THE EFFECTS OF CIRCADIAN RHYTHMICITY OF SALIVARY CORTISOL AND TESTOSTERONE ON MAXIMAL ISOMETRIC FORCE, MAXIMAL DYNAMIC FORCE, AND POWER OUTPUT

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ABSTRACT

Teo, W, McGuigan, MR, and Newton, MJ. The effects of circadian rhythmicity of salivary cortisol and testosterone on maximal isometric force, maximal dynamic force, and power output. J Strength Cond Res 25(6): 1538–1545, 2011—The study investigated the effects of circadian rhythm of cortisol (C) and testosterone (T) on maximal force production (Fpeak) and power output (Ppeak). Twenty male university students (mean age = 23.8 ± 3.6 years, height = 177.5 ± 6.4 cm, weight = 78.9 ± 11.2 kg) performed 4 time-of-day testing sessions consisting of countermovement jumps (CMJs), squat jumps (SJ), isometric midthigh pulls (IMTPs), and a 1-repetition maximum (1RM) squat. Saliva samples were collected at 0800, 1200, 1600, and 2000 hours to assess T and C levels on each testing day. Session rate-of-perceived exertion (RPE) scores were collected after each session. The results showed that Fpeak and Ppeak presented a clear circadian rhythm in CMJ and IMTP but not in SJ. One repetition maximum squat did not display a clear circadian rhythm. Session RPE scores collected at 0800 and 2000 hours were significantly (p ≤ 0.05) higher than those obtained at 1200 and 1600 hours. Salivary T and C displayed a clear circadian rhythm with highest values at 0800 hours and lowest at 2000 hours; however, no significant correlation was found between T and C with Fpeak and Ppeak. A very strong correlation was found between Tarud with Fpeak of CMJ and IMTP and Ppeak of CMJ (r = 0.86, r = 0.84 and r = 0.8, p ≤ 0.001). The study showed the existence of a circadian rhythm in Fpeak and Ppeak in CMJ and IMTP. The evidence suggests that strength and power training or testing should be scheduled later during the day. The use of Tarud seemed to be a more effective indicator of physical performance than hormonal measures, and the use of session RPE should also be closely monitored because it may present a circadian rhythm.

KEY WORDS time of day, isometric midthigh pull, aural temperature, session rating-of-perceived exertion

INTRODUCTION

The effects of steroidal hormones, cortisol (C) and testosterone, (T) play an important role in the development and success of athletic performance. One of the main functions of T is to maintain anabolism of the body by means of promoting protein synthesis within the muscular system (11). Considered to be a catabolic hormone, C is often referred to as a "stress" hormone, and previous studies have documented that prolonged elevation of C levels have been associated with a higher risk of muscular atrophy and strength deficits (23,28). Because of the nature of their functions, and their importance to strength and power athletes, many studies have investigated the impact of the anabolic hormone T and the catabolic hormone C on strength and hypertrophic adaptations (28,31).

Both T and C display a distinct circadian rhythm with morning peaks and evening troughs (14). As such, researchers have previously tried to use different training modalities to manipulate the circadian rhythm of T and C to create a more favorable anabolic environment in athletes at a particular time. A study by Kraemer et al. (21) looked at the effects of an acute bout of heavy resistance training in the morning on the circadian rhythm of salivary T. The results indicated that an acute bout of heavy resistance training in the morning did not alter the circadian rhythm of salivary T for the remainder of the day. Another study by Sedlak et al. (34) investigated the effects of a 10-week specific time-of-day resistance training protocol on the circadian rhythm of both T and C. Their findings mirrored the results from Kraemer et al. (21) when they found that performing resistance training at a specific
time of the day did not induce any significant difference in the diurnal variation of T and C even after 10 weeks of training.

However, little is known about the effects of the circadian rhythm of salivary T and C on strength and power measurements. To date, there have been no studies looking at the relationship between the acute circadian variations of both T and C on strength and power production. Because of the circadian nature of both of these hormones and the impact T and C have on physical performance, fluctuations in both steroid hormones may provide an indication of an individual’s anabolic status in relation to circadian fluctuations in physical performance.

The use of saliva samples to determine T and C concentrations has been shown to be a popular method in recent years. The fact that collection of salivary samples is noninvasive and a relatively simple analysis procedure makes it an attractive alternative to collecting blood samples for serum analysis. Earlier studies have demonstrated that salivary samples are not only highly correlated with serum specimens, but intra-assay variations of salivary samples are also highly reliable. Its ability to show the circadian rhythm of hormones and its reliability even with larger sampling frequencies makes it a simple yet effective analytical tool.

The fluctuations in force and power outputs have the potential to affect the outcome of many sporting performances. Events occurring outside of the optimal time frame may potentially have an adverse effect on performance. This suggests not only an understanding of the circadian variation in sports performance but also the mechanisms that cause such variations, to be a practical consideration for both athletes and coaches. Therefore, the purpose of the study was to establish a relationship between the diurnal variations of T and C with the circadian rhythm of strength and power performance, and secondly, to examine whether perceived exertion would be affected by time of day. We hypothesized that a relationship would exist between the circadian rhythm of salivary T and C with strength and power performances and that there would be a time-of-day effect on perceived exertion.

**Methods**

**Experimental Approach to the Problem**

To determine if a relationship exists between the diurnal variations of T and C and strength performances, 4 different times of the day (0800, 1200, 1600, and 2000 hours) were chosen for strength and power testing. Saliva samples were collected immediately before each testing session to investigate the relationship of T and C with the various strength and power measures. The rationale for using these 4 time points is that they would provide a clear indication of the variation of strength and power performances through the day. In addition, the assessments selected (countermovement jumps [CMJ’s], squat jumps [SJ’s], isometric mid-thigh pull [IMTP], and 1 repetition maximum [1RM] squats) for the present study were chosen because they are commonly used by strength and conditioning coaches to determine strength and power in athletes. The Borg CR-10 rate-of-perceived exertion (RPE) scale was measured 15–30 minutes after the testing session to determine if perceived exertion was also affected by time of day.

**Subjects**

Twenty male university students (mean age = 23.8 ± 3.6 years, height = 177.5 ± 6.4 cm, weight = 78.9 ± 11.2 kg) were recruited as subjects for the study. All subjects had a minimum of 1 year of recreational resistance training experience and were resistance training at least 3 times a week before commencing the study. Subjects were free from any lower limb and back injury within the 6 months before participation of the study, and all were deemed as evening chronotypes through the use of the Horne & Ostberg (H&O) Questionnaire. The study was approved by the Edith Cowan University Human Research Ethics Committee. All subjects were also informed of the experimental risks associated with the research and signed an informed consent document before the start of the study.

**Procedures**

The experimental protocol was conducted over a period of 6 sessions. The first session was used for the collection of the subject’s physical data and chronotype. Height and weight were collected, and aural temperature \( T_{aural} \) was recorded using a digital tympanic thermometer (Thermoscan Pro 3000, Braun, Kronberg, Germany). The evaluation of the subject’s chronotype was also performed using the H&O questionnaire. All subjects were instructed to provide saliva samples at 4 different times of the day (0800, 1200, 1600, and 2000 hours) on a separate day, which included minimal physical activity. The saliva samples were subsequently used for analysis of resting C and T levels and were also used to compare the diurnal fluctuation of C and T for each subject.

The second testing session was used as a familiarization session for subjects and also to establish baseline strength and power measurements. Before the commencement of each testing session, food and water consumption was recorded to ensure that all subjects were not fasted or dehydrated. All subjects were also required to perform 2 sets of 10 jumps at 40 and 60% of their maximal effort as a warm-up. The session consisted of 4 different tests for the assessment of strength and power in the following order: 3 trials of CMJs, 3 trials of SJs, 3 trials of IMTP, and a 1RM squat. A mandatory rest period of 3 minutes was given between each test and between each 1RM squat trial. The weight recorded for the 1RM squat was the last load successfully lifted using the proper technique, just before an unsuccessful attempt. At the conclusion of the session, all subjects were requested to sit quietly for a minimum of 15 minutes before a session RPE score was collected using a Borg’s CR-10 RPE scale.

The test procedures of the third to sixth sessions were kept identical to the familiarization session with the exception of
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the starting times and were performed 1 week after the familiarization session to allow for the recovery of any delayed onset of muscle soreness. All subjects were instructed to wake up at 0630 hours on all testing sessions to standardize the amount of time spent awake and the starting times for each of the 4 testing sessions were randomized. Each testing session was also separated by a rest period of at least 48 hours so as to avoid any carry-over fatigue from the previous testing session. Saliva samples from the 4 time points (0800, 1200, 1600, and 2000 hours) were taken on the testing days to determine the CR of T and C. All measurements taken at 1600 hours were used as a control and compared against the other time points. A thermometer (Hygrometer PMRH120, TempTec, Adelaide, South Australia) was used to ensure that the ambient temperature of the laboratory was kept constant throughout the study.

Salivary Hormone Analysis
Saliva samples (at least 1 ml) were collected by passively drooling directly into a plastic tube. The samples were placed immediately into a refrigerator and frozen at −80°C for subsequent analysis. Both salivary C (µg·dl⁻¹) and T (pg·ml⁻¹) were analyzed in duplicate by an enzyme-linked immunosorbent assay (Salimetrics, State College, PA, USA) using a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA). The procedure used to determine salivary C and T concentrations were followed strictly in accordance with the manufacturer’s instructions. A microplate data acquisition and analysis software (SoftMax Pro 5, Molecular Devices) was used to determine the salivary concentration of both the hormones. Test–retest reliability using this technique to determine salivary hormone concentrations resulted in an ICC of 0.84 and a CV% of 3.5% for C and R ≥ 0.71 and a CV% of 16% for T.

Dynamic Power Analysis
Analysis of dynamic power was conducted using SJ and CMJ protocols (24). This involved the subjects performing unweighted SJ and CMJ without the aid of an arm swing. This was standardized by having the subject hold a lightweight bar (plastic bar weighing <1.0 kg) on their shoulders.

The SJ involved the subject flexing their knee to an angle of approximately 90°, maintaining that angle for 4 seconds, and jumping maximally on the command “go.” The CMJ was performed under the same conditions, but it involved a rapid flexion and extension of the knee joint at a self selected depth instead of maintaining a 90° for 4 seconds before jumping. Each jump consisted of 3 attempts, and the maximum power value of the 3 trials was used for subsequent analysis.

Vertical ground reaction force was analyzed using a 200-Hz force platform (Fitness Technologies, 400 Series, Adelaide, South Australia). Analysis was performed using the Ballistic Measurement System software (Fitness Technologies, 400 Series) collecting at 200 Hz. Reliability of these procedures has been previously established in our laboratory with an ICC of R ≥ 0.98 and a CV% of <3% for both CMJ and SJ (22).

Maximal Isometric Strength
Maximal isometric strength was measured using the IMTP exercise (25,38). Vertical ground reaction force was assessed using a force platform (Fitness Technologies, 400 Series). The force data were then analyzed using the Ballistic Measurement System software (Fitness Technologies).

Subjects were instructed to pull on an immovable bar (fixed into a power rack) as fast and as hard as they could and maintain a maximal effort for 5 seconds. Each subject performed 3 5-second trials with a 3-minute rest period between each trial. The highest value of the 3 trials was used for subsequent data analysis. The bar height was set such that the knee angle of the subject was 150° (full leg extension = 180°) (Figure 3). Force–time curves were analyzed during the IMTP to locate the maximum isometric strength and the maximum rate-of-force development (RFD) of each subject. Test–retest reliability of the IMTP was shown to be high with an ICC of R ≥ 0.96 and a CV% of 2.3% (26).

Dynamic Strength Analysis
Lower body dynamic strength was analyzed using a 1RM squat. Subjects were instructed to perform 1 warm-up set at approximately 30% of their estimated 1RM. After the warm-up set, loading was increased based on the subject’s feedback of level of repetition intensity allowing 1RM to be achieved within 3 maximal trials. A rest period of 3 minutes was provided between each trial.

The 1RM squat required the subjects to rest the bar on the trapezius while standing with legs fully extended, and then squat down to a depth where the greater trochanter of the femur was in line with the knee. Quality and depth of the squat were assessed by the investigator, and verbal feedback was provided once the subjects have achieved the required depth. The subjects then lifted the weight until the legs returned to the fully extended position. The test–retest reliability of this method of 1RM testing in our laboratory is high (R ≥ 0.98) (26).

Statistical Analyses
All statistical data were analyzed using SPSS for windows version 15.0 (SPSS Inc, Seattle, WA, USA). One-way analysis of variance with paired T-test post hoc treatment was conducted to determine if and where changes occurred between conditions. An alpha level of p ≤ 0.05 was applied as the criterion for statistical significance. Cohen’s formula for effect size (ES) was used, and the results were based on the following criteria: >0.7, large; 0.3–0.69 moderate; <0.3 small (5). Correlation between T and C concentrations, T_{max}, strength, and power variables were measured using Pearson’s product moment correlation coefficient. Cohen (5) ranked the meaningfulness of the correlation as r= trivial (0.0), small (0.1), moderate (0.3), strong (0.5), very strong (0.7), near perfect (0.9), and perfect (1.0).

RESULTS
The results from force and power measures from CMJ, SJ, IMTP, and 1RM squat at the different time of day are
provided in Figure 1. Results from the present study showed the presence of a clear circadian rhythm in $F_{\text{peak}}$ in CMJ and IMTP, respectively. Peak force measurements at 0800, 1200, and 2000 hours were significantly ($p < 0.05$) lower compared to 1600 hours in both CMJ and IMTP. Effect size calculations showed a large difference (ES = 0.80 and 0.88) in CMJ and IMTP, respectively, when comparing 0800 hours with 1600 hours. Both 1200 and 2000 hours only displayed moderate differences (ES = 0.32–0.45) when compared to 1600 hours. A significant ($p < 0.001$) difference in $P_{\text{peak}}$ output was also present in CMJ when comparing 0800, 1200, and 2000 hours to 1600 hours. There was a large difference (ES = 0.95) at 0800 hours; however, differences were found to be moderate (ES = 0.34 and 0.38) at 1200 and 2000 hours, respectively. The RFD$_{\text{peak}}$ in CMJ and IMTP also displayed a circadian rhythm with lower ($p < 0.05$) values obtained at 0800 and 2000 hours. Differences were found to be large (ES = 0.70 and 0.95) at 0800 hours and moderate (ES = 0.16–0.67) at 1200 and 2000 hours. With the exception of the values obtained at 0800 hours, force and power measurements did not attain significance at the other time points in SJ and 1RM squat. Figure 2 shows a clear circadian rhythm in salivary T and C with elevated values at 0800 hours and a nadir at 2000 hours. No significant correlation was found between T and C with $F_{\text{peak}}$ and $P_{\text{peak}}$. Results from Figure 3 showed that session RPE scores
collected at 0800 and 2000 hours were found to be significantly higher \((p < 0.001\) and \(p < 0.05\)) when compared to 1600 hours. Figure 4 showed that a clear circadian rhythm was found for \(T_{aural}\) with measurements at 0800 and 1200 hours significantly \((p < 0.001)\) lower than at 1600 hours. The results also showed a very strong correlation between \(T_{aural}\) with \(F_{peak}\) and \(P_{peak}\) of CMJ and \(F_{peak}\) of IMTP \((r = 0.86, r = 0.8, r = 0.84, p \leq 0.001)\).

**DISCUSSION**

The purpose of the study was to investigate the effects of circadian rhythm of salivary C and T on strength and power performances. From the study, the main findings from the jump analysis indicated that CMJ performance displayed a very distinct circadian rhythm in \(F_{peak}\), \(P_{peak}\), and \(RFD_{peak}\) (Figure 1). On the contrary, performance variables collected from SJ did not display a similar pattern. For SJ, there was a trend for a circadian rhythm in \(F_{peak}\), \(P_{peak}\), and \(RFD_{peak}\); however, those variables did not reach statistical significance. From these results, there seems to be an indication of increased sensitivity to a time-of-day effect in CMJ when compared to SJ. The findings are in agreement with the other studies that have also reported a failure in establishing a circadian rhythm in force and power variables for SJ \((13,30)\). Bernard et al. \((4)\) and Giacomoni and Garnett \((13)\) suggest that stored elasticity in musculotendinous tissues may be sensitive to a time-of-day effect, thus partly contributing to a fluctuation in force. Results from their study reported the existence of a circadian rhythm in power production with nadirs at 0800 hours and peaks at 1600 hours in CMJ, but no diurnal variation was found in SJ performance using a similar testing protocol.

The 1RM squat was another measure that did not reveal a clear circadian trend (Figure 1), and only values obtained at 0800 hours showed a significant \((p \leq 0.05)\) decrease in the amount of weight lifted when compared to the peak at 1600 hours. Similar to the SJ, it seems that the elimination of any significant use of an elastic component has a blunting effect on the diurnal variation of muscular performance. Although a significant \((p \leq 0.05)\) difference was observed between 0800 and 1600 hours in the SJ and 1RM squat, these protocols do not seem to be as sensitive to a diurnal change when compared to the CMJ. On the basis of these results, it appears that the time-of-day sensitivity of the elastic component within the muscle and tendons could play a larger role in the fluctuation of force production.

The importance of maximal isometric strength in certain athletic populations (i.e., sprint cyclists and wrestlers) has been demonstrated in previous studies \((26,38)\). Maximal isometric strength produced using the IMTP has been found to be highly correlated with 1RM tests and also with certain dynamic characteristics \((23)\). Thus, establishing a circadian
rhythm using the IMTP method may provide useful information for strength and conditioning coaches with regard to the circadian nature of isometric strength. To the best of our knowledge, this was the first time that the IMTP was employed to investigate CR in isometric strength. Previously, most studies focusing on isometric strength used a single leg extension or an elbow flexion protocol to determine isometric strength.

There have been limited data on the use of multijoint movements in the analysis of CR in isometric strength. The results from the current study have shown the existence of a strong CR in $F_{\text{peak}}$ and $RFD_{\text{peak}}$ for the IMTP with a peak at 1600 hours and a nadir at 0800 hours (Figure 1). These findings are in line with results from the past studies using single-joint movements to assess isometric strength (33,37). It seems that the inclusion of multiple joint movement have no blunting effect of the circadian rhythm of isometric force production and therefore the IMTP seems to be an effective test for assessing the circadian nature of overall maximal isometric strength.

To determine the circadian rhythm of T and C, saliva samples were analyzed using enzyme-linked immunosorbent assays. To date, no studies have tried to establish a relationship between the circadian rhythm of T and C with the CR of maximal force and power. Salivary analysis revealed a circadian rhythm in T and C similar to those found in previous studies (14,27). Measurements taken at 0800 hours were found to be the highest and lowest values were obtained at 2000 hours for both T and C (Figure 2). Earlier studies have reported a significant correlation between maximal force production with T and C after a prolonged period of strength training (1,17). Although this seems to be the case in longer training studies, the results from the current research failed to show any significant relationships for T and C with $F_{\text{peak}}$ and $P_{\text{peak}}$. It would seem that diurnal variations in T and C did not provide a clear representation of force and power fluctuations. The results from the study agreed with Häkkinen et al. (16), who previously hypothesized that acute changes in serum hormonal levels did not necessarily indicate changes in performance capabilities. Acute changes in serum hormones, however, were reported to be more indicative of the magnitude of physiological stress of the body (32).

Although no correlation was found with T and C, findings from the study showed a very strong correlation between $T_{\text{actin}}$ with $F_{\text{peak}}$ and $P_{\text{peak}}$ of CMJ and $F_{\text{peak}}$ of IMTP ($r = 0.86, r = 0.8$ and $r = 0.84, p < 0.001$). The study showed a strong circadian pattern in $T_{\text{actin}}$ with peaks at 1600 hours and lows at 0800 hours (Figure 3). The proposition that physical performance and body temperature were linked concomitantly was raised in previous studies (2,15). The results of the current study mirrored the work of Racinais et al. (29) in which they proposed that the increase in force production was because of the rise in core body temperature in the afternoon. Their study showed an increase in skeletal muscle contractility resulting from an increased ambient and body temperature. In an earlier study, Racinais et al. (30) also investigated the effects of environmental temperature on the ability to produce power. They found a similar concomitant increase in $P_{\text{peak}}$ output in CMJ and SJ with increased body temperature in a neutral environment (20 ± 1°C). An increase in body temperature was proposed to have facilitated the actin–myosin crossbridging process, thus, increasing the force and strain on the myosin heads attached to the actin filament (8).

Lastly, the study investigated whether perceived exertion would be affected by time of day. From the current study, the use of session RPE seems to display a strong circadian trend (Figure 4) suggesting a possible indication of a CR in sensory drive from the motor cortex to the sensory cortex. A possible cause for increased perceptual loading at different time of day could be explained by various stressors experienced at the various time points. A study by Willis et al. (39) looking at the effects of morningness–eveningness on anxiety and cardiovascular response to stress reported that levels of anxiety were higher ($p \leq 0.05$) in the morning than in the afternoon for both “morning” and “evening” chronotypes. Another study by Kudielka et al. (32) also reported a higher level of psychological and physiological stress response in the morning than in the evening. A higher psychological and physiological stress level experienced in the mornings may have an influence over perceptual loading, thus increasing the level of perceived exertion felt in the mornings.

A limitation of the study would be the lack of monitoring of the subject’s sleep behavioral patterns. As a control measure of this study, all subjects chosen were “evening” chronotypes. Evening chronotypes, however, have the preference for activities occurring later during the day and have reported later sleeping times (20). Although the current study stipulated that all subjects had to have at least 7 hours of sleep and have a standardized waking up time of 0630 hours, this arrangement may not have been what they are used to before the testing sessions. The effects of a sudden change in acute sleeping habits remain unclear; however, this sudden change could have exerted an influence on the circadian rhythm of endogenous melatonin and C production. As such, it would be an important consideration in future studies to take into account the behavioral patterns of each subject before commencing any studies investigating circadian rhythms.

The strong relationship between body temperature and actin–myosin facilitation could also possibly explain the diurnal trend found in session RPE scores. In the current study, all of the subjects that participated in the study were found to be of “evening” chronotypes. From a psychophysiological standpoint, individuals that are of “evening” chronotypes have a tendency for performing activities later during the day (10,19). Baehr et al. (3) also reported that “evening” chronotypes experience a later temperature maximum than “morning” chronotypes. A lower muscle temperature in the morning and night could have had an effect on muscle mechanics and also the elastic compliancy.
of the musculotendinous unit. This could possibly be a contributing factor in the attenuation of force production in the morning and at night, thus, giving rise to an increased perception of exertion at those time points.

To fully understand the true circadian representation of physical performance, it would have been ideal to include more testing time points to give a more accurate representation of the circadian rhythm in physical performance and hormone fluctuation. The current study has shown that 4 time points were adequate to display a significant difference in physiological systems. In previous studies, a similar or lesser amount of time points has been used to demonstrate the effects of circadian rhythms on physical performance to much success (12,35). This, however, is not to say that peak physical performance is always going to occur at 1600 hours, but it is merely to reflect the fluctuations of the circadian system. Each individual will present different biological rhythms from one another making it very difficult to clearly define when specifically the most ideal time for optimal performance is. Further investigations to include more time points are needed to establish a clearer representation on the influence of the circadian system on physical performances. Only then can coaches and athletes accurately pinpoint when the most optimal time of training will be.

In conclusion, a circadian rhythm in $F_{\text{peak}}$, $P_{\text{peak}}$, and RFD$_{\text{peak}}$ has been shown to exist for CMJ and IMTP testing protocols. The results of the study found a significant positive relationship between $F_{\text{peak}}$ and $P_{\text{peak}}$ with $T_{\text{met}}$ with regard to CMJ and IMTP. There was, however, no significant relationship found between mean T and C concentrations with strength and power variables. Although the results obtained from SJ and 1RM squat did not reveal any significant circadian rhythm, there appeared to be an appreciable trend for SJ to follow the pattern exhibited by CMJ, and a much weaker but still existent trend for 1RM squat. The use of session RPE also revealed that subjects perceived a harder exertion at 0800 and 2000 hours.

**Practical Applications**

The current study has shown that a CR exists for $F_{\text{peak}}$, $P_{\text{peak}}$, and RFD$_{\text{peak}}$ in CMJ and the IMTP. These findings suggest that, to elicit maximal expression of strength and power, coaches and athletes should consider scheduling training or testing sessions later during the day. In events where competitions start in the mornings, adequate warm-ups should be performed before increase of body temperature for optimal performances. Results from this work also have implications for athletes with regard to pre and posttesting batteries. Pre and postmeasurements are employed to gauge the effectiveness of a training intervention, and this research suggests that they should be performed temporally as close as possible to avoid the possibility of any circadian effect on performance measures. Data from this study also indicate that the use of session RPE by strength and conditioning professionals should be monitored closely as perceived exertion seems to present a circadian trend.

**References**


