SALIVARY HORMONE RESPONSE TO MAXIMAL EXERCISE AT TWO TIME POINTS DURING THE DAY

Lawrence D Hayes[1,2], Fergal M Grace[1], J LonKilgore[1], John D Young[3], Julien S Baker[1]
[1]*Institute of Clinical Exercise and Health Science, University of the West of Scotland*
[2]*School of Human Sciences, London Metropolitan University*
[3]*Department of Life and Environment, University of the West of Scotland*

Original scientific paper

Abstract
The aim of the present study was to establish a relationship between the diurnal variations of testosterone and cortisol with the circadian rhythm of strength and power performance. Changes in salivary cortisol and salivary testosterone were measured after two different modes of maximal exercise (back squat and maximal 5 m sprint) at two different times of day to assess diurnal fluctuations. Seventeen physically active males volunteered as subjects. A randomized cross-over design was utilized and participants were allocated to a maximal back squat protocol at 09:00 and 17:00 h, and a maximal 5 m sprint protocol at 09:00 and 17:00 h separated by at least 48 h. Saliva samples were collected before exercise, at 5 and 60 min post exercise. Exercise performance displayed no time of day effect. No significant effect of exercise mode or time of day was observed in cortisol or testosterone concentrations. Cortisol concentrations were higher in the morning ($p<0.001$). Testosterone did not exhibit a significant time of day effect however, higher levels tended to be observed at 09:00 h. The data suggest that non weight trained individuals do not display a time of day effect for maximum squat or 5 m sprint performance, or the subsequent salivary hormonal response.

Keywords *Cortisol, Diurnal variation, Power, Sprinting, Strength, Testosterone.*

INTRODUCTION

Both cortisol and testosterone are considered to be of high importance in training adaptations in men (Jacks, Sowash, Anning, McGloughlin & Andres, 2002; Pearen, Ryall, Lynch & Muscat, 2009; Ronnestad, Nygaard & Raastad, 2011; Timon et al., 2009). Testosterone is a signal for protein synthesis in muscle tissue with the result being increased muscle mass and strength (Hayes, Bickerstaff & Baker, 2010). Considered to be a catabolic hormone, cortisol is often referred to as a "stress" hormone, and prolonged elevation of cortisol levels are associated with a higher risk of muscular atrophy and strength deficits (Pearten et al., 2009). The balance and timing of anabolic versus catabolic factors and the availability of dietary protein are considered essential to at least one aspect of muscle adaptation, namely hypertrophy (Hayes et al., 2010). Changes in the systemic hormonal milieu are frequently measured since they have been suggested to influence (Jacks et al., 2012) and/or predict (Ronnestad et al., 2011) adaptations to resistance exercise.

Both testosterone and cortisol exhibit circadian rhythmicity with peak concentrations in the morning, around the commencement of diurnal activity, and reduced concentrations in the evening and overnight (Touitou & Haus, 2000). The increase in testosterone at this time may be an attempt to counteract the stimulatory effect of cortisol on skeletal protein degradation (Hayes et al., 2010). Researchers have previously implemented different training modalities in an attempt to alter the circadian rhythm of cortisol and testosterone to create a more favorable anabolic environment. Kraemer et al. (2001) investigated the effects of an acute bout of heavy resistance exercise in the morning on the circadian rhythm of salivary testosterone. The results indicated that an acute bout of heavy resistance training in the morning did not alter the circadian rhythm of salivary testosterone for the remainder of the day. Sedliak, Finni, Cheng, Kraemer and Haekkinen (2007) reported similar findings in that circadian rhythms of cortisol and testosterone were unaltered after a 10-week specific time-of-day resistance training protocol.

Many measures of physical performance display patterns that closely mirror daily variations in core body temperature, which peak in the early evening hours in diurnally active individuals (Hayes et al., 2010). Similarly, a spectrum of physiological measures related to human exercise have been measured at multiple time points throughout the day and results suggest that acrophase of performance usually occurs between ~15:30 and 20:30 h, with the amplitudes ranging from 2 to 11% of the daily mean (Pearen et al., 2009). Diurnal variations have been shown to alter metabolic function across the ATP-Phosphocreate (PCr) (Reilly et al., 2007), anaerobic (Masamoto, Larson, Gates & Faigenbaum, 2003), and aerobic (Nicolas, Gauthier, Trouillet & Davenne, 2008) energy systems. In a practical setting, strength and power output measures have been shown to be

higher in the late afternoon (16:00-20:00 h) than morning (Comfort, Bullock & Pearson, 2012).

The aim of the present investigation was to establish a relationship between the diurnal variations of testosterone and cortisol with the circadian rhythm of strength and power performance. It was hypothesized that maximal exercise performance (back squat and 5 m sprint) would be greater in the evening compared to the morning and correlated to steroidal hormone concentrations.

MATERIALS AND METHODS

Participants

Seventeen male university students 20.2 ± 2.8 years, with a stature of 177.3 ± 7.3 cm, and body mass of 74.7 ± 12.4 kg participated. All participants were physically active but not weight trained, free from musculo-skeletal injuries and disease, having abstained from alcohol, caffeine and exercise for 24 h prior to the investigation. Participants woke up at ~07:30 h and consumed a basic breakfast (cereal or toast) according to self-reporting. Exclusion criteria included poor sleep quality, recent shift work, extreme chronotype as determined by the Horne-Ostberg Morningness-Eveningness Questionnaire (Horne & Ostberg, 1967) indicated by a score >69 or <31, or travel across multiple time zones. The investigation met the ethical standards as outlined previously (Hammouda et al., 2011) and was approved by the University of the West of Scotland Ethics Committee.

After three initial familiarization trials, participants reported to the laboratory at ~08:30 or ~16:30 h ± 5 min. A randomized crossover design was used to assign time of day and mode of exercise for each participant and each session.

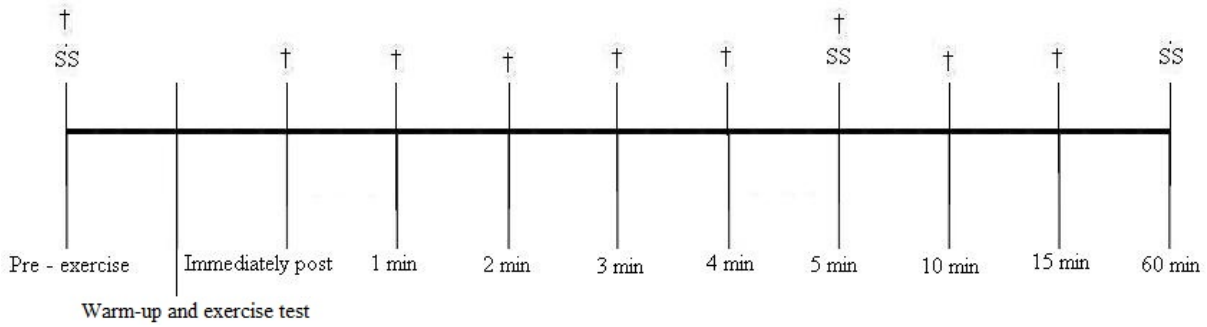

FIGURE 1. Schematic representation of protocol. SS = Saliva sample. † = Cardiovascular measures (Blood pressure and heart rate measurement).

Participants rested in a seated position for 5 min to allow baseline measures to be collected, including resting testosterone and cortisol (Figure 1). Tympanic membrane temperature was measured using a clinical-grade infrared thermometer (Thermoscan, Braun, Germany) whilst at rest. Once baseline sampling was completed, uniform and mode specific warm-ups preceded testing. Performance testing occurred at either 09:00 h or 17:00 h. These times were chosen as our previous work (Hayes et al., 2012) suggested that 09:00 h and 17:00 h were times of peak catabolism and anabolism respectively as defined by the testosterone/cortisol ratio. Participants completed a 5 m maximal sprint and a 1 repetition maximum (RM) squat at each time of day for a total of four testing sessions. These measures were utilized as back squat and 5 m sprint are commonly used for assessing strength and power in physiology research (Comfort et al., 2012; Masamoto et al., 2003; Teo, McGuigan & Newton, 2011). Blood pressure and heart rate were measured using an automated heart rate monitor and sphygmomanometer (Omron, Amsterdam, NL). Rate pressure product (RPP) was calculated by multiplying systolic blood pressure and heart rate to predict myocardial oxygen consumption as myocardial oxygen consumption can indicate stress and recovery from exercise (Atkinson, Jones & Ainslie, 2010). Testing sessions were separated by at least 48 h in order to allow complete recovery between maximal efforts (Kraemer et al., 2001).

Procedures

Prior to testing, participants were familiarized with warm-up, experimental, and measurement protocols on three occasions. A warm-up consisting of three submaximal 5 m sprints preceded participants performing three maximal 5 m sprints, each separated from the next by at least 1 min of recovery. Sprint times were recorded using automated photocells (Microgate, Bolzano, IT) adjusted to the height of the participant's hip per manufacturer's

specification. The first set of photocells was placed in front of a wall to exclude the effect of momentum. The best sprint time of the three trials was used for analyses.

Before 1RM testing, participants performed a series of submaximal warm-up sets of eight, five, two, and one repetitions with increasing load (as used by Jacks et al. [2002]). This procedure was constant across both 1RM testing sessions. All measurements were taken by the same test administrator to ensure consistency. 1RM was assessed on back squat as described previously (Baechle & Earle, 2002). Briefly, an Olympic standard barbell (York Barbell Company, York, PA) was placed above the posterior deltoids at the base of the neck. Participants lowered their body downward, bending at the hips, knees, and ankles until the top of the thighs were parallel to the floor then recovered to standing. Participants rested for at least 4 min between each of the 1RM trials. The 1RM was recorded as the maximum load lifted through the full range of motion, using good form, and only for one repetition. 1RM was attempted to be ascertained within eight test sets. If the load was lifted with the proper form, the weight was increased by approximately 1–10 kg depending on perceived exertion. As perceived effort increased, the increase in weight increment became progressively smaller until the participant failed. On average, the 1RM was determined within six trials. Failure was defined as a lift falling short of the full range of motion in two sequential attempts spaced 4 min apart. Throughout all testing procedures, a tester-to-participant ratio of 1:1 was maintained and all testing took place in a university strength and conditioning center.

Saliva collection and analysis

Whole salivary samples of approximately 1.8 mL were collected via expectoration into graduated 2 mL cryovials (Salimetrics, State College, PA). To prevent potential blood contamination of saliva resulting in an overestimation of hormone concentrations, participants were advised to avoid brushing their teeth and drinking hot fluids 2 h prior to reporting to the study venue. Saliva samples were collected and transported to a freezer immediately where they remained at -80 şC until assay. Samples were assayed in duplicate (without separation or extraction) for cortisol and testosterone using commercially available immunoassay protocols (Salimetrics, State College, PA). All samples were assayed for salivary cortisol using a high-sensitivity enzyme immunoassay (EIA) with a lower limit of sensitivity of <0.19 nmol/L, and average intra- and inter-assay coefficients of variation 4.13% and 8.89%, respectively. Saliva samples were assayed for testosterone using EIA with a lower limit of sensitivity of 5.20 pmol/L, and average intra- and inter-assay coefficients of variation less than 10% and 15%, respectively.

Statistical analysis

Data were analyzed using SPSS (version 18) (IBM North America, New York, NY, USA). Analysis of variance (ANOVA), and Pearson's correlation were used to analyze participant descriptive and experimental data. Hormonal and cardiovascular data were analyzed with an exercise type (squat vs. sprint) x time of day (09:00 vs. 17:00 h) x timepoint (pre and post exercise) repeated measures ANOVA, with a Tukey's hsd to determine pair-wise differences. A dependent t-test determined the effect of time of day on performance variables (1RM and sprint time). Significance was set *a priori* at $p \leq 0.05$.

RESULTS

Pearson's correlation revealed no relationship between maximal exercise performance (back squat and 5 m sprint) and testosterone or cortisol.

TABLE 1. Exercise performance for both protocols at each time of day (mean ± SD).

	1RM Squat (kg)	5 m Sprint (s)
09:00 h	112.5 ± 18.7	1.30 ± 0.07
17:00 h	119.4 ± 23.2	1.28 ± 0.07

1RM squat performance was higher at 17:00 h compared to 09:00 h (Table 1), however results did not reach statistical significance. There was also a tendency for improved sprint performance at 17:00 h compared to 09:00 h, although findings did not reach statistical significance ($p=.124$) and therefore the null hypothesis must be accepted. Resting and post exercise blood pressure, heart rate and RPP did not display circadian variation or significant differences between exercise conditions. Tympanic membrane temperature was not significantly different between any trials.

TABLE 2. Mean salivary hormone concentrations during the investigation (* denotes significant difference from pre exercise values, † denotes significant difference from 09:00 h values).

	Variable	Pre Exercise	5 min post exercise	60 min post exercise
Squat at 09:00 h	Cortisol (nmol/L)	9.84 ± 0.17	11.12 ± 0.26*	5.24 ± 0.09*
	Testosterone (pmol/L)	430.78 ± 32.86	469.05 ± 70.69	365.93 ± 71.68*
Squat at 17:00 h	Cortisol (nmol/L)	4.76 ± 0.11†	4.59 ± 0.08†	3.26 ± 0.06*†
	Testosterone (pmol/L)	413.61 ± 34.60	455.65 ± 27.39	349.47 ± 34.84*
Sprint at 09:00 h	Cortisol (nmol/L)	10.60 ± 0.16	10.81 ± 0.24	4.51 ± 0.08*
	Testosterone (pmol/L)	482.96 ± 25.63	478.59 ± 43.35	394.07 ± 40.70*
Sprint at 17:00 h	Cortisol (nmol/L)	5.09 ± 0.15†	4.09 ± 0.05*†	3.50 ± 0.08*†
	Testosterone (pmol/L)	345.89 ± 30.45†	410.76 ± 31.79*†	338.51 ± 25.18†

Elevated salivary hormone concentrations were observed in the 09:00 h trials during both exercise modes ($p<0.05$, Table 2). Time of day had a significant effect on cortisol and testosterone with higher values present in the morning ($p<0.05$) however, no significant differences were observed for exercise type. There was a reduction in mean salivary cortisol concentrations 60 min post exercise compared to pre and 5 min post values ($p<0.01$). Tukey's post hoc analysis revealed that this was only significant for 09:00 h tests. Resting and post exercise blood pressure and RPP did not display circadian variation in this instance.

DISCUSSION

In the present study, two sampling times were investigated (09:00 and 17:00 h). The findings indicated a variation of approximately 7% of the daily mean 1RM. This is consistent with previous research (Comfort et al., 2012), however statistical significance was not observed due to heterogeneity of participants. Sprint performance displayed a reduced variation (2.2% of the daily mean) which did not reach significance. This is in agreement with the findings of Blonc, Perrot, Racinais, Aussepe and Hue (2010) who did not observe a significant "circadian specificity" of 10 m sprint before or after time of day specific training. In a recent study, Hammouda and colleagues (2010) reported improved sprint performance at 17:00 h when compared to 07:00 h, contradicting the findings of Blonc et al. (2010) Interestingly, the study by Blonc and colleagues (2010) was conducted in a tropical environment and it is possible that the higher ambient temperatures negated the effect of the temporal variation in body temperature, which normally peaks in the early evening in diurnally active individuals. One possible explanation for the lack of circadian specificity observed in the present study may be that 5 m is too short a distance for diurnal variation to be observed. However, this explanation is at best tenuous as a 10 m sprint, 1RM squat, and countermovement jump are respectively longer, approximately the same, and shorter in duration than the 5 m sprint and these tests have shown circadian specificity (Teo et al., 2011).

Teo et al. (2011) demonstrated a circadian rhythm for countermovement jump but not a squat jump when countermovement was prohibited. In the present study, 5 m sprint was commenced from standing with a wall immediately behind the participants to exclude the effect of momentum, making it more similar to the latter of the two tests. Bernard, Giacomoni, Gavarry, Seymat and Falgairette (1998) and Giacomoni and Garnett (2002) suggested that stored elasticity in musculotendinous tissues may be sensitive to time of day effect, thus partly contributing to a diurnal fluctuation in force generation capacity. It seems that the elimination of any significant use of an elastic component has a blunting effect on the diurnal variation of muscular performance.

Despite 1RM squat including a substantial elastic component in conversion of movement direction at the bottom of the squat technique, results from the present investigation did not exhibit circadian specificity. 1RM results did however vary more than the sprint results. It is suggested that this may be due to the heterogeneity of the sample as inexperienced weight lifters may not demonstrate diurnal variation of back squat performance. Teo et al. (2011) suggested measures such as 1RM and squat jump do not appear to be as sensitive to diurnal change compared to countermovement jump. It is apparent that diurnal variation in stretch-contraction characteristics of skeletal muscle requires further research before conclusions can be made.

Salivary hormone analysis revealed diurnal variation in testosterone and cortisol similar to those found in previous studies (Hayes et al., 2012; Timon et al., 2009), with highest values observed in the morning for both hormones (Table 2). The results from the present investigation agreed with Hakkinen et al. (1988) in suggesting that acute changes in serum hormone levels did not necessarily indicate changes in performance capabilities as no correlation was observed between hormone concentrations and performance parameters. As well as individual hormone levels not influencing exercise performance, exercise mode and capabilities did not affect individual hormone concentrations.

Acute changes in serum hormones are suggested to be indicative of the magnitude of physiological stress placed on the body (Ronnestad et al., 2011). Changes in testosterone/cortisol are indicative of anabolic and catabolic status respectively. As cortisol was reduced in the 17:00 h trials when compared to testosterone, it is probable that the participants were less catabolic than at 09:00 h and therefore may have been less physiologically stressed, or in other words, more prepared for performance. This may have both training and performance ramifications.

Resting and post exercise blood pressure and RPP did not display circadian variation in this instance. This is in agreement with the findings of Jones, George, Edwards and Atkinson (2008) who observed no significant differences in RPP between conditions (nocturnal or daytime sleep) or time of day. This may have implications for cardiac rehabilitation patients and practitioners.

PRACTICAL ASPECTS

The results of the present investigation suggest that time of day does not affect hormonal response to, or performance in, a 1RM squat or a 5 m sprint protocol in non weight trained individuals. Additionally, a maximal squat or 5 m sprint protocol does not affect the circadian pattern of salivary cortisol or testosterone as the values observed in the present investigation were similar to those at the same time of day as our previous work (Hayes et al., 2012). This is in agreement with the findings of Kraemer and colleagues (2001) who observed that heavy resistance exercise did not influence circadian rhythm of salivary testosterone in men. From a practical aspect, practitioners seeking (or aiming to avoid) hypertrophy in non weight-trained populations can perform these activities at any time of day without concern for diurnal variation. This is further supported by RPP response to exercise being statistically indifferent across both times of day.

To fully understand the true diurnal variation of physical performance, future works should include more testing time points to give a more accurate representation of the diurnal variation of physical performance and hormonal fluctuation. Future research may also consider that individuals present differently timed biological rhythms and sleep/wake rhythms, adding to the difficulty in clearly defining ideal times for training progress and improved performance.

REFERENCES

1. Atkinson, G., Jones, H., & Ainslie, P. N. (2010). Circadian variation in the circulatory responses to exercise: relevance to the morning peaks in strokes and cardiac events. *European Journal of Applied Physiology,* 108: 15-29.
2. Baechle, T., & Earle, R. (2002). Essentials of strength and conditioning (2nd edition). Champaign, IL: Human Kinetics.
3. Bernard, T., Giacomoni, M., Gavarry, O., Seymat, M., & Falgairette, G. (1998). Time-of-day effects in maximal anaerobic leg exercise. *European Journal of Applied Physiology, 77,* 133-138.
4. Blonc, S., Perrot, S., Racinais, S., Aussepe, S., & Hue, O. (2010). Effects of 5 weeks of training at the same time of day on the diurnal variations of maximal muscle power performance. *Journal of Strength and Conditioning Research, 24,* 23-29.
5. Comfort, P., Bullock, N., & Pearson, S. J. (2012). A comparison of maximal squat strength and 5-, 10-, and 20-meter sprint times, in athletes and recreationally trained men. *Journal of Strength and Conditioning Research, 26,* 937-940.
6. Drust, B., Waterhouse, J., Atkinson, G., Edwards, B., & Reilly, T. (2005). Circadian rhythms in sports performance - An update. *Chronobiology International, 22,* 21-44, 2005.
7. Giacomoni, M., & Garnett, C. (2002). Interaction of time of day and menstrual cycle phase on muscle function in physically active women. *Journal of Sports Science, 20,* 36-37.
8. Hakkinen, K., Pakarinen, A., Alen, M., Kauhanen, H., & Komi, P. V. (1988). Daily hormonal and neuromuscular responses to intensive strength training in 1 week. *International Journal of Sports Medicine, 9,* 422-428.

9. Hammouda, O., Chtourou, H., Chahed, H., Ferchichi, S., Kallel, C., Miled, A., et al. (2011). Diurnal variations of plasma homocysteine, total antioxidant status, and biological markers of muscle injury during repeated sprint: effect on performance and muscle fatigue? a pilot study. *Chronobiology International, 28,* 958-967.
10. Harriss, D. J., & Atkinson, G. (2011). Update - Ethical Standards in Sport and Exercise Science Research. *International Journal of Sports Medicine, 32,* 819-821, 2011.
11. Hayes, L. D., Bickerstaff, G. F., & Baker, J. S. (2010). Interactions of cortisol, testosterone, and resistance training: influence of circadian rhythms. *Chronobiology International, 27,* 675-705.
12. Hayes, L. D., Grace, F. M., Kilgore, J. L., Young, J. D., & Baker, J. S. (2012). Diurnal variation of cortisol, testosterone, and their ratio in apparently healthy males. *Sport Scientific and Practical Aspects, 12,* 5-13.
13. Horne, J. A., & Ostberg, O. (1976). A self assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology, 4,* 97-110.
14. Jacks, D. E., Sowash, J., Anning, J., McGloughlin, T., & Andres, F. (2002). Effect of exercise at three exercise intensities on salivary cortisol. *Journal of Strength and Conditioning Research, 16,* 286-289, 2002.
15. Jones, H., George, K., Edwards, B., & Atkinson, G. (2008). Effects of Time of Day on Post-Exercise Blood Pressure: Circadian or Sleep-Related Influences? *Chronobiology International, 25,* 987-998, 2008.
16. Kraemer, W.J., Loebel, C.C., Volek, J.S., Ratamess, N.A., Newton, R.U., Wickham, R.B., et al. (2001). The effect of heavy resistance exercise on the circadian rhythm of salivary testosterone in men. *European Journal of Applied Physiology, 84,* 13-18.
17. Masamoto, N., Larson, R., Gates, T., & Faigenbaum, A. (2003). Acute effects of plyometric exercise on maximum squat performance in male athletes. *Journal of Strength and Conditioning Research, 17,* 68-71.
18. Nicolas, A., Gauthier, A., Trouillet, J., & Davenne, D. (2008). The influence of circadian rhythm during a sustained submaximal exercise and on recovery process. *Journal of Electromyography and Kinesiology, 18,* 284-290.
19. Pearen, M. A., Ryall, J. G., Lynch, G. S., & Muscat, G. E. O. (2009). Expression profiling of skeletal muscle following acute and chronic beta(2)-adrenergic stimulation: implications for hypertrophy, metabolism and circadian rhythm. *Genomics, 10,* 448.
20. Peeters, G. M., Van Schloor, N. M., Van Rossum, E. F., Visser, M., & Lips, P. (2008). The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. *Clinical Endocrinology, 69,* 673-682.
21. Reilly, T., Atkinson, G., Edwards, B., Waterhouse, J., Farrelly, K., & Fairhurst, E. (2007). Diurnal variation in temperature, mental and physical performance, and tasks specifically related to football (soccer). *Chronobiology International, 24,* 507-519.
22. Ronnestad, B. R., Nygaard, H., & Raastad, T. (2011). Physiological elevation of endogenous hormones results in superior strength training adaptation. *European Journal of Applied Physiology, 111,* 2249-2259.
23. Rubinow, D. R., Roca, C.A., Schmidt, P.J., Danaceau, M.A., Putnam, K., Cizza, G., Chrousos, G., & Nieman, L. (2005). Testosterone suppression of CRH-stimulated cortisol in men. *Neuropsychopharmacology, 30,* 1906-1912.
24. Sedliak, M., Finni, T., Cheng, S., Kraemer, W. J., & Haekkinen, K. (2007) Effect of time-of-day-specific strength training on serum hormone concentrations and isometric strength in men. *Chronobiology International, 24,* 1159-1177.
25. Teo, W., McGuigan, R., & Newton, M. J. (2011). The effects of circadian rhythmicity of salivary cortisol and testosterone on maximal isometric force, maximal dynamic force, and power output. *Journal of Strength and Conditioning Research, 25,* 1538-1545.
26. Timon, R., Olcina, G., Tomas-Carus, P., Munoz, D., Toribio, F., Raimundo, A., et al. (2009). Urinary steroid profile after the completion of concentric and concentric/eccentric trials with the same total workload. *Journal of Physiology & Biochemistry, 65,* 105-112.
27. Touitou, Y., & Haus, E. (2000). Alterations with aging of the endocrine and neuroendocrine circadian system in humans. *Chronobiology International, 17,* 369-390.

Corresponding Author:
Lawrence Hayes
School of Human Sciences
London Metropolitan University
Holloway Road, N7 8DB.
E-mail: L.Hayes@Londonmet.ac.uk

Received: 15. January 2013
Accepted: 23. April 2013