

SALIVARY BIOMARKER ASSESSMENT OF ELITE COLLEGIATE BASKETBALL  
PLAYERS ACROSS AN NCAA SEASON

By

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## ABSTRACT

The ratio between testosterone and cortisol (TC) has been used to monitor training stress and performance in athletes. **PURPOSE:** To monitor free testosterone (T), cortisol (C), and the ratio of testosterone to cortisol (TC) in elite NCAA Division I basketball athletes, weekly, throughout an entire season. **METHODS:** Twenty-two athletes (12 male, 10 female) gave a salivary sample before an afternoon practice in the middle of each week for 30 consecutive weeks, beginning in the pre-season and ending one week after the end of post-season competition. Salivary samples were assayed for T and C. Additionally, a composite value composed of Z-scores (COMP) for weekly practice minutes, game minutes, resistance training repetitions, academic stress, and travel stress was used in an attempt to quantify weekly cumulative stress so that an increase in COMP suggested an increase in cumulative stress. One-way RM ANOVA with LSD pairwise comparisons were used to determine which weekly values were different ( $\alpha=.05$ ) from the season average. **RESULTS:** For T, 10 weeks were different from baseline (5.1 nmol/L) for men while 4 weeks were different from baseline (2.2 nmol/L) for women. For C, 11 weeks were different from baseline (9.0 nmol/L) for men while 4 weeks were different from baseline (6.5 nmol/L) for women. For TC, weeks 7 ( $p=.007$ ), 17 ( $p=.007$ ), and 25 ( $p=.005$ ) were different from baseline (TC=0.69) for men while weeks 6 ( $p=.004$ ), 16 ( $p=.024$ ), 24 ( $p=.003$ ), and 27 ( $p=.008$ ) were significantly different from baseline (TC=0.42) for women. **CONCLUSIONS:** The large increase in TC at Wk7 (men) suggested that these athletes were recovered from stressful pre-season training and physiologically prepared for the first week of regular season competition. The decrease in TC at Wk17 (men), despite the current win-streak, suggests that the lengthy season was having a physical effect on the student-athletes. Despite a brief 3-game losing streak during Wks20-21, TC was not significantly impacted (men). Finally,

following a decrease in TC before post-season competition and a trend towards a decrease in Wk28 ( $p=.073$ ; 1.5SD below baseline), the athletes were able to return to hormonal baseline one week after the end of the season (men). For women, during Wk6, one week prior to the first exhibition game, TC was below baseline and corresponded with an increase in COMP. However, TC returned to baseline by Wk7, which was the first week of exhibition play. During Wk16, which was collected the week after holiday break, TC was below baseline despite a decrease in COMP. During Week 24, TC was below baseline and corresponded with an increase in COMP. During Wk27, which was collected immediately before the team's first match of the NCAA tournament, TC was below baseline and corresponded with a decrease in COMP. However, the athletes returned to baseline during the tournament and remained at baseline up to and beyond their 4<sup>th</sup> round elimination. While these athletes experienced significant decreases in TC throughout the season, the overall hormonal profile appeared to remain stable compared to baseline despite constant variation in cumulative student-athlete stress and also suggested that the athletes were able to return to hormonal baseline by the end of the season. **PRACTICAL APPLICATIONS:** The methods of this study can be used for monitoring fatigue management by assessing how one's athletes adapt to stressful pre-season training and whether or not they recover in time for regular season play, in addition to how the athletes handle the stressors of the competitive season, and is useful for female athletes.

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## REVIEW OF LITERATURE

The following chapter will serve as a review of the current literature related to testosterone, cortisol, the ratio of testosterone to cortisol, and how these hormones relate to basketball performance. The review will begin with a general introduction to testosterone and cortisol. Next, the ratio of testosterone to cortisol, the analysis of salivary concentration versus blood concentration, basketball physiology, and basketball endocrinology will be thoroughly discussed with the goal of determining what gaps, if any, exist in the literature regarding elite NCAA Division I basketball performance and endocrine monitoring.

### Testosterone

Testosterone (T), a steroid hormone, is the primary androgen in males, with only about 2% free and available in the circulation, with the rest being bound to proteins such as sex hormone-binding globulin (SHBG) and albumin (Fry & Hoffman, 2008; Goodman, 2009). For testosterone to have an effect, it must be unbound and it must bind to androgen receptors, which can be found in numerous different tissues throughout the body (Goodman, 2009). Testosterone is primarily formed in the testis via steroidogenesis, where it aids in development of the testis and sperm production (Goodman, 2009). To a lesser extent, testosterone is also produced in the adrenals of both men and women (Goodman, 2009). Women typically produce approximately 10% of the testosterone that men do, and the majority of their testosterone is from the ovaries (Fry & Hoffman, 2008).

Leydig cells, found in bunches between the seminiferous tubules in the testis, produce testosterone after being stimulated by luteinizing hormone (LH) (Goodman, 2009). When LH

receptors in the Leydig cells are activated, cyclic AMP is formed (Goodman, 2009). Then, protein kinase A is activated, which leads to the phosphorylation of proteins that cause steroidogenesis (Goodman, 2009). After steroidogenesis, the body makes pregnenolone from cholesterol by cleaving the cholesterol side chain during the rate-limiting step in steroid hormone production (Goodman, 2009). After that, there are 2 different pathways:  $\Delta$ -4 and  $\Delta$ -5 (Goodman, 2009).

The  $\Delta$ -4 pathway starts with pregnenolone being converted to progesterone, which will then form 17 OH-Progesterone via 17 $\alpha$  Hydroxylase (Goodman, 2009). Next, 17 OH-Progesterone will form androstenedione (Goodman, 2009). Finally, androstenedione will utilize 17 $\beta$ -OH steroid dehydrogenase to make testosterone (Goodman, 2009). The  $\Delta$ -5 pathway starts with pregnenolone forming 17 OH-Pregnenolone (Goodman, 2009). From there, 17 OH-Pregnenolone can join into the  $\Delta$ -4 pathway by forming 17 OH-Progesterone or it can continue the  $\Delta$ -5 pathway by forming DHEA (Goodman, 2009). Next, DHEA can join into the  $\Delta$ -4 pathway by forming androstenedione or it can continue the  $\Delta$ -5 pathway by forming androstenediol (Goodman, 2009). Finally, androstenediol will make testosterone, which can be degraded to estradiol via aromatase (Goodman, 2009).

Testosterone has various responses to different modes of exercise, both acutely and chronically, in different populations. During endurance exercise, testosterone does not change with lower intensities, but increases near maximal intensities (Fry & Hoffman, 2008). However, if the duration is long enough, then testosterone will decrease before the end of the event (Cumming et al., 1986; Fry & Hoffman, 2008). Additionally, increases in T have been observed

after wrestling bouts, with even greater increases experienced by the winners, suggesting that T is impacted by winning and losing during athletic contests (Fry et al., 2011).

Regarding resistance training, several studies (Fahey et al., 1976; Gotschalk et al., 1997; Kraemer et al., 1990; Weiss et al., 1983) have demonstrated that, in response to resistance exercise with moderate to high volumes, testosterone increases. Larger testosterone responses have been observed with heavy, multi-joint, large-muscle mass movements, including high-power movements, such as snatches, cleans, and jerks (Kraemer et al., 1992), as compared to lighter movements (Harber et al., 2004; Kraemer et al., 1990). However, if the load is so heavy that adequate volume cannot be achieved, then the testosterone response will be minimized (Hakkinen & Pakarinen, 1993).

### Cortisol

Cortisol (C), a steroid hormone, is secreted by the adrenal cortex during times of physical or psychological stress to increase the glucose concentration, thus ensuring adequate energy (Fry & Hoffman, 2008; Goodman, 2009). As a glucocorticoid, cortisol plays an essential role in maintaining carbohydrate stores (Goodman, 2009). Cortisol can be considered a catabolic hormone since it will catabolize protein into amino acids for energy when necessary (Fry & Hoffman, 2008).

Similar to testosterone, cortisol is formed from cholesterol. Adrenocorticotrophic hormone (ACTH) is secreted by the anterior pituitary gland and signals production of cortisol. After steroidogenesis, the body makes pregnenolone from cholesterol by cleaving the cholesterol side chain during the rate-limiting step in steroid hormone production (Goodman, 2009). Next,

17 $\alpha$ -pregnenolone is converted to 17 $\alpha$ -hydroxy-progesterone, which is then converted to 11-deoxycortisol, before finally being converted to cortisol.

Cortisol has various responses to different modes of exercise, both acutely and chronically, in different populations. Regarding acute aerobic exercise, cortisol tends to increase most during higher-intensity training, and continues to rise throughout an event (Brandenburger & Follenius, 1975; Fry & Hoffman, 2008). Chronically, this cortisol response may lessen as a training adaptation (Fry & Hoffman, 2008; Winder et al., 1979). Large increases in cortisol have been seen from the beginning to the end of a wrestling match (Fry et al., 2011).

Similarly to the acute response to endurance exercise, cortisol has a larger acute response to resistance exercise than testosterone (Kraemer et al., 1990). Also similar to testosterone, larger cortisol responses have been observed with heavy, multi-joint, large-muscle mass movements, including high-power movements, such as snatches, cleans, and jerks (Kraemer et al., 1992). Likely due to increased metabolic requirements, cortisol is elevated to a greater extent when rest periods are kept shorter (Kraemer et al., 1990). While there are potential negative effects of cortisol from a catabolic standpoint, it also appears that cortisol plays a crucial role in tissue remodeling, and thus is a necessary response to resistance exercise (Fry & Hoffman, 2008).

#### Testosterone/Cortisol Ratio

The testosterone/cortisol ratio (T/C) is the amount of the hormone “testosterone,” normally in its unbound or free form, found in a blood or salivary sample divided by the amount of the hormone “cortisol” found in the same blood or salivary sample. Typically, in response to

one stressful training session, T will increase or decrease slightly, while cortisol will experience a significantly higher increase, thus leading to a decrease in T/C (Fry & Hoffman, 2008).

However, while T and C can be affected by winning and losing in important wrestling matches, T/C is not necessarily impacted (Fry et al., 2011), suggesting that even though an acute exercise bout was stressful, the ratio is not always impacted significantly. Chronically, as training stress increases over time, T/C tends to decrease. With a taper or reduction in training stress, T/C will usually return to baseline or increase beyond baseline due to super-compensation (Fry & Hoffman, 2008; Winchester, 2009).

The testosterone/cortisol ratio is considered an important endocrine marker, but it is an oversimplification which does not necessarily imply causality (Kraemer & Ratamess, 2005). Studies suggest that strength, power, and athletic preparedness are positively correlated with the testosterone/cortisol ratio (T/C) (Alen et al., 1988; Fry & Kraemer, 1997; Fry et al., 1994; Fry et al., 1993; Haff et al., 2008; Hakkinen et al., 1987; Hakkinen et al., 1988; Hakkinen et al., 1985; Moore & Fry, 2007; Winchester, 2009; Wu et al., 2008). The aim of these studies was not to show the relationship between T/C and one bout of exercise, but rather to determine the effect of a certain training program on T/C, since this ratio is often used as a marker of cumulative training stress (Fry & Hoffman, 2008).

One study (Haff et al., 2008) found that T/C was positively correlated with performance in isometric and dynamic mid-thigh pull with trained weightlifters, demonstrating that T/C is positively correlated to force production, and thereby related to strength. Other studies have shown a relationship between weightlifting performance and T/C in elite and sub-elite weightlifters, where T/C was positively correlated with performance (Fry et al., 1994; Fry et al.,

1993; Hakkinen et al., 1987). In other words, when a weightlifter's individual T/C was higher, their relative performance was higher. These studies monitored T/C and performance over time, as a group.

A case study involving an elite weightlifter showed how T/C changed throughout a contest preparation training period; his T/C went up and down, depending on his total training volume at that point in time (Wu et al., 2008). At an international competition, the athlete's T/C was lower than his best, and his performance was also less than his best, indicating the possibility that the decrease in performance was related to the decrease in T/C, which may have indicated that the training volume should have been reduced earlier in contest preparation (Wu et al., 2008). The athlete's T/C was steadily increasing as the subject finished peaking and tapered the volume down for the competition, but T/C did not return to the highest baseline level (Wu et al., 2008). Similarly, Obminski and Wisniewska looked at Olympic weightlifters preparing for the Olympic Games (2008). They found that their athletes improved their performances over a training cycle despite drops in T/C, drops in testosterone, and increases in cortisol, suggesting that T/C may indicate stress without impacting performance in some elite athletes (Obminski & Wisniewska, 2008).

While many studies have addressed the relationship between T/C and training stress in strength and power athletes, there have also been studies observing changes in T/C with athletes from sports with competing demands (i.e. anaerobic power and muscular endurance). Kraemer and colleagues (2004) were able to use T/C to predict performance in the upcoming season for collegiate division I soccer players. They determined that collegiate division I male soccer players starting a competitive season with low T and elevated C may experience reductions in

performance during that season, and that this phenomenon is worse for starters than for non-starters (Kraemer et al., 2004). The authors concluded that athletes should have a conditioning program that does not result in acute overtraining prior to preseason, as the athletes may not recover quickly enough (Kraemer et al., 2004). While this study did not monitor hormonal changes throughout a season, it does suggest that stressful pre-season training may have an effect on later performance in team sport athletes. Vervoorn & colleagues (1992) found a significant negative correlation ( $r = -.98$ ) between training volume and T/C in elite Olympic rowers during a high-volume peaking period, so that as training volume increased, T/C decreased, and vice-versa. Fry and colleagues (2011) found that T/C is not necessarily impacted by a stressful wrestling bout, despite changes in both T and C. While these studies indicate that T, C, and T/C may relate to stressful training in rowers, wrestlers, and soccer players, the nature of the studies leave several questions unanswered about some team sports, such as basketball.

#### Testosterone/Cortisol Ratio: Monitoring Female Athletes

Although T and T/C have traditionally been used to evaluate performance in males, as women average only 10% T production of males, there are data (Cardinale & Stone, 2006; Cook & Beaven, 2013; Cook et al., 2012; Edwards & Kurlander, 2010; Hamilton et al., 2009; Healy et al., 2014; Nunes et al., 2011) to suggest that T is also related to performance in elite female athletes. One study (Healy et al., 2014) evaluated hormonal profiles from 693 elite athletes (454 male, 239 female) and found that 13.7% of women had high levels with complete overlap between the sexes, demonstrating that elite female athletes may be more impacted by testosterone than the average female (Healy et al., 2014). Additionally, Cook and colleagues found that international-level female athletes from several Olympic sports had levels of T that were approximately double their national-level teammates (Cook et al., 2012). Cardinale and

Stone (2006) observed a positive correlation between vertical jump height and testosterone in elite female athletes. In female collegiate volleyball and tennis players, T was increased in anticipation of competition and also during sport practice (Edwards & Kurlander, 2010) while elite female wrestlers also experienced an increase in T from pre to post-bout (Hamilton et al., 2009). In elite female basketball players, increases in T/C correlated with increases in improvement to an off-season resistance training program (Nunes et al., 2011). Considering the current literature, further research directed towards using the T/C ratio to monitor performance in female athletes is justified.

It is interesting to note that T may vary throughout the menstrual cycle. However, research is currently inconsistent in this area (Battaglia et al., 2008; Oka et al., 1988; Salonia et al., 2008). One study (Battaglia et al., 2008) observed no changes in T throughout the menstrual cycle, while another (Salonia et al., 2008) saw an increase in T during the ovulatory phase, although both studies evaluated non-athletes. Another study (Oka et al., 1988) saw the greatest increase in T during the luteal phase (during the 20th through 26th days after menses) in women with a regular menstrual cycle, but also saw the highest increases in T during and surrounding ovulation (approximately the 14<sup>th</sup> day after menses) in women with an irregular menstrual cycle, suggesting that menstrual irregularity can affect T production in women. These studies suggest that further research is needed to understand the impact of the menstrual cycle on salivary T concentrations in elite female athletes, as it may potentially impact T levels during a monitoring study. Additionally, one study (Edwards & O'Neal, 2009) found that responses to exercise are not statistically different between athletes using oral contraception and those who are not.

## Salivary Analysis

One issue with the aforementioned studies is that, to date, the majority of studies attempting to use T/C to monitor athletic performance have used serum or plasma to determine T/C. However, saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (Baxendale et al., 1982; Johnson et al., 1987; Luisi et al., 1980; Papacosta & Nassis, 2011; Wang et al., 1981). Using saliva instead of serum would allow researchers to collect samples simultaneously from a group of athletes in a non-laboratory setting, which would cause minimal interference in the athletes' training. Additionally, while it would be difficult to do weekly blood draws with elite athletes, it is more probable that a researcher will be able to collect weekly saliva samples with elite athletes, allowing a week-to-week monitoring program across an entire competitive season.

Several studies have used salivary hormones to monitor performance in elite athletes. One study (Winchester et al., 2009) has used salivary T/C to monitor performance in elite college throwers and to determine how T/C and anaerobic performance are affected by a peak-taper cycle. Elite college throwers were tested for T/C, peak force and rate of force development (isometric mid-thigh pull), and standing broad jump on three separate occasions with two weeks in between each testing session. The first testing session was after 48 hours of rest, the second testing session was after two weeks of "peaking" where volume and intensity were both temporarily increased, and the third testing was after two weeks of "tapering" where volume and intensity were reduced. Significant positive correlations were observed between testosterone and the ratio of testosterone to cortisol with performance measures at all three time-points. Significant negative correlations were observed between cortisol and performance measures at all three time-points. Performance decreased after the peaking phase and then super-compensated

to above baseline after the taper, with hormonal concentrations matching the changes in performance (Winchester et al., 2009). With non-functional overreaching, performance and hormonal markers merely return to baseline after a taper (Moore & Fry, 2007), suggesting that T/C may be a way to help determine the effectiveness of a peak-taper cycle, as well.

While Winchester and colleagues (2009) were able to monitor performance in anaerobic strength and power athletes from an individual sport, data on the use of salivary hormones to monitor performance in team sports is scant. Nelson and colleagues (2008) used salivary T/C to successfully predict which collegiate division I football players would respond best to a resistance training program, and found that those with the highest T/C had the greatest adaptation to the resistance training program. However, they did not address the stresses of a competitive football season. Fortunately, a few studies have used salivary hormones to determine the stressfulness of practice and competition for team sports (Haneishi et al., 2007; Kraemer et al., 2009).

One study (Haneishi et al., 2007) found that salivary cortisol levels increased for starters and non-starters from before to immediately after a regular season match in female collegiate division 1 soccer players, although the increase was greater for starters. No differences were found for salivary cortisol before and after a practice session, indicating that the stress of competition was greater than the stress of practice (Haneishi et al., 2007). This data conflicts with the results of another study that showed neither T nor C being affected by a collegiate division 1 American football game in male athletes (Kraemer et al., 2009), although that study used blood samples 18-20 hours later. In contrast, during the female soccer study (Haneishi et al., 2007), salivary samples were taken thirty minutes before play and ten minutes after. Ease of

collection in the field indicates that saliva may be the optimal tool for measuring the hormonal response to athletic competition.

### Basketball Physiology

Basketball is a sport which is popular world-wide, and often involves numerous stressors for in-season elite players, including (but not necessarily limited to) practicing every day (sometimes twice per day), playing 1-2 competitive games against other opponents weekly, and other tournaments and conditioning sessions (Ziv & Lidor, 2009). Elite collegiate basketball players also have the additional stress of being a full-time student and travelling, training, and competing during the academic year. Programming for elite basketball players often includes preparation, competition, and transition phases, with the goal of constantly developing the athletes from a physical, technical, tactical, and psychological standpoint (Ziv & Lidor, 2009). The development of agility, endurance, and strength are considered to be of great importance for these athletes (Ziv & Lidor, 2009).

Several studies (Ackland et al., 1997; Carter et al., 2005; LaMonte et al., 1999; Latin et al., 1994; Ostojic et al., 2006; Sallet et al., 2004; Smith & Thomas, 1991) have attempted to determine anthropometric and physiological characteristics of male and female basketball players of differing levels of athletic ability. Male basketball centers have been found to have larger body masses and higher percent body-fat than guards (Latin et al., 1994; Ostojic et al., 2006; Sallet et al., 2004). In female elite basketball players, the best teams had players who were both taller in height and had a greater distance of arm-span (Ackland et al., 1997; Carter et al., 2005). Centers tend to have not only a higher body-fat percentage than guards, but also a larger absolute amount of fat-free mass, indicating that they are not only larger in general with more

body-fat, but also have more skeletal muscle compared to guards (LaMonte et al., 1999; Smith & Thomas, 1991).

Despite not being exclusively classified as an endurance sport, aerobic endurance plays a role in basketball performance (Ziv & Lidor, 2009). Therefore, several studies (McArdle et al., 1971; Narazaki et al., 2008; Riezebos et al., 1983; Rodriguez-Alonso et al., 2003; Smith & Thomas, 1991; Vaccaro et al., 1979) have attempted to assess cardiopulmonary function in basketball players via  $VO_{2max}$  testing. Only 3 studies (Cormery et al., 2008; Apostolidis et al., 2004; Gocentas et al., 2004), all with male basketball players, have looked at ventilator threshold, and found ranges 50.4-77.6 percent of  $VO_{2max}$ . An analysis of men's studies suggested that male basketball players typically have  $VO_{2max}$  ranging from 50-60 mL $O_2$ /kg/min, and that this range has not changed in the past 40 years (Ziv & Lidor, 2009). This variable was not always as consistent in female basketball players as it was in males.

In 1971, McArdle and colleagues found that female collegiate basketball players had  $VO_{2max}$  values (35.5 mL $O_2$ /kg/min) that were very similar to non-athlete college students at 33.6 mL $O_2$ /kg/min (McArdle et al., 1971). However, after the installment of Title IX, several studies (Narazaki et al., 2008; Riezebos et al., 1983; Rodriguez-Alonso et al., 2003; Smith & Thomas, 1991; Vaccaro et al., 1979) saw vast improvements in  $VO_{2max}$ , ranging from 44.0 to 54.0 mL $O_2$ /kg/min, with higher values found in athletes of international caliber and no differences across studies in maximal heart rate. The improvements in  $VO_{2max}$  after Title IX are thought to be caused by increases in women's involvement in sport and the incorporation of more intense strength and conditioning programs, especially when considering that a similar trend was not observed in men's  $VO_{2max}$  (Ziv & Lidor, 2009).

In addition to aerobic ability, anaerobic power also appears to be an important determinant of basketball performance (Ziv & Lidor, 2009). Vertical jump height is an expression of anaerobic power which is specific to basketball (Ziv & Lidor, 2009). Female basketball players typically have a countermovement vertical jump height of over 40 cm (Bale, 1991; LaMonte et al., 1999; Smith & Thomas, 1991; Ziv & Lidor, 2009) while male basketball players typically have a value over 60 cm (Hoffman, 1996; Ziv & Lidor, 2009). While vertical jump height values do not seem to differ between different positions, vertical jump power is higher in centers than guards for female players (Bale, 1991), which is likely explained by the higher body mass in centers. In both men and women, the best players on each team for each position had higher vertical jump heights than their teammates (Hoare, 2008).

Another important physiological component of basketball performance is metabolic conditioning, which can be assessed via blood lactate (Ziv & Lidor, 2009). One study (Ben Abdelkrim et al., 2007) found that guards had higher blood lactate levels than centers. Another study (McInnes et al., 1995) saw that basketball players have significantly elevated levels of blood lactate during competition, suggesting that glycogen metabolism plays an important role in basketball performance. While blood lactate levels are useful and can suggest the importance of anaerobic pathways in basketball, it must be acknowledged that these values do not indicate how much relative stress is coming from anaerobic mechanisms versus aerobic mechanisms (Ziv & Lidor, 2009).

### Basketball Endocrine Monitoring

Only a few studies (Hoffman et al., 1999; Schelling et al., 2009) have attempted to monitor testosterone, cortisol, and T/C in elite basketball players. One study (Hoffman et al.,

1999) observed changes in T, C, and T/C in elite professional basketball players during a 4-week training camp. While they found significant increases in C from the beginning of the camp to the end, they did not observe changes in T or T/C (Hoffman et al., 1999). Schelling et al (2009) sampled serum testosterone and cortisol from elite professional basketball players at 8 time-points throughout a competitive season (4-6 weeks apart, always at 0800 hours, 24-36 hours after a competition). They found that testosterone levels and T/C were significantly lower at the later collection dates, while cortisol remained the same throughout the season (Schelling et al., 2009).

Currently, 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 all aspects of stress (i.e. competition, practice, resistance training, academic, travel) weekly throughout an entire season in elite National Collegiate Association Division I basketball athletes. Additionally, to our knowledge, no study has observed T, C, or T/C during a basketball season for elite female basketball athletes. A recent review on basketball (Ziv & Lidor, 2009) made the following conclusion: “More studies of hormonal and biochemical markers of overtraining that are evaluated over an entire basketball season are warranted. Such objective markers of overtraining can help coaches to start tapering early enough to prevent overtraining from developing.”

However, Fry and colleagues (1998) have demonstrated that T/C is not necessarily impacted despite onset of overtraining syndrome. Therefore, while changes in T/C may reflect acute fatigue-management, we cannot make statements about overtraining syndrome with this population by simply monitoring T/C, despite the suggestion by Ziv and Lidor (2009). Regardless, attempting to monitor fatigue management is still worthwhile, as changes in T/C

may not only affect performance, but may also affect athlete health and student-athlete academic performance. Cortisol has been demonstrated to relate to academic preparation (Murphy et al., 2010) as well as presence of academic exams in college students (Murphy et al., 2010; Singh et al., 2012) while testosterone decreased and cortisol increased in response to a stressful test in college students (Maestriperi et al., 2010) which further suggests the need for hormonal monitoring in collegiate athletes. Thus, a gap exists in the literature which can and should be addressed by monitoring testosterone and cortisol weekly throughout an entire season of elite collegiate basketball.

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## **MANUSCRIPTS**

The following section will provide the two manuscripts derived from the dissertation research. Each manuscript will contain introduction, methods, results, discussion, and conclusions sections. The first manuscript addresses salivary biomarkers in male collegiate basketball athletes, while the second manuscript addresses the same problem in females.

# **SALIVARY BIOMARKER MONITORING OF ELITE COLLEGIATE MALE BASKETBALL PLAYERS ACROSS AN NCAA DIVISION I SEASON**

## **INTRODUCTION**

The testosterone/cortisol ratio (T/C) is the amount of the hormone “testosterone,” normally in its unbound or free form, found in a blood or salivary sample divided by the amount of the hormone “cortisol” found in the same blood or salivary sample. Studies suggest that strength, power, and athletic preparedness are positively correlated with T/C in male athletes (Alen et al., 1988; Fry & Kraemer, 1997; Fry et al., 1994; Fry et al., 1993; Haff et al., 2008; Hakkinen et al., 1987; Hakkinen et al., 1988; Hakkinen et al., 1985; Winchester, 2009; Wu et al., 2008). The testosterone/cortisol ratio is considered an important endocrine marker, but it is suggested to be an oversimplification which does not necessarily imply causality (Kraemer & Ratamess, 2005). Still, it may be useful for monitoring fatigue management in athletes.

Typically, in response to one stressful training session, T will increase or decrease slightly, while cortisol will experience a significantly higher increase, thus leading to a decrease in T/C (Fry & Hoffman, 2008). As training stress increases over time, T/C tends to decrease. With a taper or reduction in training stress, T/C will usually return to baseline or increase beyond baseline due to super-compensation (Fry & Hoffman, 2008; Winchester, 2009). However, with non-functional overreaching, performance and hormonal markers merely return to baseline after a taper (Moore & Fry, 2007), suggesting that T/C may be a way to help determine the effectiveness of a peak-taper cycle, as well.

While many studies have addressed the relationship between T/C and training stress in strength and power athletes, there have also been studies (Fry et al., 2011; Hoffman et al., 1999; Kraemer et al., 2004; Moore & Fry, 2007; Nelson et al., 2008; Schelling et al., 2009; Vervoorn et al., 2002) observing changes in T/C with athletes from sports with competing demands (i.e. anaerobic power and muscular endurance). Kraemer and colleagues (2004) were able to use T/C to predict performance in the upcoming season for collegiate division I soccer players. They determined that collegiate division I male soccer players starting a competitive season with low T and elevated C may experience reductions in performance during that season, and that this phenomenon is worse for starters than for non-starters (Kraemer et al., 2004). Vervoorn & colleagues (1992) found a significant negative correlation ( $r = -.98$ ) between training volume and T/C in elite Olympic rowers during a high-volume peaking period, so that as training volume increased, T/C decreased, and vice-versa. Fry and colleagues (2011) found that T/C is not necessarily impacted by a stressful wrestling bout, despite changes in both T and C. Another study (Nelson et al., 2008) used salivary T/C to successfully predict which collegiate division I football players would respond best to a resistance training program, and found that those with the highest T/C had the greatest adaptation to the resistance training program. However, they did not address the stresses of a competitive football season.

Only a few studies (Hoffman et al., 1999; Schelling et al., 2009) have attempted to monitor testosterone, cortisol, and T/C in elite male basketball players. One study (Hoffman et al., 1999) observed changes in T, C, and T/C in elite professional basketball players during a 4-week training camp. While they found significant increases in C from the beginning of the camp to the end, they did not observe changes in T or T/C (Hoffman et al., 1999). Schelling et al (2009) sampled serum testosterone and cortisol from elite professional basketball players at 8

time-points throughout a competitive season (4-6 weeks apart, always at 0800 hours, 24-36 hours after a competition). They found that testosterone levels and T/C were significantly lower at the later collection dates, while cortisol remained the same throughout the season (Schelling et al., 2009).

To date, the majority of studies attempting to use T/C to monitor athletic performance have used serum or plasma to determine T/C. However, saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (Baxendale et al., 1982; Johnson et al., 1987; Luisi et al., 1980; Papacosta & Nassis, 2011; Wang et al., 1981). Using saliva instead of serum allows researchers to collect samples simultaneously from a group of athletes in a non-laboratory setting, which would cause minimal interference in the athletes' training, thus, it is more probable that a researcher will be able to collect weekly saliva samples with elite athletes, allowing a week-to-week monitoring program across an entire competitive season.

Currently, 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 all aspects of stress (i.e. competition, practice, resistance training, academic, travel) weekly throughout an entire season in elite National Collegiate Association Division I basketball athletes. Therefore, the purpose of this study is to monitor free testosterone, cortisol, and the ratio of testosterone to cortisol in elite female NCAA Division I basketball athletes, weekly, throughout an entire season, and compare hormonal changes to variations in other measures of stress (practice minutes, game minutes, resistance training repetitions, academic stress, and travel stress).

## METHODS

### Experimental Approach to the Problem

This study was observational in nature. The athletes performed their normal duties as assigned, but each give a salivary sample immediately upon arriving for a regularly-scheduled afternoon practice, once weekly, from pre-season to after post-season during the entire 2012-2013 season. Changes in salivary hormones were then compared to changes in other measures of objective (minutes played in competition, minutes played in practice, repetitions in the weight-room) and subjective (travel and academic) stress to see if any statistically-significant relationships exist.

### Saliva Collection

Twelve male Elite National Collegiate Athletic Association Division I basketball athletes gave a salivary sample before an afternoon practice in the middle of each week for 27 different weeks within a period consisting of 30 consecutive weeks, beginning in the pre-season and ending one week after the end of post-season competition. All saliva collections were conducted at rest, in the afternoon, in the middle of the week. Subjects were instructed not to eat, brush their teeth, or drink anything other than water for one hour prior to meeting. When the subjects arrived for mid-week afternoon practice, they were instructed to rinse their mouths out with water and sit quietly. Then, subjects held an oral swab (Salimetrics Oral Swab, Salimetrics, PA, USA) in their mouths for 2 minutes before releasing the swab into a centrifuge tube. Samples were frozen at -80° C and stored for later analysis.

## Salivary Analysis

Saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (Baxendale et al., 1982; Johnson et al., 1987; Luisi et al., 1980; Papacosta & Nassis, 2011; Wang et al., 1981). Using saliva instead of serum allowed simultaneous data collection, in-the-field, in a non-invasive manner. For the salivary testosterone enzyme immunoassay kits (Salimetrics, PA, USA), a 96-well microtitre plate coated with rabbit testosterone antibodies is used to analyze the saliva samples. Testosterone found in the pre-determined standards which accompany the kit and testosterone in the salivary samples compete with a testosterone conjugate (testosterone linked to horseradish peroxidase) for the antibody binding sites within the wells of the microtitre plate. After a one hour incubation, unbound components are washed away. After the wash, the substrate tetramethylbenzidine (TMB) is added, and bound testosterone peroxidase is measured by the reaction of the peroxidase enzyme on the TMB, which produces a blue color. The plate is again incubated, this time for 30 minutes in the dark. A yellow color is formed after stopping the reaction using 2-molar sulfuric acid. The darkness of the yellow is inversely related to the amount of testosterone present, so that a darker yellow well has lower testosterone than a paler shade of yellow. Optical density is then read on a standard plate reader at 450 nm. The amount of testosterone peroxidase detected is inversely proportional to the amount of testosterone present.

For the salivary cortisol enzyme immunoassay kits (Salimetrics, PA, USA), a 96-well microtitre plate coated with coated with monoclonal antibodies to cortisol. Cortisol found in the pre-determined standards which accompany the kit and cortisol in the salivary samples compete with a cortisol conjugate (cortisol linked to horseradish peroxidase) for the antibody binding sites within the wells of the microtitre plate. After a one hour incubation, unbound components are

washed away. After the wash, the substrate tetramethylbenzidine (TMB) is added, and bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the TMB, which produces a blue color. The plate is again incubated, this time for 30 minutes in the dark. A yellow color is formed after stopping the reaction using 2-molar sulfuric acid. The darkness of the yellow is inversely related to the amount of cortisol present, so that a darker yellow well has lower cortisol than a paler shade of yellow. Optical density is then read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present.

A separate assay was used for each player with 12 assays per hormone, for 24 assays in total, with samples, standards, and controls all added in duplicate. Assay plates were read in a plate reader (KC4, Biotek Instruments, USA). The minimal concentration that can be distinguished from 0 with this assay is less than 0.03 nmol/L and 0.2 nmol/L for T and C, respectively. Correlations with serum T and C for these assays are strong ( $r = .96, p < .001$ ;  $r = .91, p < .0001$ ; for T and C, respectively). Expected salivary ranges are 2.2-8.6 nmol/L and ND-8.5 nmol/L for T and C, respectively. Inter- and intra-assay variation was 4.2% and 3.7%, respectively, for T, and 3.6% and 3.2%, respectively, for C.

### Composite Stress

The following data was also recorded throughout the season: playing time (minutes), practice time (minutes), resistance-training volume (reps), travel stress (scale), and academic stress (scale). The travel scale was determined by the coaches so 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, because the coaches felt that these

numbers were fair representatives of how stressful the travel would be for the athletes. Travel scores were averaged for each week. The academic stress scale was generalized so that a score of “0” was given when classes were not held, a score of “2” was given during normal weeks, and a score of “4” was given during exams. Academic scores were averaged for each week. Additionally, a composite value composed of Z-scores (COMP) for playing time, practice time, resistance-training volume, travel stress, and academic stress was used in an attempt to quantify weekly cumulative stress so that an increase in COMP suggested an increase in cumulative stress. All variables were converted to Z-scores to standardize each player’s values, and a comprehensive score.

### Statistical Analysis

Of 324 team samples for the 30-week period, 20 individual samples were missing due to players missing practice. As has been previously suggested (Borg & Gall, 1983), missing values were replaced with the team average for that week. One-way repeated-measures analysis of variance with LSD pairwise comparisons were used to determine which weekly values (T, C, T/C, COMP) were different from the season average. Pearson correlations were also used to help determine relationships between team hormones and team COMP throughout the season. Significance was determined *a priori* ( $\alpha = .05$ ).

## **RESULTS**

The overall ANOVA models were significant for T ( $F(27, 297) = 2.832, p < .001$ ), C ( $F(27, 297) = 5.957, p < .001$ ), T/C ( $F(27, 297) = 2.660, p < .001$ ), and COMP ( $F(27, 297) = 335.465, p < .001$ ). For T, 10 weeks were different from baseline (5.1 nmol/L). For C, 11 weeks

were different from baseline (9.0 nmol/L). For T/C, weeks 7 ( $p=.007$ ), 17 ( $p=.007$ ), and 25 ( $p=.005$ ) were different from baseline ( $T/C=0.69$ ). A significant negative correlation ( $r = -0.47$ ;  $r_{crit} = 0.38$ ;  $p < .05$ ) was observed between T/C and game minutes. No other correlations were statistically significant. Refer to Tables 1-2 and Figures 1-4 for all data.

Samples were missing for weeks 3 (3), 7 (1), 11 (1), 12 (1), 13 (2), 15 (2), 16 (1), 19 (2), 21 (2), 24 (1), 25 (1), and 30 (3). During Week 7, at the start of regular season play, T/C was more than 3 standard deviations (SD) above baseline while COMP was significantly below baseline. During Week 17, which was leading into a streak of important conference matches, T/C was more than 2.5 standard deviations below baseline while COMP was not different from baseline. During Week 25, which was one week before the conference tournament, T/C was more than one standard deviation below baseline while COMP was significantly above baseline.

Table 1: Weekly Hormonal Levels (X±SD)

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	15
T (nmol/L)	5.2±0.6	5.0±1.2	5.0±0.9	5.8±2.4	5.7±2.3	4.7±1.3	5.1±3.1	5.1±1.5	5.1±1.7	5.0±0.9	5.2±0.9	5.1±0.9	5.8±1.1*	5.9±1.0*
C (nmol/L)	9.5±6.4	7.4±2.7*	7.2±4.4	9.6±4.8	9.2±5.2	6.9±2.7**	5.5±3.3**	8.0±3.9	8.5±6.8	6.9±2.6**	9.0±4.6	8.9±4.1	11.6±5.1	13.1±5.8*
T/C	.73±.39	.76±.31	.85±.35	.71±.33	.74±.32	.78±.31	1.10±.50**	.73±.27	.77±.32	.79±.25	.70±.31	.67±.27	.58±.25	.53±.26
Week	16	17	18	19	20	21	22	24	25	26	27	28	30	
T (nmol/L)	3.7±0.8**	6.9±2.5*	4.9±0.7	5.0±1.7	5.0±1.6	4.0±0.8**	4.0±1.2*	3.9±0.6**	4.1±0.5**	5.9±4.2	4.3±0.7*	6.2±1.4*	4.9±1.1	
C (nmol/L)	6.9±3.1	20.3±8.9**	7.7±2.4	7.8±4.0	7.8±3.2	7.6±3.0	6.9±2.8*	6.9±2.1**	8.1±3.0	10.5±6.1	6.4±2.2**	17.7±12.6*	7.1±3.1*	
T/C	.60±.20	.41±.25**	.70±.27	.73±.29	.73±.25	.58±.23	.62±.19	.61±.19	.56±.17**	.63±.36	.75±.25	.50±.34	.78±.30	

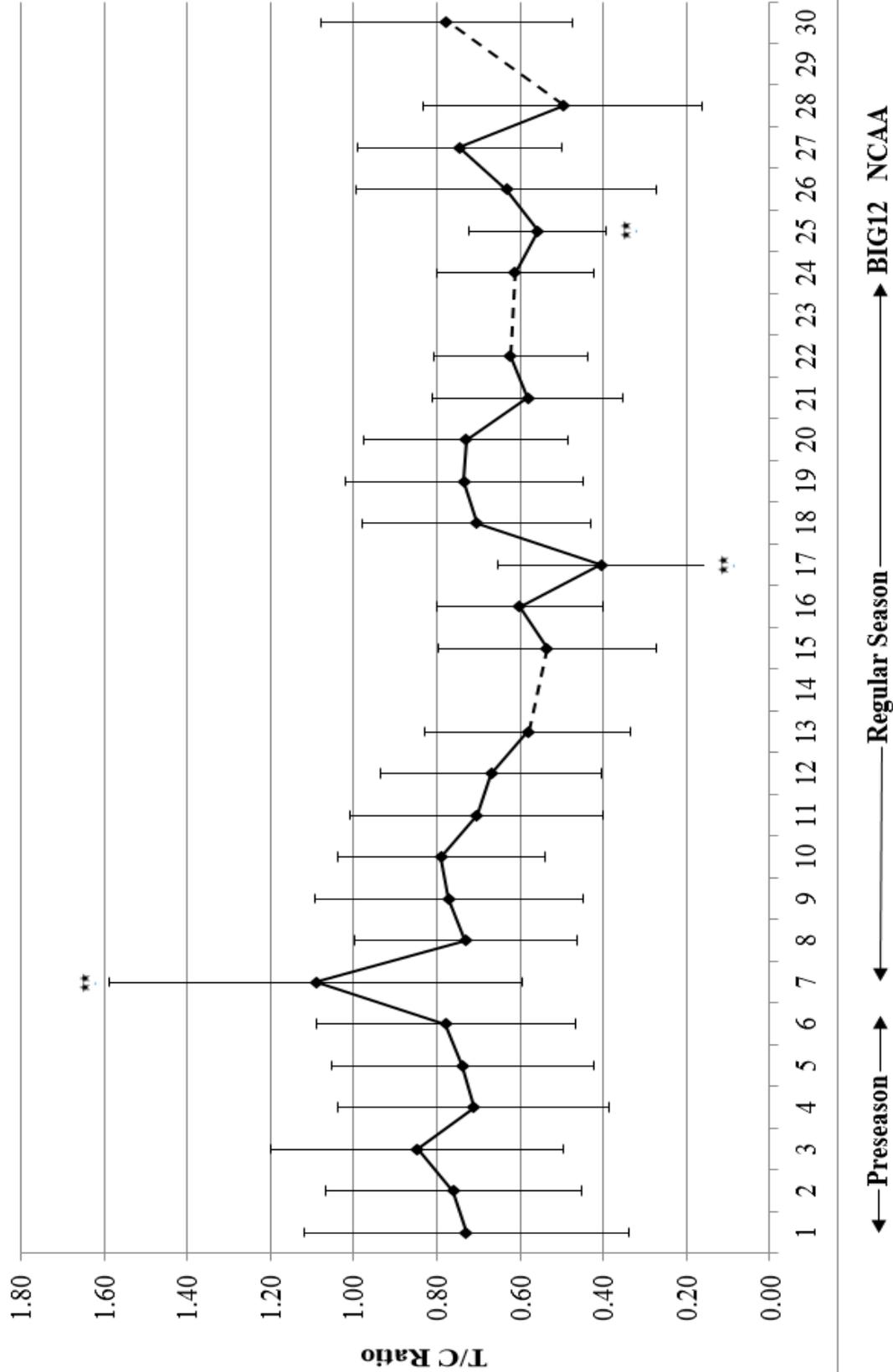
\*p<.05 \*\*p<.01

Table 2: Correlations Between Composite Stress Data and Hormones

	Composite	Game Minutes	Practice Minutes	RT Volume (Reps)	Academic	Travel
Testosterone	-.02	-.13	.13	.16	.02	-.31
Cortisol	.13	.18	.19	.21	-.16	-.07
T/C Ratio	-.17	-.47*	-.06	-.13	.21	-.16

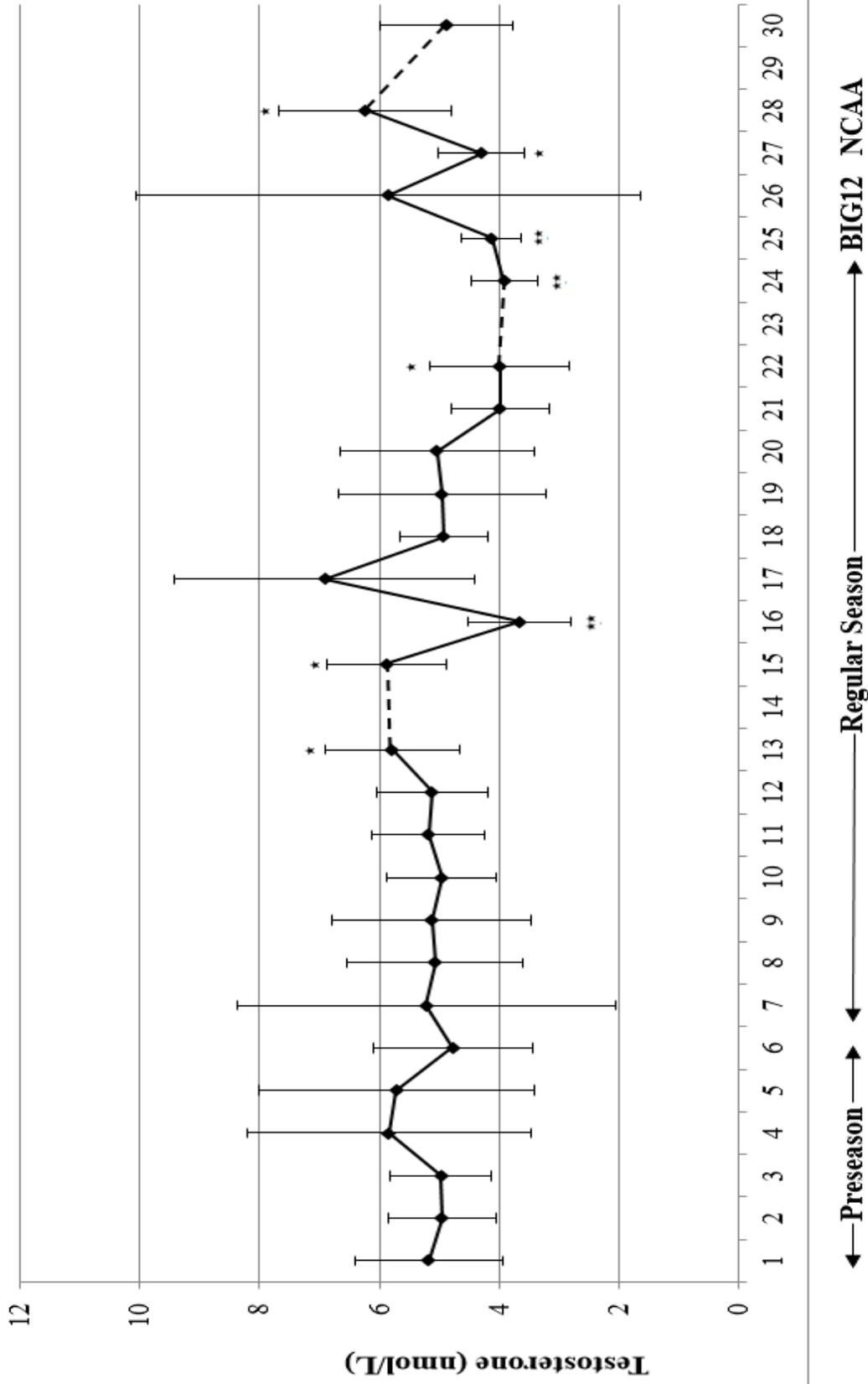
\* $p < .05$ ;  $r_{crit} = .38$

**Figure 1: Testosterone/Cortisol Ratio**



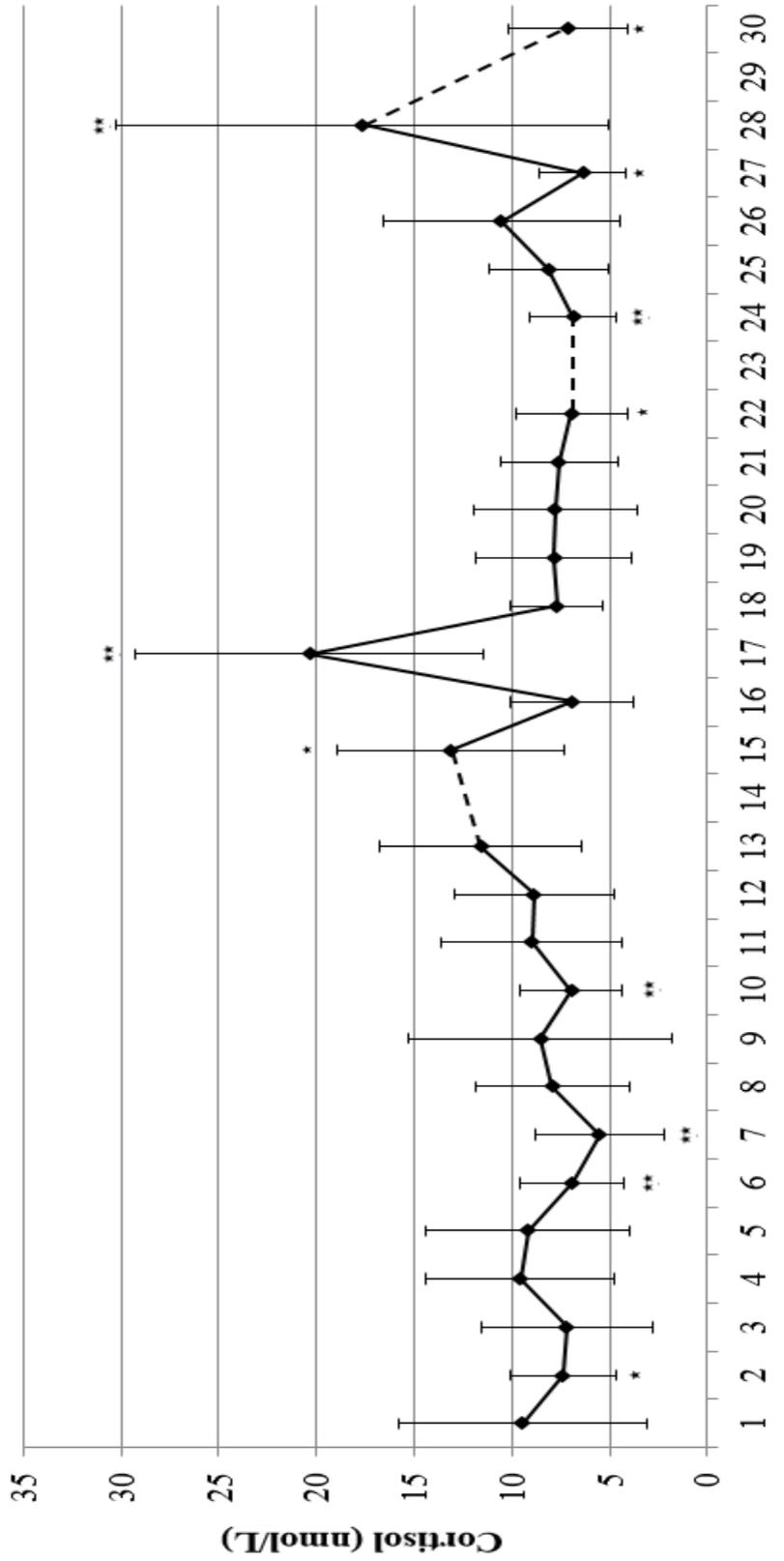
\* $p < .05$ ; \*\* $p < .01$

**Figure 2: Testosterone**



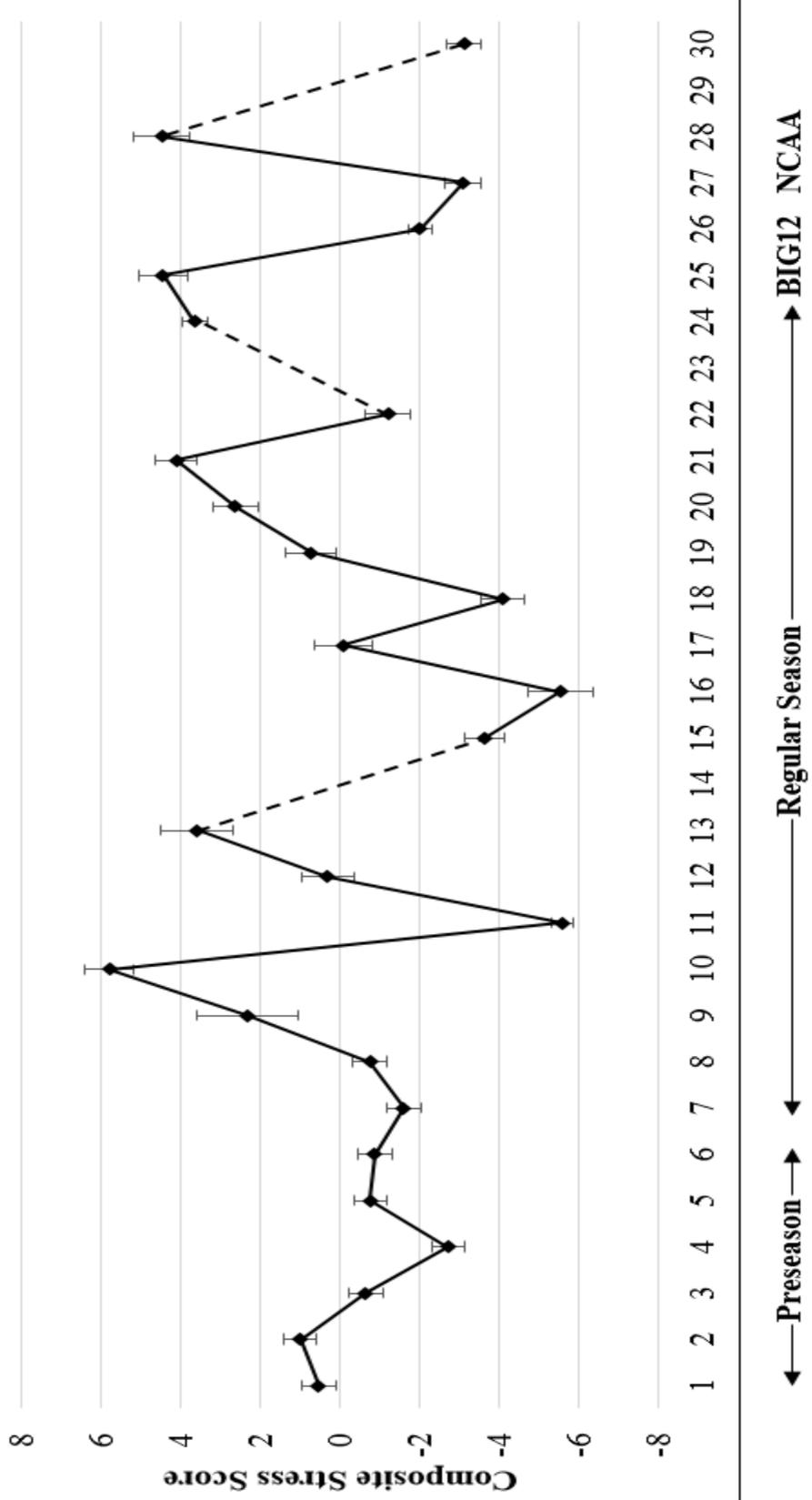
\* $p < .05$ ; \*\* $p < .01$

Figure 3: Cortisol



\* $p < .05$ ; \*\* $p < .01$

**Figure 4: Composite Stress Score**



For all weeks,  $p < .01$  (besides Wk17:  $p = .984$ )

## DISCUSSION

The results of this study demonstrate that this was an appropriate method for those who are attempting to use T/C to monitor fatigue management of elite male National Collegiate Athletic Association Division I basketball athletes. 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 that group of samples was collected. The coaches reported an intentional peak-taper cycle during the weeks leading up to Week 7, which corresponded with a significant decrease in COMP. Specifically, the components of COMP that caused its significant decrease were resistance training volume and practice minutes, which were decreased below baseline; this may have led to the increase in T/C.

The present study was similar to Hoffman et al (1999) in that C was elevated by the stressful preseason training camp while T and T/C did not change during the training camp. It would be interesting to see if the athletes in Hoffman et al's (1999) study saw a similar increase in T/C following a taper as observed in the present study. In contrast to the results of Schelling et al (2009), the athletes in the present study did not see an increase in T after a taper despite an increase in T/C. However, Schelling and colleagues (2009) measured total testosterone, while the present study measured free testosterone. Therefore, it is possible that the difference may have been due to total versus free T, suggesting that they may respond differently to a taper in basketball athletes. It is also possible that the results differed due to the timing of the taper, the type of athlete (professional versus collegiate), or another factor.

The present study is consistent with other studies with athletes (Fry & Hoffman, 2008; Winchester, 2009) which saw T/C return to baseline after a taper in one case or super-compensate above baseline after a taper in another study. Similarly to Winchester et al. (2009), T/C was elevated above baseline after a taper, suggesting a super-compensatory effect of the peak-taper cycle. Interestingly, the study by Winchester and colleagues (2009) used NCAA Division I nationally-qualified track and field throwers and jumpers, which are strictly strength and power athletes, yet they experienced a similar response to a peak-taper cycle as elite NCAA Division I basketball athletes, who have different physiological demands. This change in T/C is important because if off-season or preseason training is too stressful, then the athletes may not recover in time for competition, or may simply not reap the benefits of the program. An example would be off-season collegiate football players who experienced non-functional overreaching, and saw performance and hormonal markers merely return to baseline after reducing training volume (Moore & Fry, 2007). That study (Moore & Fry, 2007) observed an ineffective attempt to increase performance and athletic preparation, whereas the results of the present study suggest an effective peak-taper cycle for these athletes.

The decrease in T/C at Week 17, despite the current win-streak, suggests that the lengthy season was having a physical effect on the student-athletes. This was further supported by the statistically-significant negative correlation between T/C and playing minutes, demonstrating that weeks with more playing time also had a decrease in T/C. However, since correlation does not imply causality, it cannot be determined whether or not increased playing time caused the decrease in T/C, rather that they both occurred simultaneously. Despite a brief 3-game losing streak during Weeks 20 and 21, T/C was not significantly impacted. 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 (Fry et al., 2011). 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 (2009), who found that testosterone levels and T/C were significantly lower at the later collection dates, while C remained the same throughout the season (Schelling et al., 2009). 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, suggesting that, while the competitive efforts of male NCAA Division I basketball athletes may be stressful, they may recover quickly from the season. Additionally, the present study may have differed from Schelling and colleagues (2009) because of the professional status of their athletes versus the student-athlete status of the athletes in the present study, suggesting that student-athletes may experience more variability in C than professional athletes.

While some may propose that a large decrease in T/C indicates a state of overtraining syndrome, Fry and colleagues (1998) have demonstrated that T/C is not necessarily impacted despite onset of overtraining syndrome. Therefore, while changes in T/C may reflect acute physical stress-management, we cannot make statements about overtraining syndrome 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 overtraining syndrome. Therefore, the potential for overtraining syndrome is not the only reason to perform a biomarker monitoring study, as coaches and scientists can use this information to evaluate effectiveness of recovery strategies and fatigue management. Additionally, the general population may consider the effects of combined life

stressors on hormonal status and attempt to reduce lifestyle stress for the purpose of improving the ratio of anabolic to catabolic hormones, thereby potentially improving health.

The methods of the present study, specifically the frequency of athlete monitoring, may explain some of the differences between this study and others (Hoffman et al., 1999; Schelling et al., 2009) that also measured hormones in elite male basketball athletes. The present study observed T, C, and T/C weekly, from preseason to after postseason, while the other studies (Hoffman et al., 1999; Schelling et al., 2009) observed only a few time-points. Hoffman and colleagues (1999) observed hormonal levels 4 times during a 4-week off-season training camp. While this was similar to the present study in that it was weekly observations, is only accounted for off-season training and not the stressors of the regular season or postseason play (Hoffman et al., 1999). Schelling et al (2009) observed 8 time-points throughout an 8-month period, stating that collections were taken every 4-6 weeks. Had they (Schelling et al., 2009) monitored their athletes weekly, they may have seen more variation in C. Therefore, studies attempting to monitor hormonal changes in elite basketball players should plan to use weekly collections to get a broader view of variation across a season.

Another important aspect of the present study was the Composite Stress Score. This is not a currently-validated measure for assessing student-athlete stress. Rather, it is a novel approach used to attempt to identify changes in cumulative stress throughout an entire season in elite male NCAA Division I basketball athletes. Interestingly, the only statistically-significant correlation between a measure of COMP and the hormones was the inverse correlation between game playing minutes and T/C, suggesting that game minutes may be a critical component of COMP. Regardless, during several weeks where COMP significantly increased or decreased

from baseline, T/C also significantly increased or decreased, demonstrating the usefulness of this measure of composite stress. Future studies could use the measure described here to monitor elite basketball players throughout a season.

## **CONCLUSIONS**

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. The methods of this study can be used for monitoring fatigue-management efforts by assessing how one's athletes adapt to stressful pre-season training and whether or not they recover in time for regular season play, in addition to how the athletes handle the stressors of the competitive season. 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 fatigue management 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 (i.e. vertical jump height) after each specimen collection, to see if changes in performance variables relate to changes in salivary hormones.

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# **SALIVARY BIOMARKER MONITORING OF ELITE COLLEGIATE FEMALE BASKETBALL PLAYERS ACROSS AN NCAA DIVISION I SEASON**

## **INTRODUCTION**

The testosterone/cortisol ratio (T/C) is the amount of the hormone “testosterone,” normally in its unbound or free form, found in a blood or salivary sample divided by the amount of the hormone “cortisol” found in the same blood or salivary sample. Studies suggest that strength, power, and athletic preparedness are positively correlated with T/C in male athletes (Alen et al., 1988; Fry & Kraemer, 1997; Fry et al., 1994; Fry et al., 1993; Haff et al., 2008; Hakkinen et al., 1987; Hakkinen et al., 1988; Hakkinen et al., 1985; Winchester, 2009; Wu et al., 2008). The testosterone/cortisol ratio is considered an important endocrine marker, but it is suggested to be an oversimplification which does not necessarily imply causality (Kraemer & Ratamess, 2005). Still, it may be useful for monitoring fatigue management in athletes.

Typically, in response to one stressful training session, T will increase or decrease slightly, while cortisol will experience a significantly higher increase, thus leading to a decrease in T/C (Fry & Hoffman, 2008). As training stress increases over time, T/C tends to decrease. With a taper or reduction in training stress, T/C will usually return to baseline or increase beyond baseline due to super-compensation (Fry & Hoffman, 2008; Winchester, 2009). However, with non-functional overreaching, performance and hormonal markers merely return to baseline after a taper (Moore & Fry, 2007), suggesting that T/C may be a way to help determine the effectiveness of a peak-taper cycle, as well.

While many studies have addressed the relationship between T/C and training stress in strength and power athletes, there have also been studies (Fry et al., 2011; Hoffman et al., 1999; Kraemer et al., 2004; Moore & Fry, 2007; Nelson et al., 2008; Schelling et al., 2009; Vervoorn et al., 2002) observing changes in T/C with athletes from sports with competing demands (i.e. anaerobic power and muscular endurance). Kraemer and colleagues (2004) were able to use T/C to predict performance in the upcoming season for collegiate division I soccer players. They determined that collegiate division I male soccer players starting a competitive season with low T and elevated C may experience reductions in performance during that season, and that this phenomenon is worse for starters than for non-starters (Kraemer et al., 2004). Vervoorn & colleagues (1992) found a significant negative correlation ( $r = -.98$ ) between training volume and T/C in elite Olympic rowers during a high-volume peaking period, so that as training volume increased, T/C decreased, and vice-versa. Fry and colleagues (2011) found that T/C is not necessarily impacted by a stressful wrestling bout, despite changes in both T and C. Another study (Nelson et al., 2008) used salivary T/C to successfully predict which collegiate division I football players would respond best to a resistance training program, and found that those with the highest T/C had the greatest adaptation to the resistance training program. However, they did not address the stresses of a competitive football season.

Only a few studies (Hoffman et al., 1999; Schelling et al., 2009) have attempted to monitor testosterone, cortisol, and T/C in elite male basketball players. One study (Hoffman et al., 1999) observed changes in T, C, and T/C in elite professional basketball players during a 4-week training camp. While they found significant increases in C from the beginning of the camp to the end, they did not observe changes in T or T/C (Hoffman et al., 1999). Schelling et al (2009) sampled serum testosterone and cortisol from elite professional basketball players at 8

time-points throughout a competitive season (4-6 weeks apart, always at 0800 hours, 24-36 hours after a competition). They found that testosterone levels and T/C were significantly lower at the later collection dates, while cortisol remained the same throughout the season (Schelling et al., 2009).

While T and T/C have traditionally been used to evaluate performance in males, as women average only 10% T production of males, there are data (Cardinale & Stone, 2006; Cook & Beaven, 2013; Cook et al., 2012; Edwards & Kurlander, 2010; Hamilton et al., 2009; Healy et al., 2014; Nunes et al., 2011) to suggest that T is also related to performance in elite female athletes. One study (Healy et al., 2014) evaluated hormonal profiles from 693 elite athletes (454 male, 239 female) and found that 13.7% of women had high levels with complete overlap between the sexes, demonstrating that elite female athletes may be more impacted by testosterone than the average female (Healy et al., 2014). Additionally, Cook and colleagues found that international-level female athletes from several Olympic sports had levels of T that were approximately double their national-level teammates (Cook et al., 2012). Cardinale and Stone (2006) observed a positive correlation between vertical jump height and testosterone in elite female athletes. In female collegiate volleyball and tennis players, T was increased in anticipation of competition and also during sport practice (Edwards & Kurlander, 2010) while elite female wrestlers also experienced an increase in T from pre to post-bout (Hamilton et al., 2009). In elite female basketball players, increases in T/C correlated with increases in improvement to an off-season resistance training program (Nunes et al., 2011). Considering these studies, further research directed towards using the T/C ratio to monitor performance in female athletes is justified.

It is interesting to note that T may vary throughout the menstrual cycle. However, research is currently inconsistent in this area (Battaglia et al., 2008; Oka et al., 1988; Salonia et al., 2008). One study (Battaglia et al., 2008) observed no changes in T throughout the menstrual cycle, while another (Salonia et al., 2008) saw an increase in T during the ovulatory phase, although both studies evaluated non-athletes. Another study (Oka et al., 1988) saw the greatest increase in T during the luteal phase (during the 20th through 26th days after menses) in women with a regular menstrual cycle, but also saw the highest increases in T during and surrounding ovulation (approximately the 14<sup>th</sup> day after menses) in women with an irregular menstrual cycle, suggesting that menstrual irregularity can affect T production in women. These studies suggest that further research is needed to understand the impact of the menstrual cycle on salivary T concentrations in elite female basketball athletes, as it may potentially impact T levels during a monitoring study. Additionally, while the use of oral contraceptives amongst the athletes was unknown, research (Edwards & O'Neal, 2009) has suggested that responses to exercise are not statistically different between athletes using oral contraception and those who are not.

To date, the majority of studies attempting to use T/C to monitor athletic performance have used serum or plasma to determine T/C. However, saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (Baxendale et al., 1982; Johnson et al., 1987; Luisi et al., 1980; Papacosta & Nassis, 2011; Wang et al., 1981). Using saliva instead of serum allows researchers to collect samples simultaneously from a group of athletes in a non-laboratory setting, which would cause minimal interference in the athletes' training, thus, it is more probable that a researcher will be able to collect weekly saliva samples with elite athletes, allowing a week-to-week monitoring program across an entire competitive season.

Currently, the literature has shown that T/C can relate to training stress in anaerobic athletes and team sport athletes with multiple physical demands, including male professional basketball players. However, no studies have addressed all aspects of stress (i.e. competition, practice, resistance training, academic, travel) weekly throughout an entire season in elite female National Collegiate Association Division I basketball athletes. Therefore, the purpose of this study is to monitor free testosterone, cortisol, and the ratio of testosterone to cortisol in elite female NCAA Division I basketball athletes, weekly, throughout an entire season, and compare hormonal changes to variations in other measures of stress (practice minutes, game minutes, resistance training repetitions, academic stress, and travel stress).

## **METHODS**

### Experimental Approach to the Problem

This study was observational in nature. The athletes performed their normal duties as assigned, but each give a salivary sample immediately upon arriving for a regularly-scheduled afternoon practice, once weekly, from pre-season to after post-season during the entire 2012-2013 season. Changes in salivary hormones were then compared to changes in other measures of objective (minutes played in competition, minutes played in practice, repetitions in the weight-room) and subjective (travel and academic) stress to see if any statistically-significant relationships exist.

### Saliva Collection

Ten female Elite National Collegiate Athletic Association Division I basketball athletes gave a salivary sample before an afternoon practice in the middle of each week for 26 different

weeks within a period consisting of 29 consecutive weeks, beginning in the pre-season and ending one week after the end of post-season competition. All saliva collections were conducted at rest, in the afternoon, in the middle of the week. Subjects were instructed not to eat, brush their teeth, or drink anything other than water for one hour prior to meeting. When the subjects arrived for mid-week afternoon practice, they were instructed to rinse their mouths out with water and sit quietly. Then, subjects held an oral swab (Salimetrics Oral Swab, Salimetrics, PA, USA) in their mouths for 2 minutes before releasing the swab into a centrifuge tube. Samples were frozen at  $-80^{\circ}\text{C}$  and stored for later analysis.

### Salivary Analysis

Saliva is a safe, reliable, non-invasive method for measuring T and C and is strongly correlated with serum values (Baxendale et al., 1982; Johnson et al., 1987; Luisi et al., 1980; Wang et al., 1981). Using saliva instead of serum allowed simultaneous data collection, in-the-field, in a non-invasive manner. For the salivary testosterone enzyme immunoassay kits (Salimetrics, PA, USA), a 96-well microtitre plate coated with rabbit testosterone antibodies is used to analyze the saliva samples. Testosterone found in the pre-determined standards which accompany the kit and testosterone in the salivary samples compete with a testosterone conjugate (testosterone linked to horseradish peroxidase) for the antibody binding sites within the wells of the microtitre plate. After a one hour incubation, unbound components are washed away. After the wash, the substrate tetramethylbenzidine (TMB) is added, and bound testosterone peroxidase is measured by the reaction of the peroxidase enzyme on the TMB, which produces a blue color. The plate is again incubated, this time for 30 minutes in the dark. A yellow color is formed after stopping the reaction using 2-molar sulfuric acid. The darkness of the yellow is inversely related to the amount of testosterone present, so that a darker yellow well has lower testosterone than a

paler shade of yellow. Optical density is then read on a standard plate reader at 450 nm. The amount of testosterone peroxidase detected is inversely proportional to the amount of testosterone present.

For the salivary cortisol enzyme immunoassay kits (Salimetrics, PA, USA), a 96-well microtitre plate coated with coated with monoclonal antibodies to cortisol. Cortisol found in the pre-determined standards which accompany the kit and cortisol in the salivary samples compete with a cortisol conjugate (cortisol linked to horseradish peroxidase) for the antibody binding sites within the wells of the microtitre plate. After a one hour incubation, unbound components are washed away. After the wash, the substrate tetramethylbenzidine (TMB) is added, and bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the TMB, which produces a blue color. The plate is again incubated, this time for 30 minutes in the dark. A yellow color is formed after stopping the reaction using 2-molar sulfuric acid. The darkness of the yellow is inversely related to the amount of cortisol present, so that a darker yellow well has lower cortisol than a paler shade of yellow. Optical density is then read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present.

A separate assay was used for each player with 10 assays per hormone, for 20 assays in total, with samples, standards, and controls all added in duplicate. Assay plates were read in a plate reader (KC4, Biotek Instruments, USA). The minimal concentration that can be distinguished from 0 with this assay is less than 0.03 nmol/L and 0.2 nmol/L for T and C, respectively. Correlations with serum T and C for these assays are strong ( $r = .96, p < .001$ ;  $r = .91, p < .0001$ ; for T and C, respectively). Expected salivary ranges are 0.4-3.0 nmol/L and ND-

9.9 nmol/L for T and C, respectively. Inter- and intra-assay variation was 4.2% and 1.7%, respectively, for T, and 5.9% and 3.2%, respectively, for C.

### Composite Stress

The following data was also recorded throughout the season: playing time (minutes), practice time (minutes), resistance-training volume (reps), travel stress (scale), academic stress (scale), and menstrual cycle. The travel scale was determined by the coaches so 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, because the coaches felt that these numbers were fair representatives of how stressful the travel would be for the athletes. Travel scores were averaged for each week. The academic stress scale was generalized so that a score of “0” was given when classes were not held, a score of “2” was given during normal weeks, and a score of “4” was given during exams. Academic scores were averaged for each week. Menstrual status was observed and a “1” was marked if currently menstruating at the time of saliva collection, while a “0” was entered when an athlete was not currently menstruating at the collection time. Finally, a composite value composed of Z-scores (COMP) for playing time, practice time, resistance-training volume, travel stress, academic stress, and menstruation was used in an attempt to quantify weekly cumulative stress so that an increase in COMP suggested an increase in cumulative stress. All variables were converted to Z-scores to standardize each player’s values, and a comprehensive score.

### Statistical Analysis

Of 260 team samples for the 29-week period, 16 individual samples were missing due to players missing practice. As has been previously suggested (Borg & Gall, 1983), missing values

were replaced with the team average for that week. One-way repeated-measures analysis of variance with LSD pairwise comparisons were used to determine which weekly values (T, C, T/C, COMP) were different from the season average. Pearson correlations were also used to help determine relationships between team hormones and team COMP throughout the season. Significance was determined *a priori* ( $\alpha = .05$ ).

## RESULTS

The overall ANOVA models were significant for T ( $F(26, 234) = 1.638, p < .05$ ), C ( $F(26, 234) = 1.848, p < .01$ ), T/C ( $F(26, 234) = 1.932, p < .01$ ), and COMP ( $F(26, 234) = 169.260, p < .001$ ). For T, 4 weeks were different from baseline (6.5 nmol/L). For C, 4 weeks were different from baseline (6.5 nmol/L). For T/C, weeks 6 ( $p=.004$ ), 16 ( $p=.024$ ), 24 ( $p=.003$ ), and 27 ( $p=.008$ ) were significantly different from baseline (0.42). No correlations were statistically significant. Refer to Tables 1-2 and Figures 1-4 for all data. During Wk6, one week prior to the first exhibition game, T/C was below baseline and corresponded with an increase in COMP. However, T/C returned to baseline by Wk7, which was the first week of exhibition play. During Wk16, which was collected the week after holiday break, T/C was below baseline despite a decrease in COMP. During Week 24, T/C was below baseline and corresponded with an increase in COMP. During Wk27, which was collected immediately before the team's first match of the NCAA tournament, T/C was below baseline and corresponded with a decrease in COMP. However, the athletes returned to baseline during the tournament and remained at baseline up to and beyond their 4th round elimination.

Table 1: Weekly Hormonal Levels ( $\bar{X} \pm SD$ )

Week	1	2	3	4	5	6	7	8	9	10	11	12	15
T (nmol/L)	2.4±0.9	2.4±1.3	2.0±0.7	2.2±0.7	2.2±0.7	2.1±0.8	1.7±0.7*	1.9±0.6	2.4±0.6	1.9±0.6	3.2±2.1	2.5±1.7	1.9±.84
C (nmol/L)	7.8±8.0	5.7±3.1	4.7±2.1**	7.3±3.3	8.8±4.7	7.0±2.0	5.4±2.5	5.2±3.0	5.6±2.4	5.3±2.2	6.5±3.4	6.2±1.9	6.7±4.8
T/C	.50±.30	.47±.18	.57±.50	.33±.12	.32±.21	.32±.14**	.39±.24	.45±.23	.48±.20	.41±.15	.55±.31	.44±.31	.37±.25
Week	16	17	18	19	20	21	22	24	25	26	27	28	29
T (nmol/L)	2.4±1.4	1.8±0.7**	2.2±0.6	2.2±0.7	2.3±0.7	2.3±1.0	1.8±0.6**	2.2±0.5	2.1±1.1	2.0±0.9	2.4±1.7	1.8±0.9**	2.4±1.4
C (nmol/L)	8.6±5.7	4.1±2.4**	6.4±2.7	5.2±2.7	5.9±2.9	5.8±1.8	6.6±2.4	11.5±5.3**	6.1±3.1	5.2±2.3	9.2±6.4	8.0±10.3	4.6±1.6*
T/C	.33±.18*	.51±.22	.41±.23	.51±.24	.45±.18	.40±.14	.33±.22	.23±.11**	.38±.15	.48±.36	.31±.17**	.40±.23	.61±.42

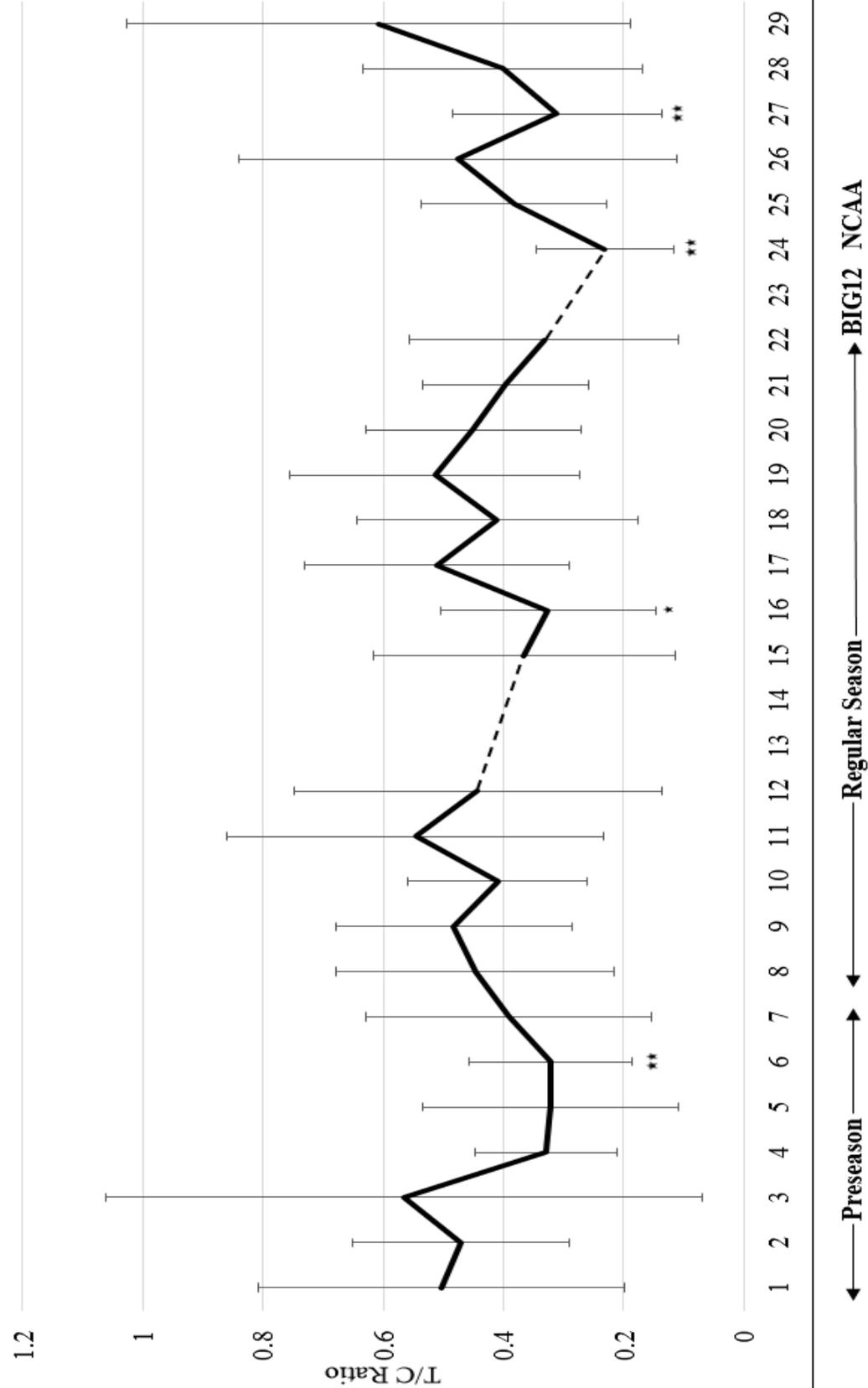
\* $p < .05$  \*\* $p < .01$

Table 2: Correlations Between Composite Stress Data and Hormones

	Composite	Game Minutes	Practice Minutes	RT Volume (Reps)	Academic	Travel
Testosterone	-.24	.03	-.24	.29	-.22	-.25
Cortisol	-.10	-.11	-.08	.18	-.14	-.07
T/C Ratio	-.19	.09	-.31	-.09	.06	-.01

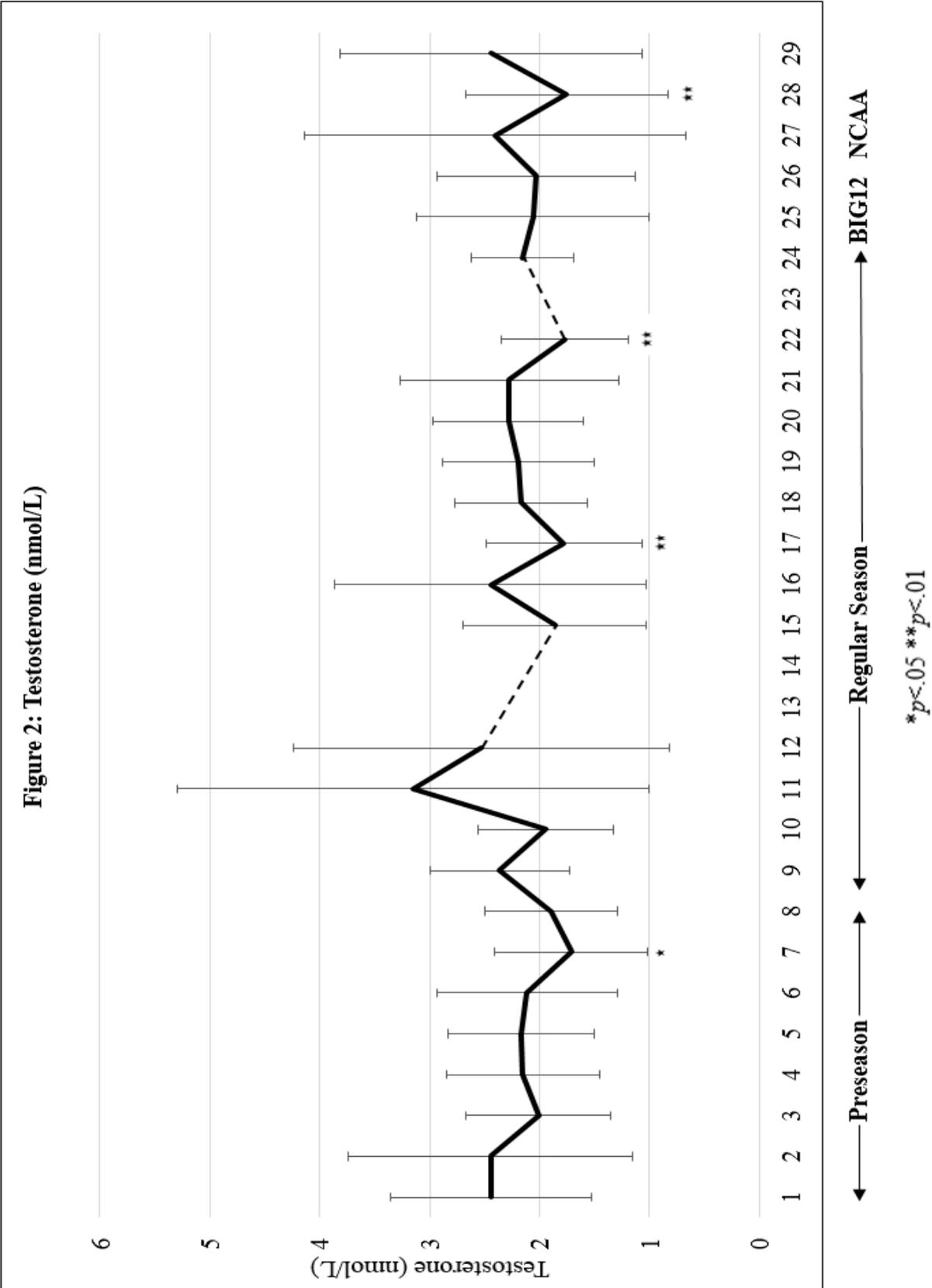
\* $p < .05$ ;  $r_{crit} = .38$

**Figure 1: Testosterone/Cortisol Ratio**

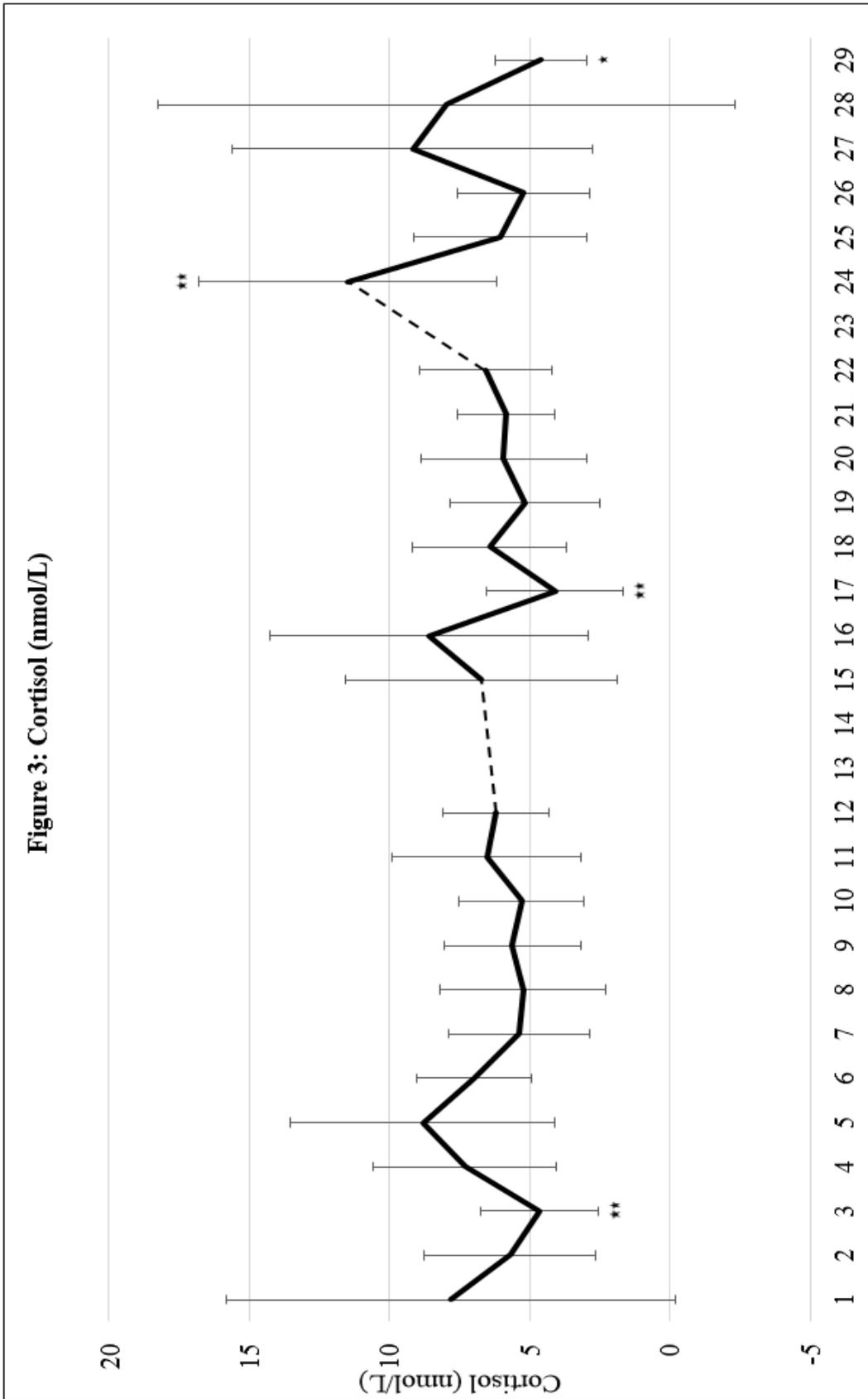


\* $p < .05$  \*\* $p < .01$

**Figure 2: Testosterone (nmol/L)**

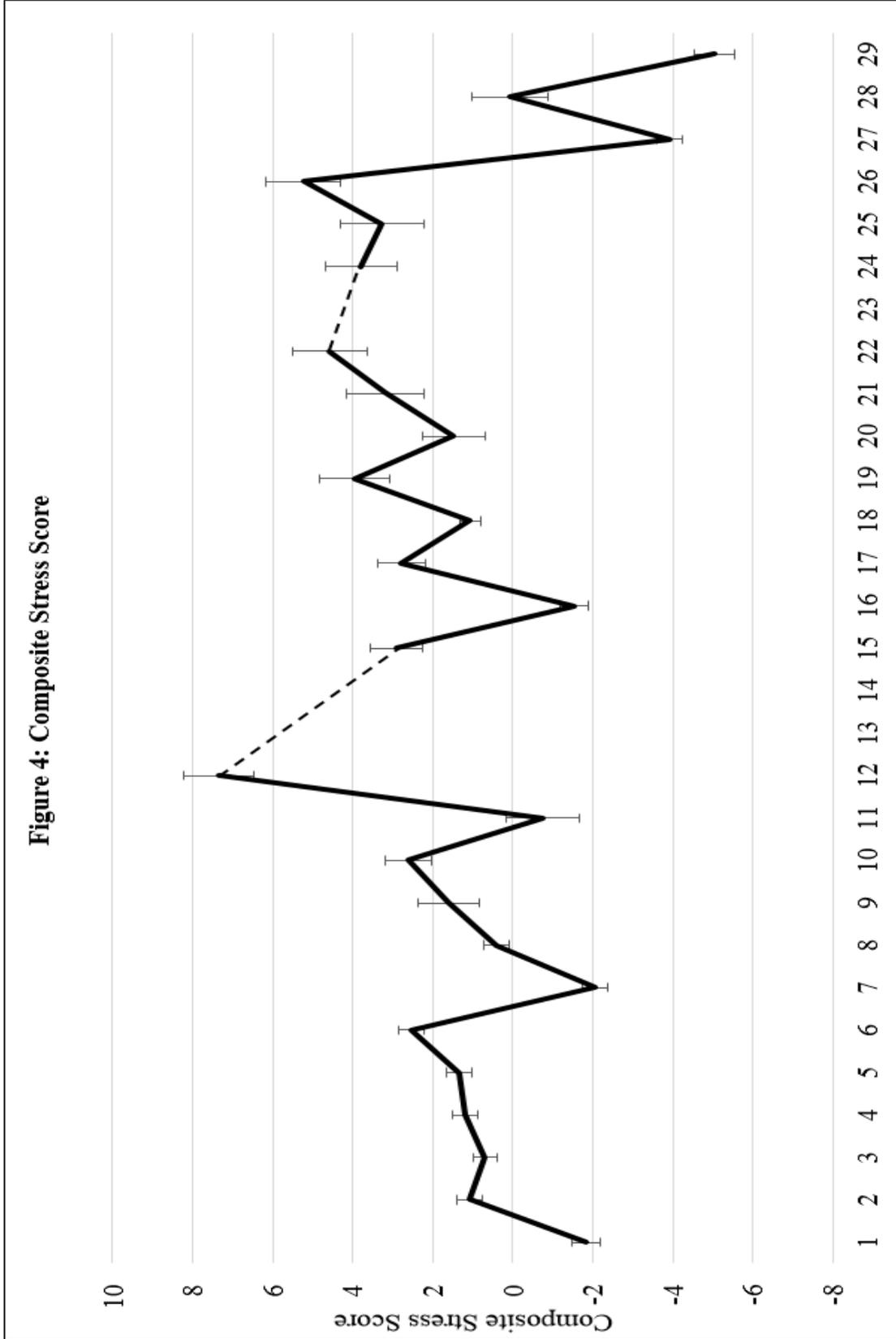


**Figure 3: Cortisol (nmol/L)**



\* $p < .05$  \*\* $p < .01$

Figure 4: Composite Stress Score



## DISCUSSION

The results of this study demonstrate that this was an appropriate method for monitoring fatigue management of elite female National Collegiate Athletic Association Division I basketball athletes. Several interesting phenomena were observed. First, the return to baseline for T/C by Week 7, the first week of exhibition play, demonstrated that the athletes were recovered from stressful preseason training and prepared to start the competition period, beginning the following week. During Week 6, there was an increase in COMP as a final effort to peak the athletes before the taper used to transition from pre-season to the competition period. Specifically, the components of COMP that caused its significant decrease during Week 7 were resistance training volume and practice minutes, which were decreased below baseline; this may have led to the increase in T/C. This is consistent with studies observing male athletes (Fry & Hoffman, 2008; Winchester, 2009) which saw T/C return to baseline or beyond after a taper. This is important because if off-season or preseason training is too stressful, then the athletes may not recover in time for competition, or may simply not reap the benefits of the program.

The present study observed opposite results of Hoffman et al (1999) in that C was not elevated by the stressful preseason training camp while T dropped from a preseason high back to baseline during camp and T/C significantly decreased during the training camp, while Hoffman and colleagues observed changes in C, but not T or T/C. It is unknown whether or not the results differed due to the biological sex of the athletes, the playing status (professional versus collegiate), or another factor. It would be interesting to see if the athletes in Hoffman et al's (1999) study saw a similar increase in T/C following a taper as observed in the present study. In contrast to the results of Schelling et al (2009), the athletes in the present study did not see an

increase in T after a taper despite a return to baseline for T/C. However, Schelling and colleagues (2009) measured total testosterone, while the present study measured free testosterone. Therefore, it is possible that the difference may have been due to total versus free T, suggesting that they may respond differently to a taper in basketball athletes. It is also possible that the results differed due to the biological sex of the athletes, timing of the taper, the type of athlete (professional versus collegiate), or another factor.

During Wk16, which was collected the week after holiday break, T/C was below baseline despite a decrease in COMP. While the increase in C during Week 16 was not statistically-significant ( $p = .229$ ) due to team variation, the team average for C during that week was greater than one standard deviation above the season mean, which may have caused the depressed ratio. This is important because, while it may be assumed that student-athletes would be refreshed and recovered after a holiday break, it is also possible that the holidays caused more stress for the athletes. For example, one study (Kornienko et al., 2013) demonstrated that salivary cortisol is impacted by perceived social standing and friendship networks, suggesting that an increase in cortisol may occur during a period of time where student-athletes feel less connected or more socially pressured, depending on individual personality characteristics. While the current study did not evaluate these characteristics in our student-athletes, it is still possible that these characteristics were impacted by the holiday break and thus influenced cortisol. Therefore, social stress should be taken into consideration when planning training and breaks for athletes. Use of the team psychologist or other stress-reduction and recovery methods during or immediately after the break may potentially benefit the student-athletes.

During Week 24, T/C was below baseline and corresponded with an increase in COMP due to increased competitions. This occurred during a 3-game losing streak amid a string of important conference match-ups, suggesting that important conference play may reduce T/C in elite female basketball athletes. During Wk27, which was collected immediately before the team's first match of the NCAA tournament, T/C was below baseline despite a decrease in COMP, suggesting an anticipatory effect. However, the athletes returned to baseline during the tournament and remained at baseline up to and beyond their 4th round elimination, suggesting that they were physiologically prepared for post-season play.

This is similar to the results of Schelling et al (2009), who found that testosterone levels and T/C were significantly lower at the later collection dates, while C remained the same throughout the season (Schelling et al., 2009). However, in the present study, C varied significantly throughout the season, although it did decrease below baseline within 1 week of the end of the post-season competition, suggesting that, while the competitive efforts of female NCAA Division I basketball athletes may be stressful, they may recover quickly from the season. Additionally, the present study may have differed from Schelling and colleagues (2009) because of the professional status of their athletes versus the student-athlete status of the athletes in the present study, suggesting that student-athletes may experience more variability in C than professional athletes. The difference also could have been due to differences in biological sex or another factor not observed in this study.

While some may propose that a large decrease in T/C indicates a state of overtraining syndrome, Fry and colleagues (1998) have demonstrated that T/C is not necessarily impacted despite onset of overtraining syndrome. Therefore, while changes in T/C may reflect acute

fatigue-management, we cannot make statements about overtraining syndrome 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 overtraining syndrome. Therefore, the potential for overtraining syndrome is not the only reason to perform a biomarker monitoring study, as coaches and scientists can use this information to evaluate effectiveness of recovery strategies and fatigue management. Additionally, the general population may consider the effects of combined life stressors on hormonal status and attempt to reduce lifestyle stress for the purpose of improving the ratio of anabolic to catabolic hormones, thereby potentially improving health.

The methods of the present study, specifically the frequency of athlete monitoring, may explain some of the differences between this study and others (Hoffman et al., 1999; Schelling et al., 2009) which observed hormones in elite basketball athletes. The present study observed T, C, and T/C weekly, from preseason to after postseason, while the other studies (Hoffman et al., 1999; Schelling et al., 2009) observed only a few time-points. Hoffman and colleagues (1999) observed hormonal levels 4 times during a 4-week off-season training camp. While this was similar to the present study in that it was weekly observations, is only accounted for off-season training and not the stressors of the regular season or postseason play (Hoffman et al., 1999). Schelling et al (2009) observed 8 time-points throughout an 8-month period, stating that collections were taken every 4-6 weeks. Had they (Schelling et al., 2009) monitored their athletes weekly, they may have seen more variation in C. Therefore, studies attempting to monitor hormonal changes in elite basketball players should plan to use weekly collections to get a broader view of variation across a season.

Another important aspect of the present study was the Composite Stress Score. This is not a currently-validated measure for assessing student-athlete stress. Rather, it is a novel approach used to attempt to identify changes in cumulative stress throughout an entire season in elite male NCAA Division I basketball athletes. No correlations between COMP and the hormones were statistically significant. However, during several weeks where COMP significantly increased or decreased from baseline, T/C also significantly increased or decreased, demonstrating the usefulness of this measure of composite stress. Future studies could use the measure described here to monitor elite basketball players throughout a season.

Another interesting phenomena involved monitoring the menstrual cycle. A non-significant negative correlation ( $r = -0.26$ ;  $r_{crit} = 0.38$ ;  $\alpha = .05$ ) was observed between T and presence of menses, suggesting the possibility that during weeks where more players were menstruating, T was at its lowest, and vice-versa. Had the correlation achieved statistical significance, it would have been consistent with research (Oka et al., 1988) demonstrating that T will typically drop to its lowest point during menstruation in healthy, regular females. It is possible that this correlation would have been different if each individual's menstrual cycle had been more closely monitored by a physician who was trained to interpret and diagnose irregular cycles, as this may have affected the data with this population. Future studies should attempt to address this component in more detail, as well as consider the implications of fluctuations in T during the menstrual cycle for both athletes and the general population.

## CONCLUSIONS

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. The methods of this study can be used for monitoring fatigue management by assessing how one's athletes adapt to stressful pre-season training and whether or not they recover in time for regular season play, in addition to how the athletes handle the stressors of the competitive season. 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 fatigue management 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 (i.e. vertical jump height) after each specimen collection, to see if changes in performance variables relate to changes in salivary hormones, in addition to more detailed monitoring of the menstrual cycle.

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