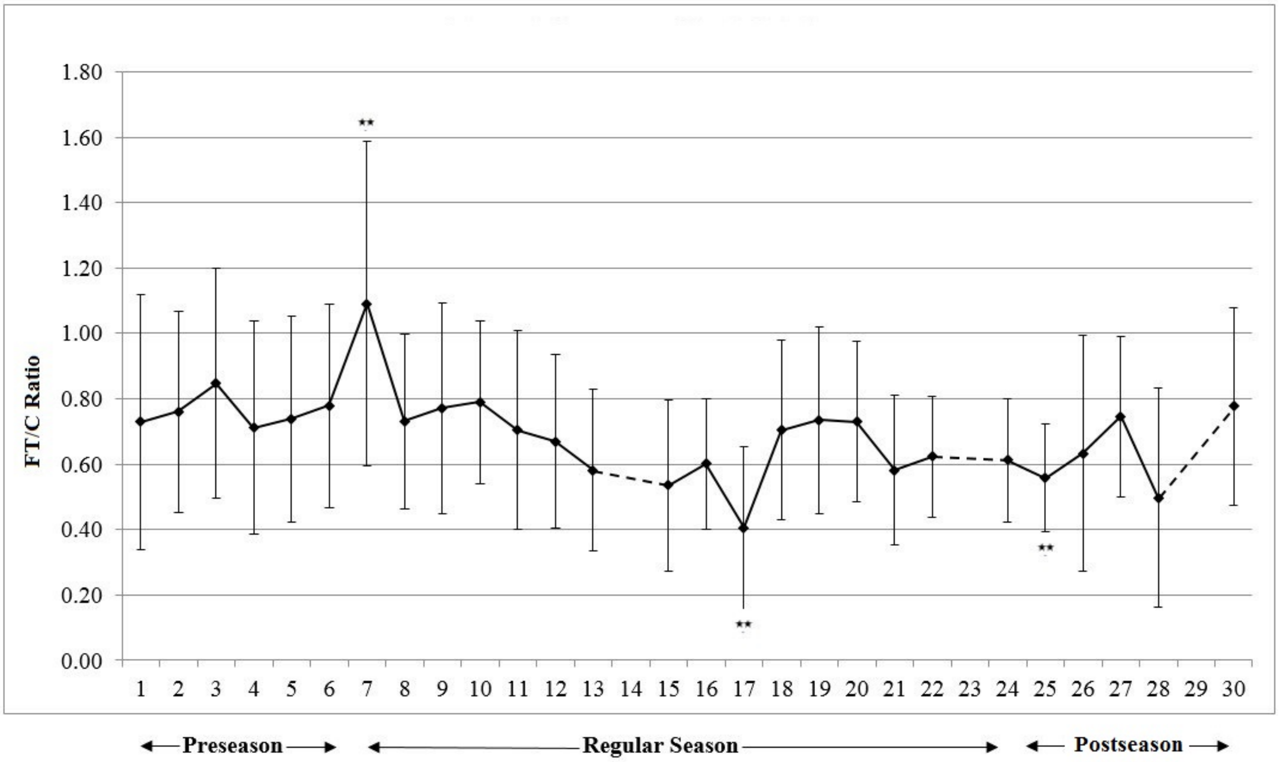

Figure 1. The free testosterone/cortisol ratio across the season (**p*<.05; ***p*<.01).

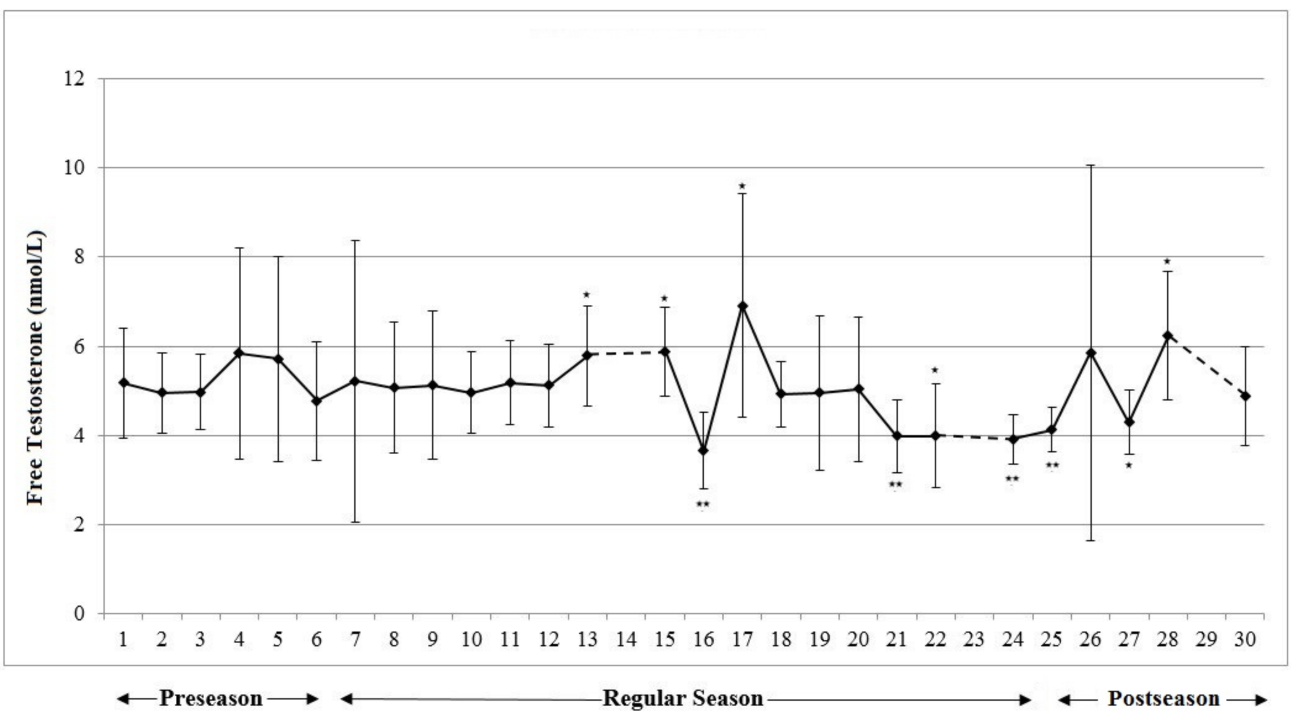

Figure 2. Testosterone concentrations across the season (**p*<.05; ***p*<.01).

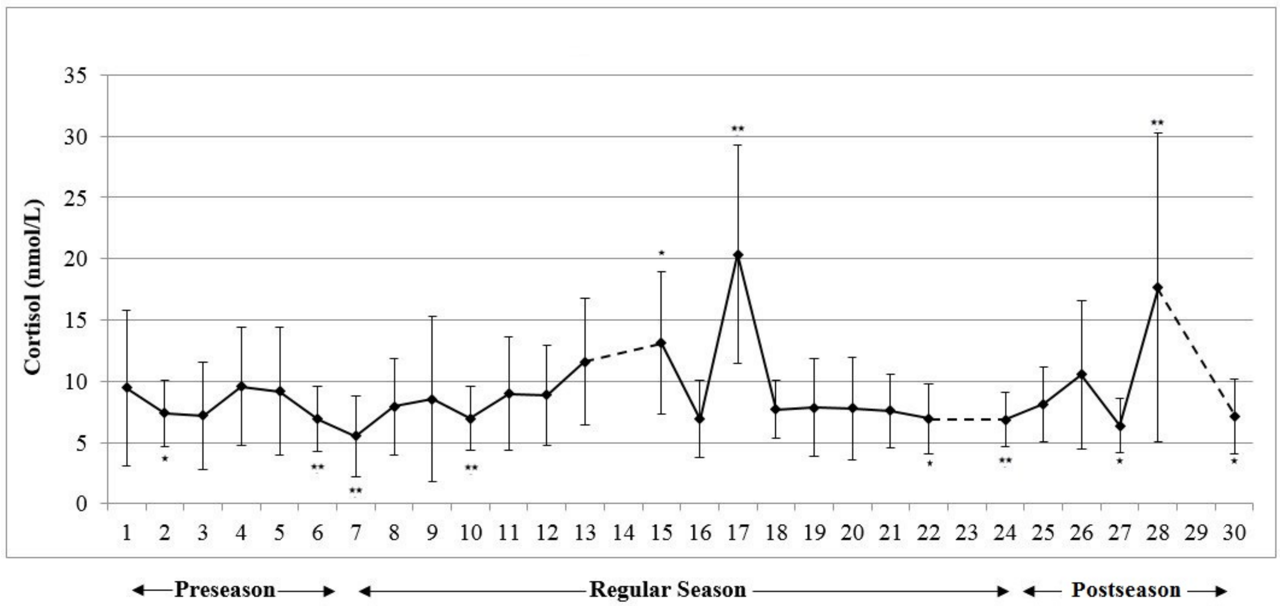

Figure 3. Cortisol concentrations across the season (*p<.05; **p<.01).

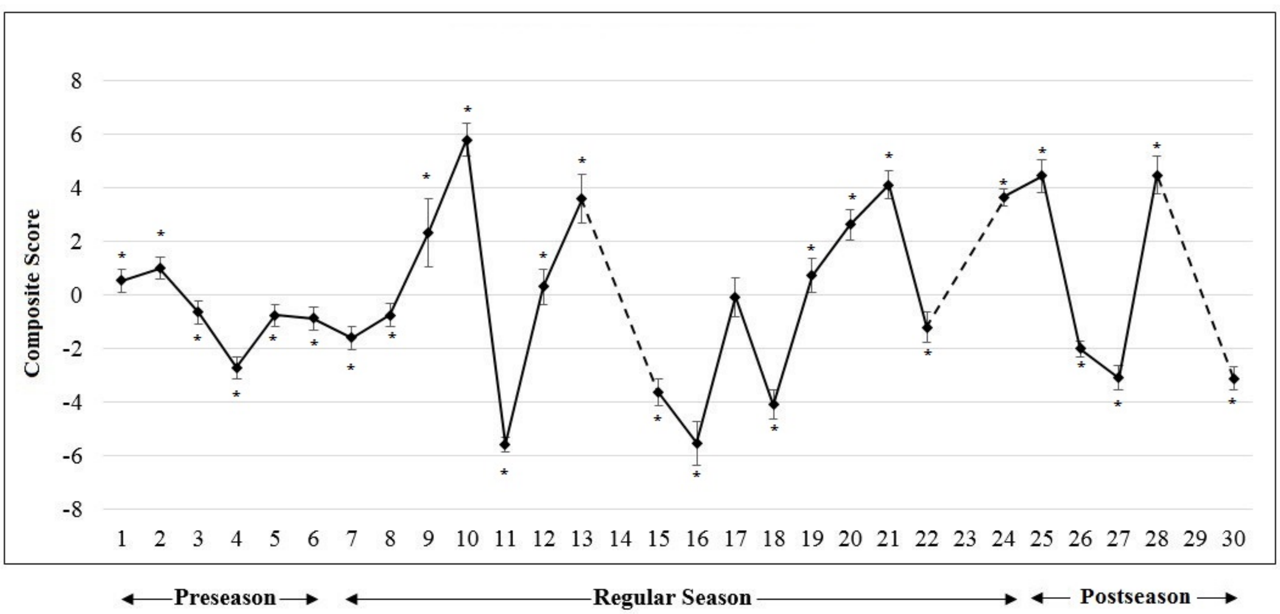

Figure 4. College Basketball Demands composite scores across the season (*p<.05).

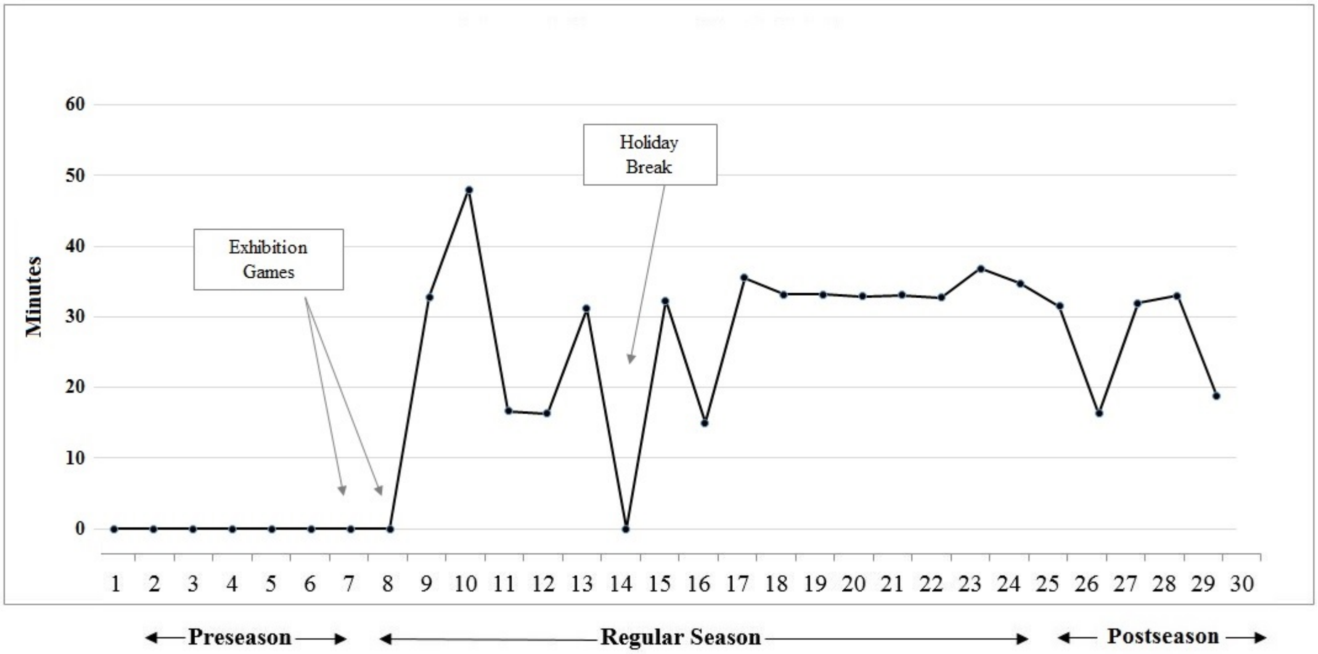

Figure 5. Weekly Game Playing Minutes (r=-.47 p<.05).

DISCUSSION

The results of the present study demonstrate that monitoring salivary testosterone and cortisol was an appropriate method for monitoring stress management of elite male National Collegiate Athletic Association Division I basketball athletes across pre-season preparation and a competitive season. Several interesting phenomena were observed. First, the large increase in T/C at Week 7 suggested that these athletes were recovered from stressful pre-season training and physiologically prepared for the first week of regular season competition, which occurred several days after those samples were collected. The coaches reported an intentional taper-peak cycle during the weeks leading up to Week 7.

The observed levels of C during the preseason of the present study were similar to Hoffman et al. (18), who reported that C was elevated by the stressful preseason training camp while T and T/C did not change during the training camp. It would have been interesting to see if the athletes in the Hoffman et al. study (18) experienced a similar increase in T/C following a taper as observed in the current investigation.

Contrary to what Schelling et al. (27) observed among professional basketball players, the athletes in the present study did not experience an increase in T after a taper despite an increase in T/C. However, that investigation measured total T (27), while the current study examined free testosterone (FT). Therefore, it is possible that the difference may have been due to the form of T measured, variations in the tapers, or perhaps even the level of athlete (professional versus collegiate).

The current investigation is consistent with reports for other athletes where T/C ratios returned to baseline after a taper (23) or super-compensated above baseline after a taper (24), suggesting a super-compensatory effect of the peak-taper cycle. Interestingly, Nelson and colleagues (24) examined NCAA Division I nationally-qualified track and field throwers and jumpers, which are strictly strength and power athletes. However, the athletes in the current investigation experienced a similar response to a taper-peak cycle, despite the different physiological demands of NCAA Division I basketball.

Monitoring changes in T/C may be important because if off-season or preseason training is too stressful, the athletes may not recover in time for optimal performance during competition, or may simply not sufficiently adapt to the preceding program. For example, Moore and Fry (23) monitored off-season collegiate football players, who experienced non-functional overreaching. Both performance and hormonal markers merely returned to baseline after a reduction in training volume (23). This may indicate an ineffective attempt to increase performance and athletic preparation (23), whereas the results of the present study suggest an effective taper-peak cycle.

The decrease in T/C at Week 17, despite a 13-game winning streak, suggests that the season was beginning to have a detrimental physiological effect on the student-athletes. This was further supported by the statistically-significant negative correlation between T/C and playing minutes during this phase of the season, demonstrating that the weeks with more playing time also had a decrease in T/C. However, this is not necessarily a causal relationship, therefore it cannot be determined whether increased playing time caused the decrease in T/C, but rather that they only both occurred simultaneously. Despite a brief three-game losing streak during Weeks 20 and 21, T/C was not significantly altered. These results are consistent with other research demonstrating that, while T and C can both be affected by winning and losing, T/C is not necessarily impacted (11). Finally, following a decrease in T/C before post-season competition and a trend towards a decrease in Week 28 (p=.073; 1.5 standard deviations below baseline), the athletes were able to return to hormonal baseline one week after the end of the season. This is similar to the results of Schelling et al. (27), who found that T levels and T/C were significantly lower at the later collection dates, while C remained the same throughout the season. However, in the present study, C varied significantly throughout the season, although it did decrease below baseline within 2 weeks of the end of the post-season competition. This suggests that while the competitive efforts of male NCAA Division I basketball athletes may be stressful, they recover quickly after the end of the season. However, as noted above, Schelling and colleagues (27) monitored professional athletes, whereas the present investigation examined student-athletes, suggesting that this population may experience more variability in C than their professional counterparts.

While some may propose that a large decrease in T/C indicates a state of overtraining syndrome, Fry and colleagues (7) have demonstrated that T/C is not necessarily affected despite the onset of an overtraining syndrome. Therefore, while changes in T/C may reflect acute physical stress, definitive statements about overtraining syndrome cannot be made with this population by simply monitoring T/C. Still, it is advisable to monitor T/C, as it may reflect the health, recovery, preparedness, and physiological status of the athletes, independent from potential diagnoses of an overtraining syndrome. Therefore, biomarker monitoring may provide coaches and scientists useful information for evaluating the effectiveness of recovery strategies and stress-management techniques.

The methods of the current study, specifically the frequency of salivary sampling, may explain some of the differences between this investigation and others (18, 27) that also measured hormones in elite male basketball athletes. The present investigation examined T, C, and T/C weekly, from preseason to after postseason, while the other research (18, 27) observed fewer time-points. Hoffman and colleagues (18) examined their athletes' hormonal levels four times during a four-week off-season training camp. While this was similar to the present study in that there were weekly observations, it only accounted for off-season training and not the stressors of the regular season or postseason play (18). Schelling et al. (27) observed eight time-points throughout an eight-month period, stating that collections were taken every four to six weeks. Had they (27) monitored weekly, more variation in C may have been observed. Therefore, studies attempting to monitor hormonal changes in elite basketball players should consider weekly collections, as done in the present investigation, in order to obtain a better representation of potential variation across a season.

The creation and use of the CBD was intended to provide additional information about how various demands and challenges experienced by the student-athletes throughout the season may relate to T/C. However, as is visually evident in Figure 4, the score was fairly volatile across the season. It did not have a close relationship with T/C, and therefore, as a composite of the various measured demands, the CBD did not provide the anticipated insight. Yet, when each of components of the CBD was examined individually, a statistically significant inverse correlation was found between T/C and game playing minutes (Table 2 and Figure 5). Future research which more closely examines this relationship should be considered.

A limitation to this investigation is the small sample size. However, the design of the study and the use of a collegiate basketball team necessitated the use of the convenience sample. Similar hormone studies utilizing basketball teams have also been limited by the number of players on the team (18, 27). Additionally, we were unable to access injury/illness or medication data, which could have impacted stress and recovery.

Strength and conditioning coaches and sport coaches should be aware of the in-season and post-season stressors of elite collegiate basketball, and should adjust training to allow for optimal recovery. For example, strength coaches working with elite basketball athletes and the team basketball coaches should consider working together to increase resistance training volume and practice minutes during preseason, but also leave enough time for reduced resistance training volume and practice minutes to affectively create a taper into the competitive period. Using data to determine the correct length and volume of the peak-taper may be valuable for different teams. Attempting to monitor stress is worthwhile, as changes in T/C may not only affect/reflect performance, but may also affect/reflect athlete health and student-athlete academic performance. Future studies should attempt to collect basic performance data (e.g., vertical jump height) after each specimen collection, to examine whether changes in performance variables relate to changes in salivary hormones.

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