Article Classification:
Report/research note

RELATIONSHIP BETWEEN SALIVARY BIOMARKERS AND EXTERNAL LOAD IN PROFESSIONAL MALE BASKETBALL PLAYERS DURING A GAME

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OPEN ACCESS

Submitted: 13 October 2023
Revised: 22 November 2023
Accepted: 06 December 2023

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Cite this article as:
Cabarkapa, D., Eserhaut, D., Ciccone, A., Cabarkapa, D., Philipp, N., Fry, A. (2023). Relationship between salivary biomarkers and external load in professional male basketball players during a game. *Journal of Applied Sports Sciences*, Vol. 2, pp. 107 - 114. DOI: *10.37393/JASS.2023.02.9*

ABSTRACT

In a sport-specific setting, the external load represents the cumulative amount of stress placed on the athlete during a specific activity, while the internal load describes the athlete's individual physiological response to that particular activity. The purpose of this study was to determine the relationship between external load measured with a tri-axial accelerometer and salivary testosterone (T), cortisol (C), and testosterone-to-cortisol ratio (T/C) as measures of internal load during a 5-on-5 basketball game. Six professional male basketball players volunteered to participate in the investigation (age=28.3±1.9 years; height=193.3±10.6 cm; body mass=90.7±10.7 kg). Upon completing a standardized warm-up protocol, athletes played a simulated basketball game composed of four 10-minute quarters with the Elam Ending. Each athlete wore a tri-axial inertial measurement unit (StriveTech, Bothell, WA, USA) embedded within tightly fitted compression shorts throughout the competitive period. The salivary samples were collected immediately upon arrival at the gym and immediately after each quarter. To determine the relationship between the external load and T, C, and T/C, separate restricted maximum likelihood linear mixed-effects models were fitted ($p<0.05$). External load demonstrated a strong positive relationship with C ($p<0.001$, $F=49.03$, $R^2=0.59$, $R^2CI=0.36-0.78$) and a strong negative relationship with T/C ($p<0.001$, $F=56.53$, $R^2=0.61$, $R^2CI=0.39-0.79$). Overall, these findings imply that C may be a valuable biomarker for assessing the summative psychophysiological stress experienced during competitive 5-on-5 basketball play as a gradual rise in C was observed along with accumulating external loads.

Keywords: *sport, performance, testosterone, cortisol, hormones*

INTRODUCTION

The term "external load" is often used to describe the sum of all mechanical or physical stress experienced by an individual during physical activity. In the field of sports and exercise science, quantifying external load often involves the use of performance-tracking technologies to monitor the workload that an individual is exposed to during training sessions or competition (Cabarkapa et al., 2023a; Cabarkapa et al., 2023b; Scott et al., 2013). Traditionally, the external load has been assessed by measuring the distance covered across different types of movement and speed zones (Rampinini et al., 2007). However, this performance analysis approach often

fails to account for the complete range of mechanical stresses that team sport players encounter, such as sudden changes in running speed, direction, and impact forces (Varley & Aughey, 2013). Various manufacturers have begun integrating high-resolution triaxial accelerometers into their devices to address this limitation, offering a more precise measure of the overall mechanical stress and external demands placed on the body during practice or competition.

Accelerometers have been widely used in physical activity research to estimate energy expenditure in real-life conditions (Halsey et al., 2011; van Heess et al., 2011). Similarly, accelerometers have been employed in a number of team sports such as netball, soccer, and basketball (Cormack et al., 2013; Montgomery et al., 2010; Scott et al., 2013a). One of the most prevalent accelerometer-derived metrics by sports science practitioners is a vector magnitude (i.e., PlayerLoad), which represents a combined measure of the instantaneous rate of change in acceleration from all three dimensions (i.e., x-, y-, and z-axis; Montgomery et al., 2010). Its effectiveness as a marker for training load has been established by comparing it with both measures of external load (e.g., distances covered) and internal load (e.g., heart rate, ratings of perceived exertion) in training and competitive environments (Casamichana et al., 2013; Scott et al., 2013b). However, relying upon a single measure of internal load, such as heart rate or rating of perceived exertion, may fail to capture the multifactorial nature of an individual athlete's response to these physiological demands (Cabarkapa et al., 2023b; Impellizzeri et al., 2019).

While external load represents the activity dosage, internal load represents an athlete's individual response to that activity (Cabarkapa et al., 2023b). As stated, this stress response is multifaceted. During strenuous exercise, exercise-related biomarkers will undergo unique task-specific changes (Cabarkapa et al., 2023c; Luebbers et al., 2022). Upon activation of the hypothalamic-pituitary-adrenal axis, the adrenal cortex secretes cortisol (C), which is considered to be the gold standard hormonal marker of stress in response to both physical and psychological stimuli (Hellhammer et al., 2009; Hogue et al., 2013; Hough et al., 2021). In addition, the androgen testosterone (T) is also altered during strenuous exercise, with notable increases observed following live competitive basketball games in young elite players (de Arruda et al., 2019). In recent years, a considerable amount of attention has been placed on examining the changes in the aforementioned hormones in basketball players during short-term and long-term training periods as well as within different phases of the competitive season (Conte & Kamarauskas, 2022; Kamarauskas & Conte, 2022; Kamarauskas et al., 2022; Kamarauskas et al., 2023;).

Due to the invasive nature of venous blood sampling, measuring hormonal concentrations via saliva offers a non-invasive and more ecologically valid methodology to determine biomarker concentrations in a sport-specific environment (Cabarkapa et al., 2023c). However, the relationships between tri-axial accelerometer-derived external load and salivary hormonal changes throughout live competitive sporting events remain underexamined in the scientific literature. Thus, the purpose of this preliminary study was to determine the relationship between external load measured with a tri-axial accelerometer and salivary T, C, and testosterone-to-cortisol ratio (T/C) during a simulated 5-on-5 professional basketball game.

MATERIALS AND METHODS

Participants

Six professional male basketball players (age = 28.3 ± 1.9 years; height = 193.3 ± 10.6 cm; body mass = 90.7 ± 10.7 kg; professional playing experience = 5.8 ± 1.7 years) volunteered to participate in the investigation. All participants were currently under or between contracts in first-tier or second-tier European professional basketball leagues (e.g., France ProA, Germany ProB) and had previous collegiate competitive experience in the United States (e.g., NCAA Division-I). No athlete reported any musculoskeletal injuries that could impair and/or limit on-court basketball playing performance. The testing procedures performed in this study were approved by the University's Institutional Review Board, and all participants signed an informed consent document.

Procedures

Upon completing a standardized warm-up protocol consisting of dynamic stretching exercises (e.g., high knees, butt-kicks, lunge-and-twist, A-skips, karaoke, and pogo jumps), athletes participated in a simulated 5-on-5 basketball game. The game consisted of four 10-minute quarters (i.e., 24-second shot clock and three-point line set at 6.75m) with the Elam Ending (i.e., the clock turns off at the first stoppage under the four-minute mark, eight points are added to the leading team score, and the team that reaches the target score wins). During the entire game, each athlete wore a tri-axial inertial measurement unit (StriveTech, Bothell, WA, USA) embedded within tightly-fitted compression shorts (i.e., 5 cm under the umbilicus). The device sampling rate was 100 Hz. All athletes played the entire game with no substitutions. The algorithm-derived external load metric obtained from the inertial measurement unit was calculated as the squared rate of change in acceleration in the x-, y-, and z-axis.

To avoid diurnal variations in salivary T and C hormonal concentrations, the testing procedures were scheduled to begin at 12:00 hours. The participants were advised not to eat, brush their teeth, or drink anything except water for 60 minutes prior to the start of the game. They were instructed to hold an oral swab (Salimetrics, State College, PA, USA) sublingually in their mouths for two minutes before releasing it into a centrifuge tube. The first salivary sample (i.e., baseline - BS) was collected immediately upon arrival at the gym and the second through fifth immediately post the first (P1Q), second (P2Q), third (P3Q), and fourth quarter (P4Q). During the data collection process, the salivary samples were kept on regular ice (0°C). After the completion of the data collection, the salivary samples were transferred into a cooler with dry ice where they stayed for 72 hours (-78.5°C) until being transferred into a chest-style laboratory freezer (-80°C; So-Low Environmental Equipment, Cincinnati, OH, USA). Before being assayed, the salivary samples were thawed for 60 minutes at room temperature (22°C). A separate enzyme-linked immunosorbent assay (ELISA; Salimetrics, State College, PA, USA) was used for each hormone (T: #1-2402 / C: #1-3002), and all samples were run in duplicates, closely following the manufacturers' recommendations. Assay plates were analyzed with a plate reader (KC4, Biotek Instruments, Winooski, VT, USA). Intra-assay and inter-assay variances for T and C were 5.6%, 5.1%, 6.2%, and 6.9%, respectively. In addition, all salivary analyses were conducted in the same laboratory by the same research technician.

Statistical Analysis

All statistical analyses were performed using R version 4.2.1 (R Core Team). As

a follow-up analysis to the previously published data set (Cabarkapa et al., 2023c), separate lme4 restricted maximum likelihood linear mixed-effects models were fitted to determine the relationship between the external load and T, C, and T/C (Bates et al., 2015). The external load was specified as a model-fixed effect and the subject as random effects. All variables were treated as continuous. Models were also fitted with the external load metric as a random effect but were found not significantly to add to the model, so they were not included in the results. Assumptions of residual normality and homoscedasticity were visually verified using Q-Q plots and model-predicted scores vs. residual plots, respectively. It was found that models were heteroscedastic, so T, C, and T/C were logarithmically transformed prior to mixed model analyses. All tables and figures report raw, non-transformed data. Fixed effect p-values were obtained using lmerTest (Kuznetsova et al., 2017). Alpha was set at $p < 0.05$. Fixed-effect effect sizes were quantified as pseudo R^2 values obtained using the Nakagawa & Schlelzeth approach (2013) via r2beta in r2glmm (Jaeger, 2017).

RESULTS

Descriptive statistics ($\bar{x} \pm SD$) for each variable examined in this study can be found in Table 1. A weak but statistically significant positive relationship was found between external load and T ($p < 0.017$, $F = 7.05$, $R^2 = 0.07$, $R^2CI = 0.00–0.36$). Similarly, a strong positive relationship was found between external load and C ($p < 0.001$, $F = 49.03$, $R^2 = 0.59$, $R^2CI = 0.36–0.78$). On the other hand, the external load revealed a strong negative relationship with T/C ($p < 0.001$, $F = 56.53$, $R^2 = 0.61$, $R^2CI = 0.39–0.79$). A graphical representation of the relationship between external load and T, C, and T/C can be found in Figure 1.

Table 1. *Descriptive statistics ($\bar{x}$±SD) for each variable examined in this study.*

Variable [unit]	Baseline	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Testosterone [nmol·L^{-1}]	0.58±0.12	0.80±0.19	1.02±0.32	1.00±0.30	0.99±0.20
Cortisol [nmol·L^{-1}]	6.72±2.53	7.89±2.58	12.31±4.67	18.03±6.58	18.81±4.67
T/C [ratio]	0.10±0.04	0.11±0.03	0.09±0.020	0.06±0.01	0.05±0.01
External Load [AU]	–	166.3±24.2	287.5±36.7	415.6±46.6	540.4±62.8

Note: T/C – testosterone-to-cortisol ratio; AU – arbitrary unit

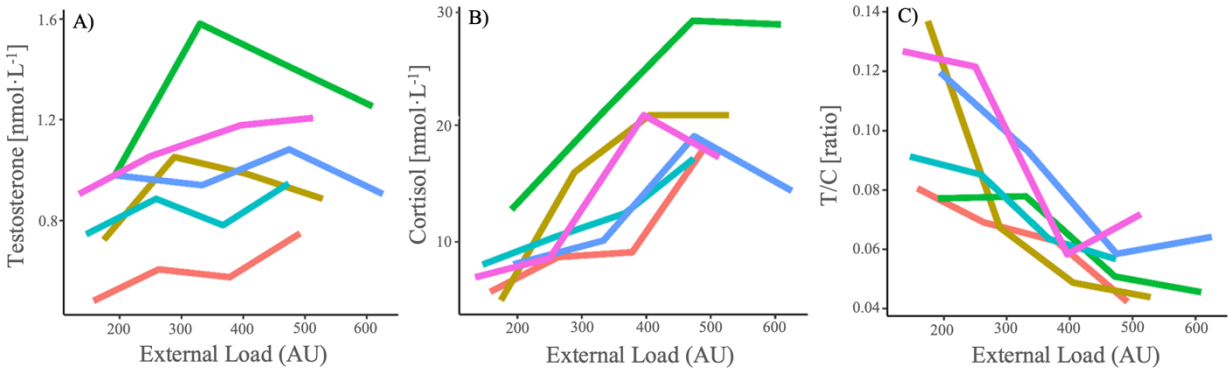

Figure 1. *Graphical representation of the relationship between external load and testosterone (A), cortisol (B), and testosterone-to-cortisol ratio (C).*

DISCUSSION

The primary aim of this study was to investigate the relationships between a measure of external load derived from a tri-axial accelerometer and salivary hormonal concentrations (i.e., T, C, and T/C) during a simulated 5-on-5 basketball game. The results of the study suggest that accelerometer-derived external load, which is the aggregate of all instantaneous rates of changes in accelerations and decelerations, may account for over half of the variance ($R^2 = 0.59$) in C elevations during competition in professional male basketball players. Additionally, external load demonstrated a moderately strong positive relationship with C ($r = 0.77$). Such an association may suggest that athletes accumulating greater external workloads during competition are also exposed to greater levels of C. Despite statistical significance, neither relationship was found for the androgen T. In addition, a moderately strong negative relationship was found between external load and T/C ($r = 0.78$). Marked T/C decreases were likely driven by the marked acute C increase observed throughout the simulated 5-on-5 basketball game. Elevations in C concentrations, paired with less pronounced elevations in T are likely to yield decreases in T/C (Cabarkapa et al., 2023c). These short-term hormonal fluctuations have been shown to reflect acute and chronic physical and psychological strains; however, inferences about an athlete's current metabolic fatigue state are less supported (de Arruda et al., 2019; Cormack et al., 2013; Hayes et al., 2015; Hough et al., 2021).

Acute fluctuations in C during strenuous exercise are well documented, with the repetitive sprint demands of basketball competition posing a unique physical demand for athletes (McInnes et al., 1995; Port, 1991). Prior reports suggest that more than 105 ± 52 high-intensity sprints can occur during a single 48-minute basketball game (McInnes et al., 1995). Significant elevations in glucocorticoid C have been reported following ten sets of 30-meter repeat sprints with 30-second rest intervals (Eryilmaz et al., 2019). Thus, the rise in C observed in this investigation is to be expected. However, the lack of a significant relationship between in-game physical demands quantified via accelerometer and T is interesting, as salivary T is known to also increase following both some forms of aerobic and anaerobic exercise (Hayes et al., 2015). Additional research on accelerometer-derived measures of external load and changes in salivary T should be performed before inferences can be made regarding the predictability of the presently used accelerometer system and this androgenic hormone.

While providing a deeper insight into the relationships between external load and salivary hormonal concentrations (i.e., T, C, and T/C), this study has some limitations. The sample size and the number of games played could have been larger. Also, individual player training history and psychological factors were not closely monitored, which could have had a potential impact on the hormonal concentrations. Moreover, future research is warranted to examine if the findings of this study are sex-specific and if they are applicable to other team sports (e.g., handball, volleyball) and competitive levels (e.g., amateur, collegiate).

CONCLUSION

Based on the findings of this study, C appears to be a valuable biomarker for assessing the summative psychophysiological stress experienced during competitive 5-on-5 basketball play, as a gradual rise in C was observed along with accumulating external loads. The relationship with an accelerometer-derived external load measure, on a quarter-to-quarter basis, may provide practitioners with additional insight into acute exercise intensity-related hormonal changes using a less invasive testing modality. Also, these findings may help strength and conditioning practitioners and sports scientists to develop and improve training regimens that adequately resemble competitive demands and ultimately optimize on-court basketball performance.

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