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Melatonin and sleep responses following exercise in elite female athletes

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ABSTRACT

To determine the melatonin concentrations and subsequent sleep indices of elite netball athletes following a training day when compared to a control day. Ten elite female netball athletes (mean ± SD; age = 23 ± 6 yrs) provided saliva samples PRE (17:15h) and POST (22:00h) a training session, and a day with no training (CONTROL). Sleep monitoring was performed using wrist actigraphy to assess total time in bed (TTB), total sleep time (TST), sleep efficiency (SE) and sleep latency (SL). Melatonin levels were significantly lower ($p < 0.05$), both PRE and POST the training condition (6.2 and 17.6 pg/mL, respectively) when compared to the CONTROL (14.8 and 24.3 pg/mL, respectively). There were no significant differences observed between conditions for any of the sleep variables. However, a small reduction in TST could be observed following the training session condition compared to the CONTROL condition. The scheduling of netball training in the evening is shown to suppress salivary melatonin levels. This may have an influence on subsequent sleep following night-time exercise.

1. Introduction

Athletes experience high training load demands and stress (Tuomilehto et al., 2016) with sleep widely regarded as important for performance and recovery (Halson, 2013). Athletes often experience poorer sleep quantity in comparison to non-athletes (Driller et al., 2017a) and reports have shown that sleep is often impaired on nights following training or competition (O'Donnell et al., 2018). Several contributing factors may cause sleep disruption, including social/media requirements (Romyn et al., 2015), competition scheduling (Fullagar et al., 2016), and increases in muscle pain and core temperature following training or competition (Oda & Shirakawa, 2014). An additional contributing factor that may impair sleep could be the suppression of melatonin following evening exercise (Monteleone et al., 1990).

Previous research has reported, disturbances to both sleep quantity and quality has implications for psychological, cognitive and physical recovery following training sessions (Fullagar et al., 2015). Given the restorative benefits provided through sleep for athletes, such as hormonal responses and cognitive performance (Davenne, 2009), disruptions in sleep indices may consequently have a negative effect on recovery and performance, which is an important consideration for those athletes that need to perform to a high standard on a weekly basis (Skein et al., 2013). Furthermore, sleep is proposed to be one of the most effective recovery strategies for elite athletes following exercise (O'Donnell et al., 2018), and previous research has shown that athletes may face unique issues that can impair their sleep, including training or competing late at night (Driller et al., 2018).

Previous research on the effect of melatonin levels following exercise has shown polarized results, with both increases (Buxton et al., 2003, Carr et al., 1981) and decreases (Buxton et al., 1997, Monteleone et al., 1990) being reported. Carr and colleagues (1981) had reported increased plasma melatonin levels in seven women following serial acute submaximal exercise. Whereas Monteleone and colleagues (1990) demonstrated reduced melatonin levels in seven male participants following nocturnal physical activity. Melatonin is a hormone that is synchronized from environmental cues, contributing to the initiation of sleep in the circadian system (Escames et al., 2012). Due to the importance of melatonin in the circadian system, its contribution to sleep initiation, and the changes in expression seen after exercise, there is a need for more investigation. In addition, the impact of exercise on biorhythms and sleep is becoming increasingly more important to understand with the increase of evening competitions and training occurring for both athletes and non-athletes.

Therefore, the aims of this study was to measure the effects of training on melatonin levels in elite female netball athletes and

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determine if there was any effect between melatonin levels and subsequent sleep indices.

2. Methods

2.1. Participants

A total of 10 elite female netball athletes (mean ± SD; age = 23 ± 6 y; body mass = 79.8 ± 8.9 kg) volunteered to participate in the study. Athletes were from the same team and were of international representative standard. The study took place during the in-season competition phase of the netball season. The athletes were free from any sleep disorders, as assessed through the Pittsburgh Sleep Quality Index (PSQI), with a global score of <5 indicating 'good sleepers' (Buysse et al., 1989). All participants provided informed written consent before taking part in this study. Ethical approval for the study was obtained through the institution's Human Research Ethics Committee (HREC#3).

2.2. Design

The current study took place over a seven-day period, whereby athletes completed one netball training session, and one rest day (CONTROL). Individual intensity for the training session was assessed through the athlete's average heart rate (Polar Electro Oy, Finland) and Rate of Perceived Exertion (RPE – Borg's 6-20 scale) (Borg, 1982, Alexiou and Coutts, 2008, Foster et al., 2001, Gaudino et al., 2015). The training session's total duration was two hours, taking place at 18:00h and concluding at 20:00h in the evening.

Saliva samples were obtained at two time points from each athlete for the training session and CONTROL days; immediately PRE (17:15h), and POST (22:00h). Athletes were instructed to collect the saliva samples in the same room, under the same lighting conditions between the two conditions. Sleep was monitored on the nights following the training session and CONTROL to assess total sleep time (TST), sleep efficiency (SE%), sleep latency (SL), and total time in bed (TTB). To control for dietary variables, athletes recorded the meals using a smartphone application (MealLogger App, Wellness Foundry, USA) for the training session and were instructed to replicate their diet for the subsequent CONTROL day.

2.3. Sleep Monitoring

Athletes were required to wear an actigraph (Readiband, Fatigue Science, Vancouver, Canada) over the duration of the study period to monitor sleep patterns. The Readiband device has been shown to have an acceptable inter-device reliability (ICC = >0.90) in a healthy adult population (Driller et al., 2016) and is commonly used in sporting teams as it is more practical and less intrusive compared to polysomnography. Athletes were instructed to wear the actigraph on the wrist they felt most comfortable (Driller et al., 2017b) continuously for the monitoring period, with the exception of time spent during on-court training sessions, or when in contact with water (e.g. showering or swimming). The raw activity scores were translated to sleep-wake scores based on computerized scoring algorithms. Sleep indices were quantified via the Fatigue Science software (Readiband, Fatigue Science, Vancouver, Canada) at a sampling rate of 16Hz.

2.4. Hormone Assessment

At each of the two time points during the two trials, athletes provided a 5 mL saliva sample by passive drool into a sterile plastic tube, with saliva samples stored at -20°C, until analysed. On the day of testing, saliva samples were thawed to room temperature and centrifuged at 3000 rpm for 15 minutes to precipitate mucins. Saliva samples were assayed using a highly sensitive Enzyme Linked Immunosorbent Assay (ELISA) for melatonin (Salimetrics, NSW, Australia), following the manufacturer's instructions. Samples were analysed in duplicate, using 100 µL of saliva per determination, with the ELISA having a lower limit of sensitivity of 1.37 pg/mL. The standard curve ranged from 0.78 pg/mL to 50.00 pg/mL, had an average intra-assay coefficient of variation (CV) of 5.7%, and an average inter-assay CV of 7.5%.

2.5. Statistical Analysis

Descriptive group statistics are shown as mean ± standard deviation unless otherwise stated. A Microsoft Excel spreadsheet was used to estimate the mean effects and 90% confidence intervals (90% CI) of all measured variables between trials (Hopkins, 2006). Magnitudes of the standardized effects were calculated using Cohen's *d* and interpreted using thresholds of 0.2, 0.6, 1.2 and 2.0 for *small, moderate, large and very large*, respectively (Hopkins et al., 2009). An effect size of < 0.2 was considered to be *trivial* and the effect was deemed *unclear* if its 90% confidence interval overlapped the thresholds for *small* positive and *small* negative effects (Batterham and Hopkins, 2006). A student's paired *t*-test was used to compare the training session and CONTROL conditions for sleep measures, and a two-way analysis of variance (ANOVA) was performed to compare the time points (PRE and POST) and the effect of conditions on salivary melatonin levels using a Statistical Package for Social Science (V.22.0, SPSS Inc., Chicago, IL), with significance set at $p \leq 0.05$.

3. Results

The athletes' mean heart rate during the training session was 145 ± 10 bpm with a mean rating of perceived exertion of 14 ± 1.

The values for the comparison between the training session and CONTROL conditions for sleep can be observed in Table 1. There was a significant interaction between time (PRE and POST) and condition (training session and CONTROL) for melatonin concentration ($p < 0.05$). A significant difference of 8.7 ± 10.4 pg/mL in melatonin was observed immediately PRE the training session compared to PRE CONTROL ($d = -0.69$, $p < 0.05$, Figure 1). There was a significant difference in melatonin levels POST the training session compared to POST CONTROL (7.4 ± 7.1, $d = -0.74$, $p < 0.05$, Figure 1).

There were no statistically significant differences observed between conditions for any of the sleep variables. However, a *small* reduction in TST could be observed following the training session condition compared to the CONTROL condition (-45 minutes, $d = 0.21$, $p > 0.05$, Table 1).

Table 1: Mean ± SD values for the measured sleep variables in a training and control environment, including the difference between training and control, P-values and Effect Sizes (±90% confidence intervals).

	Control	Training	Raw Difference (Control - Training)	Control - Training Effect Size
Total Sleep Time (TST) (h:mm)	8:46 ± 1:03	8:01 ± 1:17	0:45 ± 0:31	0.21 ±0.25 *Small*
Sleep Efficiency (SE) (%)	82.1 ± 8.9	85.3 ± 7.2	-3.4 ± 11.9	-0.35 ±0.90 *Unclear*
Total Time in Bed (TTB) (h:mm)	10:36 ± 2:09	9:56 ± 1:48	0:40 ± 1:56	0.19 ±0.60 *Unclear*
Sleep Latency (SL) (min)	27.5 ± 34.7	38.5 ± 29.3	-10.8 ± 33.8	-0.34 ±0.71 *Unclear*

[#] Significant difference between conditions ($p < 0.05$)

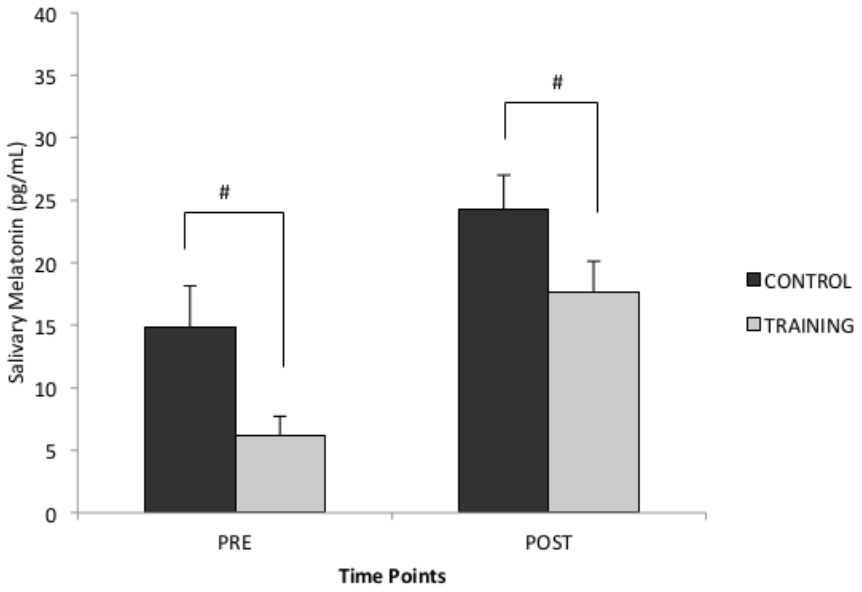

Figure 1: Salivary melatonin concentrations (pg/mL) PRE and POST a training and control day. [#] indicates a significant difference between conditions ($p < 0.05$).

4. Discussion

This study presents novel findings on the melatonin response to training and rest days and examines the subsequent sleep indices in an elite female athlete population. The main findings from the study were significantly lower salivary melatonin levels on a training day, both PRE and POST, when compared to the same time points on a rest day. Whist there was no significant differences for the sleep indices, a general trend towards impaired SL and TST in the training session trial when compared to the control was observed. These findings provide the first evidence that female athletes express lower levels of melatonin both PRE and POST training, when compared to a rest day, which contribute to the *small* trend in impaired sleep that was seen.

The results of the current study are consistent with previous investigations that have examined the suppressed melatonin response following exercise (Monteleone et al., 1990, Buxton et al., 1997). A study in healthy male participants (n=7) assessed the melatonin response following nocturnal physical activity (Monteleone et al., 1990), reporting plasma melatonin levels were significantly suppressed at 23:00 pm ($p < 0.02$), 1:00 am ($p < 0.01$), and at 2:00 am ($p < 0.03$) following a 22:40pm exercise session compared to the control condition. Similarly, a study in moderately trained males (n=8) assessing the melatonin response following differing intensity and durations of nocturnal exercise

(Buxton et al., 1997), reported a phase delay of plasma melatonin secretion following both a three hour low intensity exercise session (63 min) and a one hour high intensity exercise session (-55 minutes) compared to a baseline non-exercising condition. Whilst our study offers support of these findings, with similarities in suppressed melatonin following exercise, it should be noted there are several differences between the protocols of the studies. Both Buxton et al. (1997) and Monteleone et al. (1990) measured melatonin in plasma, in comparison to the current study where melatonin was measured through saliva. In addition, the time where exercise was initiated, differs substantially between all three studies; 1:00 am (Buxton et al., 1997), 22:40 pm (Monteleone et al., 1990), and 18:00 pm in the current study. Lastly, the duration and intensity of the exercise in each study is variable. Each of these factors make it difficult to draw decisive conclusions between the three studies. However, it is clear, from each study that exercise does have a major impact upon melatonin levels, which in turn will affect human biorhythms and physiological processes related to sleep regulation.

In regards to objective sleep metrics, the results of the current study support previous research that has assessed sleep following evening competition (Shearer et al., 2015, Juliff et al., 2018). Although not statistically significant, a *small* difference was observed following training compared to control for TST (-45 minutes), which was similar to the TST reductions from an evening game observed by Juliff and colleagues (2018) in 42 netball athletes. The physical exertion that occurs from late night training and competition may cause disruptions to circadian rhythms, in turn causing a phase delay in melatonin production, and delayed sleep onset (Shearer et al., 2015). It is positive to observe that the *small* reduction in TST (8:01 h:min) observed following the training session compared to CONTROL remained within the recommended 7 – 9 hours of sleep duration for the general population (Hirshkowitz et al., 2015). Indeed, it is somewhat surprising that the sleep duration in both conditions in the current study (8:01 and 8:46 for the training session and CONTROL) is higher than that described in previous research in elite netballers (O'Donnell et al., 2018, Juliff et al. 2018). O'Donnell et al. (2018) reported sleep durations of 6:46 the night of a netball match and 7:23 the next night following a match. Similarly, Juliff et al. (2018) reported total sleep durations of ~7:20 in the week leading up to a netball tournament. While not statistically significant, a *small* trend was also observed for delayed SL in the current study following training (38.5 minutes) compared to control (27.5 minutes). Interestingly, the athletes SE showed a *small* trend to better sleep following the training compared to the control (3.4%). These findings demonstrate that the impact of exercise (and anticipation of exercise) on the melatonin biorhythm does appear to elicit changes in sleep duration and sleep latency.

Considering the continuous level of training demands athletes' experience, any disruption they face regarding sleep would be disadvantageous. Previous research has highlighted the importance of implementing sleep hygiene education and strategies (Fullagar et al., 2016, O'Donnell & Driller, 2017) into athlete's routines, as an aid to maintain or improve sleep indices specifically during and following competition. A strategy that may be beneficial to elite athletes is the implementation of meditation following nocturnal exercise. A study by Tooley et al. (2000) investigated the melatonin response in 17 male and female participants following a meditation period. Results reported that the participants' plasma melatonin levels were significantly higher post the meditation period compared to the same time on the control night. Other studies investigating melatonin and health (Szewczyk-Golec et al., 2015) has highlighted the implications of suppressed melatonin on general well being. These studies highlight the need to better understand the effects of nocturnal exercise and its interaction with melatonin, especially given the suppression of melatonin levels seen in our studies and their potential effect on sleep quality.

There were a number of limitations with this study, which may have influenced the results. Exposure to artificial light was not controlled, which has been shown to suppress melatonin levels (Anisimov et al., 2012) and cannot be discounted as having an influence on the results. However, the data was collected in the athletes' home environment(s) during the CONTROL trial, therefore it would have detracted from the ecological validity of the results if this was performed in a laboratory. A further limitation of the current study was the lack of control for the menstrual cycle phases for each individual athlete. This may have influenced melatonin levels by the change in body temperature that occurs across the different phases (Cagnacci et al., 1996). Other limitations were the small sample size and the small number of measured time points where saliva was collected, meaning the persistence of the effects of exercise cannot be extrapolated. Regardless of this, a significant impact of exercise on melatonin levels was shown and highlights the need for more research in this area with highly trained athletes.

5. Conclusion

The results of the current study indicate that the training environment resulted in significantly suppressed melatonin levels with a trend towards impaired sleep indices when compared to a control day in female athletes. Given adequate sleep is crucial for aiding in the psychological and physiological recovery of an athlete, as well as the potential health implications of the disrupted melatonin biorhythms, the findings from this study highlight the importance for future research on the interactions of nocturnal exercise and subsequent melatonin levels.

6. Practical Applications

Results from the current study may be used by coaching staff and practitioners working with elite athletes to incorporate adequate recovery time into training schedules to account for the impaired sleep indices experienced following evening exercise. This may include scheduling longer sleep-in time following evening training sessions.

Conflict of Interest

The authors declare no conflict of interest.

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