Original Research

Acute Effects of 24-h Sleep Deprivation on Salivary Cortisol and Testosterone Concentrations and Testosterone to Cortisol Ratio Following Supplementation with Caffeine or Placebo

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ABSTRACT

International Journal of Exercise Science 10(1): 108-120, 2017. Caffeine has become a popular ergogenic aid amongst athletes and usage to improve athletic performance has been well documented. The effect of caffeine on anabolic and catabolic hormones in a sleep-deprived state has had little investigation to date. The purpose of the current study was to investigate the potential of caffeine to offset the effects, if any, of short-term sleep deprivation and exercise on an athlete's testosterone and cortisol concentrations via salivary technique. Eleven competitive male athletes volunteered to be part of this prospective double-blinded study. Three test days were scheduled for each athlete; one non-sleep deprived, one sleep-deprived with caffeine supplementation (6 mg.kg^{-1}) and one sleep-deprived with placebo ingestion. Sleep deprivation was defined as 24-h without sleep. Each test day was composed of 2 aerobic components: a modified Hoff test and a Yo-Yo test. Testosterone and cortisol concentrations were measured via salivary analysis at 4 different time-points; T1 to T4, representing baseline, and pre- and post-aerobic components, respectively. Overall no significant differences were detected comparing the different sleep states for testosterone or cortisol concentrations. A trend existed whereby the sleep-deprived with caffeine ingestion state mirrored the non-sleep deprived state for cortisol concentration. Therefore, caffeine supplementation may have potential benefits for athletes during short-term aerobic exercise when sleep-deprived. An increase in mean testosterone concentration post-aerobic exercise was only observed in the sleep-deprived with caffeine ingestion state.

KEY WORDS: Hormone, Sleep Deprivation, Caffeine, Ergogenic-Aid, Aerobic.

INTRODUCTION

Sleep deprivation is defined as a state in which adequate sleep has not been attained (19). Acute sleep deprivation has well known effects on mood, cognitive function and rate of

perceived exertion. In order to help combat the effects of sleep deprivation, athlete and non-athletes alike frequently use caffeine.

The International Olympic Committee recently lifted the partial ban on caffeine, hence making the supplement more attractable to athletes for performance enhancement (3). Cook *et al.* reported increased voluntary workload in professional athletes following caffeine supplementation in a sleep-restricted state (7). Spriet *et al.* and Desbrow *et al.* reported greater improvement in endurance performance following supplementation with 6 compared to 3 and 9 mg.kg^{-1} of caffeine (9, 15).

Peak cortisol concentrations are highest in the morning time in normal individuals. Thereafter cortisol concentration drops off slowly throughout the day (8). Cortisol is considered a catabolic hormone responsible for muscle proteolysis, stimulation of gluconeogenesis, and mobilization of FFAs. It is the major glucocorticoid released from the adrenal cortex and rises in times of "stress". Researchers have reported increased cortisol concentrations in a sleep deprived state when compared to baseline (18, 20).

Regarding testosterone concentrations, the majority of its daily release occurs when an individual is asleep (14). A lack of sleep will therefore result in a decrease in testosterone concentration compared to baseline (14). Testosterone is considered an anabolic hormone, responsible for several functions including development of secondary sexual characteristics in the young male, development of lean muscle mass, bone density and sex-drive (4). Studies (2, 12) investigating testosterone concentration in the sleep deprived participant have documented a significant decrease in testosterone concentration when compared to the non-sleep restricted participant.

By assessing testosterone and cortisol, the ratio of testosterone to cortisol (T/C) can be calculated. The T/C ratio was originally examined as a means of diagnosing overtraining syndrome (OTS) in athletes (10). A high ratio reflects an anabolic state, whereas a low ratio reflects a catabolic state. However, the T/C ratio was found to more accurately reflect a means of assessing current training load rather than as a means of diagnosing OTS (10). The current study aims to investigate changes in the T/C ratio induced by sleep deprivation.

Limited studies exist assessing the effects of caffeine on testosterone and cortisol. Beaven *et al.* documented an increase in testosterone concentration as a result of caffeine supplementation (3). Interestingly, the same study also revealed a marked rise in cortisol concentration, thus maintaining a high T/C ratio. Importantly this study was not performed in the sleep deprived status. Therefore, several factors are at play in determining the effects of sleep deprivation on testosterone and cortisol concentrations. Our hypothesis is that there will be an alteration in the T/C ratio as a result of sleep deprivation and that caffeine supplementation may alter these effects.

METHODS

Participants
Inclusion criteria were, healthy male amateur games players as assessed by subjective questionnaire and medical examination, actively participating in a competitive sport on a seasonal basis and aged 18-35 yr. Athletes were excluded from the study if they did not participate in competitive games, were female, or if they were deemed unfit to participate medically. In total, 11 athletes were enrolled into the current study.

Data in Table 1 presents participants' age, height, mass, percent body fat and body mass index data. Body fat composition was assessed via skinfold caliper. Percentage body fat data were consistent with the assumed normal range for games players. The Trinity College Dublin Research Ethics Committee granted ethical approval for the study in May 2015.

Table 1. Mean ± SD anthropometric data of participants (n=9)

	Expected range (Mean ± SD)	Results (Mean ± SD)
Height (cm)	176 ± 6	178 ± 8
Age (yr)	24 ± 6	25 ± 4
Mass (kg)	80.0 ± 8.2	80.2 ± 10.4
BMI (kg.m^{-2})	23.4 ± 1.8	24.1 ± 2.3
Body fat (%)	15.0 ± 4.2	15.1 ± 5.6

Protocol
Each participant required 4 test sessions; namely, non-sleep deprived familiarization session, a non-sleep deprived night (NSD) with no supplementation, a sleep-deprived night (SDC) with caffeine supplementation (6mg.kg^{-1}) and a sleep deprived night (SDP) with placebo supplementation. Each test day was separated by at least one week to facilitate adequate recovery between sessions. All testing was performed indoors in a gymnasium to minimize environmental factors influencing test data.

The modified Hoff test was based on the originally devised Hoff test (11). The modified Hoff test involves each participant dribbling a size 5 O'Neills football (O'Neills, Dublin, Ireland) around a series of cones and hurdles, the total distance covered in 10-min was recorded. The original test was modified to fit the exercise hall available for the current study. Each participant was followed by a tester to ensure all cones and hurdles were adequately cleared. Testers also replaced any cones or hurdles which were moved while the participant was completing the course. The first section involves dribbling around 11 cones spaced 2-m apart. The second part involves dribbling backwards with the ball until a pair of cones 10-m away was reached. The participant then turned and dribbled to the third section. The third section involves dribbling the ball through three by 1-m wide, 70-cm high hurdles and hopping over them, hurdles was spaced 4-m apart. The fourth section involves a 2.5-m sprint to a cone and then a 9-m sprint to the next cone. The fifth section involves dribbling from side to side at an

angle around a series of 12 cones. The final section was an 11-m sprint to the finish line. Each circuit equated to 150-m and participants completed as many circuits as possible in the allotted time.

The Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1) was used to assess the aerobic fitness of participants (13) This involved using a CD player to play a copy of the testing procedure. Four cones were put in a line across the hall at 3 set intervals. The distance between the first and second sets of cones was 20-m. The third set of cones were placed 5-m behind the first set of cones. Participants lined up on the first set of cones and were required to complete shuttles from the first to the second set of cones and back in times instructed by the CD. The area between the first and third set of cones formed a jog / walk area. A fixed time of 10-s separated each shuttle run. Participants completed as many shuttles as possible. Participants received a verbal warning when they did not complete a successful shuttle in the allocated time. The test terminated when they received a second verbal warning. Each participant's final level was recorded and distance completed was calculated.

A maximum of 4 participants attended on any test day, and to ensure a competitive environment competed in pairs based on performance data collected during their familiarization session. The modified Hoff test could only accommodate 2 participants at a time. Consequently, each group of 4 participants was sub-divided into 2 sub-groups: sub-group A and sub-group B.

For the purpose of this study, acute sleep deprivation was defined as remaining awake, with no sleep, for a period of 24-h prior to commencing testing. Participants arrived in Dublin on the night prior to testing and remained at an independent accommodation for the night prior. A member of the test team accompanied all participants once they arrived. Strict supervision of participants occurred in order to ensure concordance of sleep deprivation. A standardized timetable of events to entertain athletes occurred each night; including video gaming and board games. On each test day, a uniform breakfast of 5 kCal.kg^{-1} body mass, consisting of cornflakes and low-fat milk was served to participants at 07:00. Breakfast content and timing was uniform for all scheduled tests.

The caffeine (anhydrous caffeine, Sigma Aldrich, MO, USA) or placebo (lactose BDH, Poole, UK) supplements were pre-measured by the project supervisor, and amounted to 6 mg per kg body mass for each individual. Neither tester nor athlete (double blinded study design) knew the content of the randomly assigned capsule. Each individual's mass, denoted by their initials to maintain anonymity, assessed during familiarization, was provided to the
project supervisor for capsule preparation. The placebo (lactose) or caffeine supplement was then accurately weighed using a laboratory grade scales and placed into either brown or white capsules at random. Consequently, the project supervisor, an individual not involved in data collection, was the only member of the team with knowledge of the contents of each athlete's assigned capsules.

Participants provided 4 saliva samples during each test session. Samples were collected via passive drooling through a 7.4 by 20 mm straw into a clean dry pre-labeled 1.5mL Eppendorf tube.

The first saliva sample (T1) was collected before ingestion of the caffeine or placebo supplement on sleep deprived nights (SDC and SDP). On the non-sleep deprived night, the first saliva sample was not followed by any supplement ingestion. The second saliva sample (T2) was collected just before the modified Hoff aerobic test, following a low-intensity 10 min warm-up. Following termination of the Hoff test another saliva sample (T3) was collected. The 4th and final sample (T4) was collected immediately following the Yo-Yo aerobic test, see Table 2. All samples (T1 to T4) were immediately placed in an on-site pre-cooled icebox maintained at 2-3°C during testing. Once testing was completed the collected samples were immediately transported to an off-site freezer, and stored frozen (-20°C) for batch analysis.

Table 2. Timetable depicting designated times for aerobic testing, supplement for SDC and SDP trials and saliva sample collection.

	Participant A/B (Sub-group A)	Participant C/D (Sub-group B)
07:00	Breakfast	Breakfast
08:19	T1 and supplement	Nil
08:39	Nil	T1 and supplement
09:10	Warm-up	Nil
09:19	T2	Nil
09:20	Commence Hoff test	Nil
09:30	Finish Hoff test, T3, commence warm-down	Warm-up
09:39	Continue warm-down	T2
09:40	Continue warm-down	Commence Hoff test
09:50	Commence Yo-Yo test	Finish Hoff test, T3 commence warm-down
10:10	Finish Yo-Yo test, T4 commence warm-down	Commence Yo-Yo test
10:28	Continue warm-down until 10:30	Finish Yo-Yo test, T4 commence warm-down

Samples were assayed for cortisol and testosterone concentrations using highly sensitive enzyme linked immunoassay (ELISA) kits (Salimetrics, PA, USA). Each test used 25 μL for singlet determination. The cortisol assay had a lower limit sensitivity of 0.007 μg.dL^{-1} and mean intra- and inter-CV of 6.8 and 15%, respectively. The testosterone assay had a lower limit sensitivity of 3.7 pg.mL^{-1}, range of 3.7 to 360 pg.mL^{-1} and mean intra- and inter-CV of 6.5 and less than 15%, respectively.

For each ELISA plate, the mean optical density (MOD) was computed from duplicate wells. This also included the non-specific binding well (NSB). In line with manufacturer guidelines NSB data were subtracted from the MOD of, standards, controls and unknowns.

The percent bound (B_1/B_0) for each standard, control and unknown, was computed by multiplying MOD by the inverse optical density for zero ($1/B_0$). The concentration of controls and their equivalent B_1/B_0 data were entered into a graphical interpolation package. For each ELISA plate, a graph of log control concentration versus B/B_0 was constructed. A 3rd order polynomial was fitted to the data and the line of best fit calculated for each ELISA plate. Coefficients of determination (r^2) exceeded 0.98 for all plates. The resultant cubic equations were used to assess concentrations of cortisol and testosterone of the unknown samples on each plate.

Statistical Analysis
Initially an *a priori* power analysis was conducted for expected outcomes with a type 1 error probability of 0.05 and a power of 0.8, this analysis indicated that n=8 would provide a statistical power of 82% (G*power v3.0.10). To avoid discrepancies between plate readings, each participant's saliva from their three test sessions were analyzed in duplicate on an ELISA plate. Each plate, for testosterone and cortisol, could accommodate three test sessions for two participants' in duplicate with 4 wells remaining for additional test standards. Any unexpectedly high or low assays were subsequently re-assayed on an additional plate. Mean hormonal data are presented and standard error of the mean (SEM). A two-way (time by test) repeated measures ANOVA was utilized for statistical comparison of hormonal data. A single factor repeated measures ANOVA was utilized to compare performance data (distance completed in Hoff and Yo-Yo tests) and area under the curve (AUC) data for cortisol and testosterone across tests. *Post-hoc* Bonferroni test quantified detected significant differences and *P* < 0.05 inferred statistical significance.

RESULTS

There were a total of 11 participants involved in the current study. Of the 11 participants involved, 2 were excluded due to inadequate quantities of saliva collected at designated time-points. The remaining 9 participant's data were analyzed. For hormonal data comparison 4 discrete time-points were involved, expressed as T1-T4, respectively. T1 infers pre-supplementation, T2 infers pre-Hoff, T3 infers post-Hoff and T4 infers post-Yo-Yo.

Globally, no significant changes were detected comparing non-sleep restricted, with sleep restricted following caffeine or placebo supplementation. However, a significant time effect was detected using *post-hoc* testing in SDP, see Figure 1. Salivary cortisol concentrations were significantly lower comparing pre- and post-Hoff with post-Yo-Yo data (T2 vs. T4; *P* < 0.05 and T3 vs. T4; *P* < 0.01).
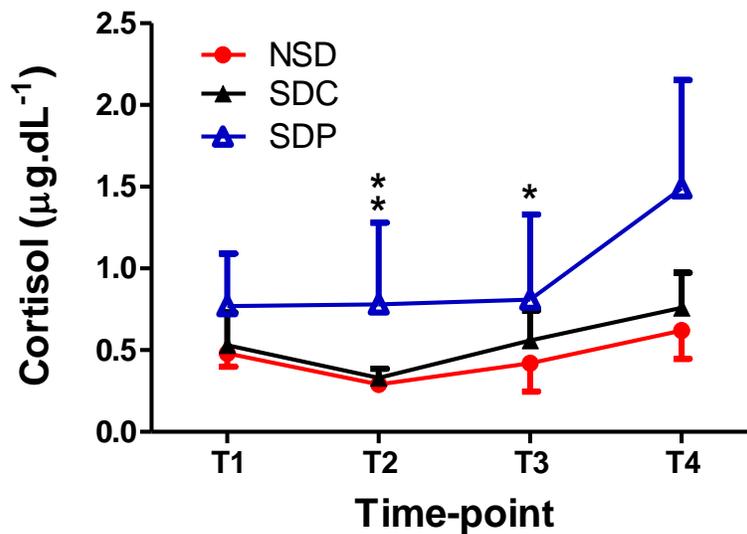
Figure 1: Mean cortisol concentration across time. T1 infers supplementation, T2 infers pre-Hoff, T3 infers post-Hoff and T4 infers post-Yo-Yo, error bars denote SEM, n=9. Asterisk (*) symbol infers significant time difference relative to T4 within SDP, *infers $P < 0.05$, **infers $P < 0.01$.

There were no significant differences detected in testosterone concentration recorded at any time-point comparing the investigated sleep states. In addition, unlike data recorded for cortisol concentration within a test session, no significant differences were detected across time within any investigated session.
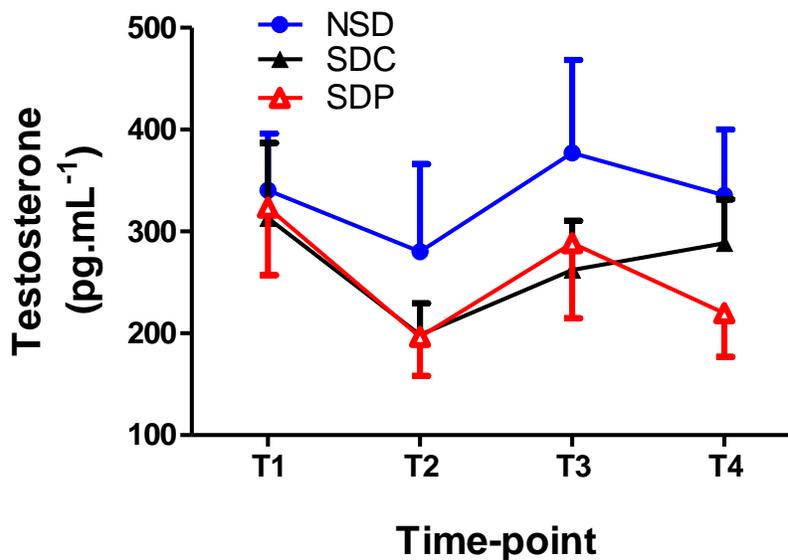

Figure 2: Mean testosterone concentration across time. T1 infers supplementation, T2 infers pre-Hoff, T3 infers post-Hoff and T4 infers post-Yo-Yo, error bar indicate SEM, n=9.

There was an overall significant time effect detected at T3 ($P < 0.05$). *Post-hoc* analysis revealed significant time effects comparing T1 and T3 and comparing T3 and T4 in the NSD state ($P < 0.05$). Within discrete time-points *post-hoc* analysis revealed a significant intervention effect comparing NSD and SDC at time-point T3 ($P < 0.05$).

The NSD state exhibited the highest testosterone to cortisol (T/C) ratio across the assessed conditions. The T/C ratio rose in the NSD state from T1 to T3, respectively, and fell to its lowest at T4. The SDP state exhibited a similar T/C response, showing a rise from T2 to T3 and a drop from T3 to T4. In contrast to the NSD state, there was a decrease in T/C ratio from T1 to T2 recorded in SDP. The SDC state exhibited a near linear decrease in T/C ratio from T1 to T4.

Comparison of computed AUC data for assayed hormones failed to detect significant differences across states, (cortisol, F=1.21, $P > 0.05$; testosterone, F=0.70, $P > 0.05$). In addition, analysis of distance data completed in the Hoff and Yo-Yo aerobic tests failed to detect significant differences ($P > 0.05$) across assessed states.
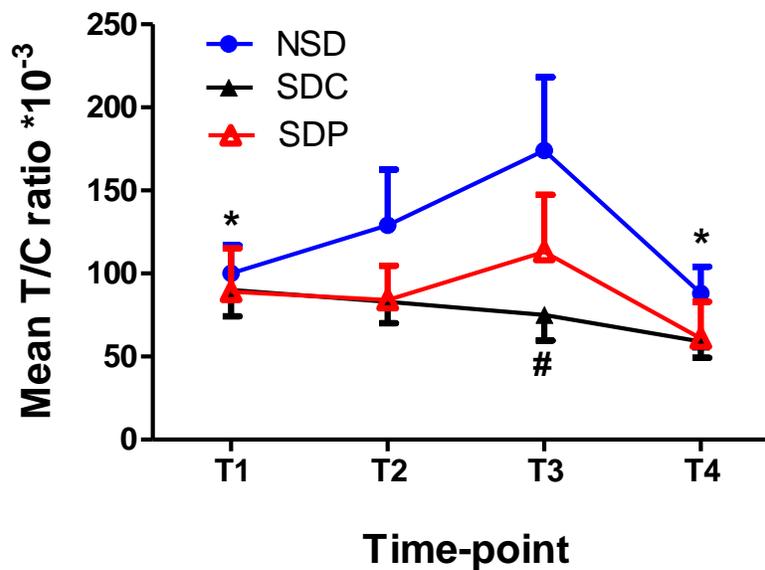

Figure 3: Mean T/C ratio across time. T1 infers supplementation, T2 infers pre-Hoff, T3 infers post-Hoff and T4 infers post-Yo-Yo, error bar denote SEM, n=9. Asterisk (*) symbol infers significant time difference relative to T3 within NSD, * infers $P < 0.05$. Hashtag (#) symbol infers significant difference at T3 comparing NSD and SDC, # infers $P < 0.05$.

DISCUSSION

Changes in mean cortisol concentration between the 3 different assessed states did not attain statistical significance for the population involved in the current study. However, there was a

significant time difference detected in the SDP state (T3 vs. T4), see Figure 1. Maximal mean cortisol concentrations were recorded in all groups at T4, following the Yo-Yo aerobic test.

Overall, the lowest cortisol concentrations were recorded in the NSD state. This would be expected and is concordant with previous research (14). The highest cortisol concentrations were recorded in SDP with the SDC state demonstrating intermediate data, see Figure 1.

The modified Hoff test is a 10-min aerobic test involving careful and skillful control of a football around a fixed circuit. The maximum sprint distance present in this modified test was just over 9-m. As skills execution was a limiting factor participants did not attain a state of true volitional exhaustion. This was highlighted by mean post-test lactate data (9.3 ± 0.6, 8.1 ± 0.8 and 8.9 ± 0.8 mmol. L^{-1} for NSD, SDP and SDC, respectively). No significant differences were recorded in Hoff distance completed across assessed states (1138 ± 18, 1143 ± 14 and 1115 ± 14 m for NSD, SDP and SDC, respectively). Although participants did not attain volitional exhaustion during this test, there were noticeable increases in cortisol data recorded at T3. Cortisol concentration at T3 was lowest in NSD, followed by SDC, with the highest concentration recorded for SDP, see Figure 1.

The final time-point, T4, exhibited the highest cortisol concentrations in all participants, regardless of assessed state. Participants at this time-point had just performed a maximal volitional aerobic exercise test (Yo-Yo test). The increase in cortisol would have been expected in all assessed states resultant from maximal exercise. In similarity with the Hoff test, no significant differences were detected in Yo-Yo distance completed across assessed states (1295 ± 6, 1325 ± 7 and 1315 ± 8 m for NSD, SDP and SDC, respectively). Nevertheless, the increases in cortisol concentration from baseline were most pronounced in SDP and SDC when compared to NSD. The addition of sleep deprivation resulted in a higher cortisol concentration at all time-points. From the results attained, it seems that the stress response that athletes were exposed to were cumulative. If the athlete was NSD and exercised they would exhibit an intermediate cortisol response. If the athlete was sleep deprived and exercised, they would exhibit a higher cortisol response. Furthermore, in the sleep-deprived states, participants who ingested caffeine (SDC) did not exhibit as extensive a cortisol increase as placebo (SDP). Therefore one could postulate that caffeine had a mild blunting effect on the endogenous cortisol response. In the current study, caffeine appeared to blunt the aerobic-exercise-induced increase in cortisol concentration following sleep deprivation.

As depicted in Figure 2, no statistically significant differences were detected across sleep states for testosterone. Furthermore, unlike data recorded for cortisol within a test session, no significant differences were detected across time within any investigated session. Maximal testosterone concentrations were recorded in the NSD state, this was evident across time-points T1-T4. This is in keeping with the expected results where sleep restriction or sleep fragmentation have been reported to negatively affect testosterone concentration (2, 12). Testosterone is thought to peak prior to REM sleep; therefore, lack of sleep will result in a decrease in total testosterone. This was evident from the results attained in the current study.

Mean testosterone concentrations across the sleep-deprived states were equivocal for the first three time-points: T1, T2 and T3, see Figure 2. Maximal data were recorded at T1 for the sleep deprived stated and were highest at T3 for the NSD state. A high testosterone concentration for both sleep deprived states relative to the other 3 time-points may be expected due to the diurnal variation of the hormone in question (peaking in the AM), as there was a dip in testosterone concentration across time from T1 (pre-supplementation) to T2 (the pre-exercise testing). Abdelmalek *et al.* depicted an increase in testosterone concentration following intermittent sprinting in sleep restricted athletes (1). This is also seen in the sleep-deprived states transitioning from T2 to T3. At this stage, there was a measurable increase in mean testosterone in the sleep-restricted states, also evident in the non-sleep restricted state.

This increase in testosterone concentration is potentially due to the non-exhausting exercise evoked in the Hoff test. It can be postulated therefore that when athletes undergo intermittent high-intensity (non-exhausting) aerobic exercise that an increase in testosterone concentration was expected. Thomas *et al.* reported overall increased testosterone concentrations in young male athletes when performing intermittent high-intensity exercise bouts involving indoor cycling (16). Testosterone has also been shown to increase linearly in response to exercise, reaching a peak once activity has ceased (21). The testosterone results attained in the current study are concordant with previous investigations. Interestingly, sleep deprivation did not appear to alter this exercise-mediated increase.

Reasons for exercise-induced increases in testosterone have been postulated by several authors. Cadouxhudson *et al.* believed that the increase was secondary to decreased testosterone clearance (6); others (11) have postulated increased testosterone secretion as a direct result of catecholamine stimulation.

The testosterone concentration at T4 did not reflect the same rise in testosterone concentration portrayed at T3. At T4 there was a decrease in testosterone concentration evident for SDP and also NSD states, see Figure 2. The Yo-Yo test induced a greater decline in testosterone concentration for SDP when compared to SDC at T4. It would seem from these results that caffeine ingestion helped to offset the decrease in testosterone expected post-exhausting aerobic exercise.

The smaller decrease recorded in testosterone concentration in SDC was in concordance with the literature. Wu (22) documented that, following caffeine supplementation (up to 6 mg.kg^{-1}), testosterone and cortisol concentrations increased significantly. This direct increase in testosterone as a result of caffeine ingestion also seems to have an effect in the sleep-deprived athlete. In this sleep-deprived state (SDC), individuals following caffeine ingestion exhibited an intermediate concentration when compared to NSD (highest concentration) and SDP (lowest concentration), see Figure 2.

Interestingly, SDC was the only sleep state that recorded an increase in mean testosterone concentration from T3 to T4. From this data one can infer that the caffeine supplementation

provided a more pronounced effect on testosterone metabolism than the exhausting Yo-Yo aerobic test. This is in keeping with data reported by Beaven *et al.* illustrating an increase in testosterone concentration after high dose caffeine ingestion (800mg) in relation to anaerobic exercise (3).

As testosterone is a potent stimulator for lean muscle formation (4), supplementation with caffeine to promote testosterone secretion would be of benefit for athletes. Improved muscle growth and recovery would enable athletes to recover quicker and ensure a more prompt return to training in a repaired / recovered state.

Overall there was a significant time difference detected when comparing NSD and SDC states ($P < 0.05$). From Figure 3, one can see that the T/C ratio was highest at all time points in the NSD state and lowest in the SDC state. This is accordance with Figures 1 and 2, respectively, which depict the NSD state as having the lowest cortisol concentrations across all 4 time points and also depict the NSD state as having double the testosterone concentrations at T3 and T4 when compared to SDC state.

As described previously a heightened testosterone concentration in the NSD state would be expected. Although it appears that caffeine may play a role in offsetting central stress responses, the overall picture points to a decrease in the T/C ratio. This can be accounted for by a more exaggerated decrease in testosterone in SDC compared to NSD. Furthermore, increased cortisol concentrations which were not evident at the T3 in the NSD state were evident in the SDC state. These findings would be consistent with data reported by Beaven *et al.* depicting an overall decrease in T/C in caffeinated rugby players (supplemented with 800 mg.kg^{-1}) due to an overall exaggerated increase in cortisol, despite having an initial increase in testosterone (3). However, athletes in their rugby study were not sleep restricted. This exaggerated increase in one hormone with respect to the other can also explain the intervention-difference noted at T3 between NSD and SDC states.

The significant time effect recorded in NSD athletes between T1 and T3 and between T3 and T4 can be explained by examining Figures 1 and 2. Testosterone increases in an almost linear fashion in the NSD state. This is in concordance with Wilkerson *et al.* (21) and Thomas *et al.* (16) who both demonstrated increased testosterone concentrations post-exercise.

Overall there were no significant differences detected comparing the sleep-state investigated for performance data, testosterone or cortisol concentrations or T/C ratio. Potential reasons for not attaining statistical significance include a small end population size and possible skewing of data by individual participants. A trend did exist whereby the SDC state mirrored the NSD state for mean cortisol concentration. This finding may have potential benefits for athletes undertaking exhausting aerobic exercise. An increase in overall mean testosterone concentration post-aerobic exercise was only observed in the SDC state, a potentially beneficial effect of caffeine supplementation. Overall further studies are required to establish whether caffeine has a significant effect on both testosterone and cortisol in the sleep deprived athlete.

This may be achieved with a larger population size, venous sampling and comparison with baseline circadian variation.

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