Title: Salivary cortisol and testosterone responses to high-intensity cycling before and after an 11-day intensified training period

Keywords Exercise · Salivary Testosterone · Salivary Cortisol · Endocrine · Endurance ·Stress

Abstract

This study examined salivary cortisol and testosterone responses to two, different high-intensity, ~30-min cycles separated by 2 h rest before and after an 11-day intensified training period. Twelve recreationally active, healthy males completed the study. Saliva samples were collected before, immediately after and 30 min after both bouts with salivary cortisol and testosterone concentrations assessed. Compared with pre-training blunted exercise-induced salivary cortisol, testosterone and cortisol/testosterone responses to both bouts post-training were observed ($p < 0.05$ for all). Comparing pre- with post-training the absolute exercise-induced salivary cortisol, testosterone and cortisol/testosterone decreased from 11.1 to 3.1 and 7.0 to 4.4 nmol·L^{-1} (cortisol), from 407 to 258 and from 473 to 274 pmol·L^{-1} (testosterone) and from 12 to 4 and 7 to 5 (cortisol/testosterone) for the first and second bouts, respectively ($P < 0.05$). No differences in the pre- and post-training RPE and HR responses during the cycles or times to fatigue were found. ($P > 0.05$). Fatigue and Burnout scores were higher post- compared with pre-training ($P < 0.05$).

These high-intensity exercise bouts can detect altered hormonal responses following intensified training. This test could assess athlete's current hormonal status, reductions in salivary cortisol and testosterone responses suggestive of increased fatigue.

Introduction

A successful training programme involves physical overload and avoids an excessive imbalance between training stress and recovery. To improve physical performance an athlete will often progressively overload the body by intensifing their physical training (by elevating volume, duration and/or intensity of training). This intensification of training can lead to a performance decrement for a limited period but following sufficient recovery (days to weeks) a "*supercompensatory*" effect may occur with the athlete exhibiting an enhanced performance when compared to baseline levels (Halson and Jeukendrup, 2004; Hooper *et al.,* 1993; Meeusen *et al.,* 2006 & 2012; O'Toole 1998). This strategy has been termed "functional overreaching" (Meeusen *et al.,* 2006 & 2012). If this intensified training continues the athlete can move into a state of "non-functional overreaching" that will lead to a reduction in physical performance that may not resume for several weeks or months. Despite the benefits of overreaching it is possible to develop the overtraining syndrome if insufficient recovery occurs (Meeusen *et al.,* 2006 & 2012). Full recovery from this syndrome may take many weeks, months or years (Meeusen *et al.,* 2006 & 2012). Signs of overreaching have been reported to occur within a period as short as 7 days of intensified training with limited recovery (Halson *et al.,* 2002). Therefore, identifying a reliable biological marker to monitor training stress would be beneficial to highlight the incidence of overreaching and aid in reducing the risk of developing the overtraining syndrome.

Resting circulating cortisol and testosterone concentrations have been examined in athletes as possible biological markers of overreaching and the overtraining syndrome (for review see Urhausen, Gabriel & Kindermann, 1995). Cortisol and testosterone taken together highlight a state of stress by indicating the body's catabolic/anabolic balance respectively. Much of this research has provided contrasting results which is likely due to the variation of training protocols, training status of the participants, measuring methods and controls for diurnal and seasonal variation of hormones used in these studies. So it is difficult to compare the studies that have been completed on this topic. However, currently there is no strong evidence that resting circulating cortisol and testosterone concentrations

and the cortisol/testosterone ratio are reliable markers of overreaching/the overtraining syndrome.

Perhaps instead of examining the resting levels of these hormones during normal training, overreaching and overtraining an examination of the exercise-induced hormonal responses may give a clearer picture of the endocrine alterations that may occur during these training states. Meeusen *et al.* (2004 & 2010) examined whether the exercise-induced responses of cortisol, adrenocorticotrophic hormone (ACTH), prolactin and growth hormone to short duration, high-intensity exercise could distinguish between normally trained and overreached athletes and athletes in a state of non-functional overreaching and the overtraining syndrome. They developed a test protocol consisting of two maximal cycling exercise bouts separated by 4 h resting recovery. A double exercise protocol was used to examine the hormonal responses to a short-duration, high-intensity cycle while also examining the effect of a short duration (4 h) recovery period on the hormone responses. Meeusen *et al.* (2004) reported that the exercise-induced responses of cortisol and ACTH concentrations to the second exercise bout of a double incremental cycle to fatigue protocol decreased by ~118% (cortisol) and ~73% (ACTH) following a 10-day training period consisting of an increased training load compared with before the training period. Athletes were classed as overreached if their performances on a cycle to fatigue bout decreased following the 10-day training camp compared with before. These findings suggest that the responses of cortisol and ACTH concentrations to short duration, high-intensity exercise are blunted following a period of intensified training. In a follow on study Meeusen *et al.* (2010) reported that the responses of ACTH and prolactin to the second maximal exercise bout of the double cycle to fatigue protocol can distinguish between non-functional overreaching and the overtraining syndrome. Athletes in a state of the overtraining syndrome showed little or no exercise-induced increases in both hormones in response to the second maximal exercise bout whereas non-functional overreached athletes showed large exercise-induced increases in both hormones (~300% (prolactin) and ~600% (ACTH) increases from pre-exercise values).

The conclusions from Meeusen *et al.* (2004 & 2010) are that the endocrine responses to short-duration, high-intensity exercise will be altered while overreached or in a state of the overtraining syndrome. In addition these alterations may be able to distinguish between states of non-functional overreaching and the overtraining syndrome. These findings are positive conclusions in the examination of the endocrine alterations in overreaching and overtraining. However, the duration and physical demand of the double cycle to fatigue protocol used by Meeusen *et al.* (2004 & 2010) may make this an impractical tool to be used in overreached athletes. Reducing the physical and time demand of this testing protocol would provide a more practical tool. Hough *et al.* (2011) reported that in a normal trained state robust increases in exercise-induced salivary cortisol and testosterone concentrations occur in response to a continuous 30-min, high-intensity cycling bout consisting of alternating blocks of 1 min at 55% maximum work rate ($\dot{W}_{max}$) and 4 min at 80% $\dot{W}_{max}$ (55/80). Robust elevations of these hormones in response to the 55/80 bout when not overreached or suffering from the overtraining syndrome should make it easier for any alterations in these hormones to be highlighted. Therefore the aim of this present study was to examine the responses of salivary cortisol and testosterone to the 55/80 cycle bout before and after an 11-day intensified training period. During this intensified training period the volume of training was increased by 143%. The majority of this increase in training volume consisted of high-intensity endurance exercise (~75% peak oxygen uptake ($\dot{V}O_{2peak}$)). This duration of the intensified training period should be sufficient to induce an overreached/overtrained state (Halson *et al.,* 2002; Jeukendrup, *et al.,* 1992; Kirwan *et al.* 1988). To measure the performance levels of the participants a cycle to fatigue at 70% $\dot{W}_{max}$ (70) (a cycle until fatigue or 30 min whichever occurs first) will also be completed 2 h after completion of the 55/80 bout (30 min cycle). In addition salivary hormone responses to the 70 bout will also be assessed. The hypothesis of this current study was that the intensified training period would induce overreaching in the participants in unison with a deterioration of performance levels in the 70 exercise bout. In addition the cortisol and testosterone responses to the 55/80 and 70 bouts would be altered comparing pre- with post-training.

Methods

Participants

Twelve recreationally active, healthy males volunteered to participate in this study. These individuals would not normally be at risk of overreaching and/or the overtraining syndrome and may be more sensitive to the intensified training compared with a group of elite athletes. The participants' anthropometric and physiological characteristics at baseline are shown in Table 1. Each participant visited the laboratory on 13 separate occasions. All study procedures were approved by the Loughborough University Ethical Advisory Committee. Following approval a full written and verbal explanation of this study and possible risks involved was given to each participant. Written informed consent to take part was obtained from each participant before testing began.

********Place Table 1 here********

Peak Oxygen Uptake ($\dot{V}O_{2\,peak}$) Assessment

On the first laboratory visit a continuous, incremental $\dot{V}O_{2\,peak}$ test was completed on a mechanically braked cycle ergometer (Monark Ergomedic 894E, Vansbro, Sweden). The test began at 95 W and the duration of each stage was 3 min. The work rate was increased at the beginning of each stage by 35 W until volitional exhaustion. Expired gas samples were collected for 1 min into Douglas bags during the final minute of each stage and during the final minute of the exercise test. Expired gas was analysed using an O_2/CO_2 analyser (Servomex 1440, Crowborough, UK) along with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for the determination of the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Heart rate (HR) was recorded continuously using short range radio telemetry (Polar F2, Polar Electro Oy, Kempele, Finland). $\dot{W}_{\max}$ was determined using the equation; $\dot{W}_{\max} = \dot{W}_{final} + (t/T)\cdot\dot{W}_{inc}$ where $\dot{W}_{final}$ is the power output during the final stage completed, t is the amount of time (s) reached in the final uncompleted stage, T is the duration of each stage (180 s), and $\dot{W}_{inc}$ is the work rate increment (35 W). This calculation was taken from

Jeukendrup *et al.* (1996). Power outputs equivalent to 55%, 70% and 80% of $\dot{W}_{max}$ for each participant were calculated and these values were used as the power outputs during the exercise trials. The work rate equivalent to 75% $\dot{V}O_{2\,peak}$ was interpolated from the relationship between $\dot{V}O_{2\,peak}$ (L·min^{-1}) and work rate (W). This value was used as the work rate during the training days.

Main Trials

REST trial

Each participant completed a resting trial (REST) within 10 days before the first exercise trial. For this trial the participant followed the schema as detailed in Figure 1 except there was no exercise completed in this trial.

Exercise trial

All participants completed two exercise trials, once before (within 3 days before)(pre-training) and 24 h after an 11-day training period which consisted of daily 1.5 h cycle bouts at 75% $\dot{V}O_{2\,peak}$ (post-training). For the exercise trials each participant followed the schema outlined in Figure 1.

*********Place Figure 1. Here********

Each participant came to the laboratory at 11:30. The exercise trials consisted of two continuous cycle bouts: (1) 30 min continuous cycling of alternating blocks of 1 min at 55% $\dot{W}_{max}$ and 4 min at 80% $\dot{W}_{max}$ (55/80); (2) cycling at 70% $\dot{W}_{max}$ for 30 min or until fatigue, whichever occurred first (70). The inclusion of the 70 bout was twofold, primarily it was to act as a performance measure but it was also added to examine the influence of the recovery period on the hormone response to exercise. It was thought that fatigue times would be close to 30 min. The purpose of stopping the trial at 30 min was to be able to compare the hormone responses to the 70 bout.

The 55/80 bout began at 12:00 and finished at 12:30. Following a 2 h resting recovery in the laboratory the 70 bout began at 14:30. HR was collected in the

final 30 s of each minute and ratings of perceived exertion (RPE) using a 6-20 Borg scale were recorded in the final 30 s of each alternating block. A 52-item Recovery-Stress questionnaire was completed at the beginning of each main trial. The Recovery-Stress questionnaire records the frequency of stress and recovery events over a period of three days and nights. Furthermore, it differentiates nonspecific and sport-specific areas of stress and recovery. The questionnaire consists of 19 stress and recovery scales in total (7 general stress; 5 general recovery; 3 sport stress and 4 sport recovery). In the Recovery-Stress questionnaire 52 there are 53 statements which the participants respond to. The participant's response covers the past 3 days/nights and each answer ranges from never (0) to always (6). Unstimulated saliva samples were collected pre-exercise, immediately post-exercise and 30 min post-exercise for both cycling bouts.

To avoid circadian rhythm and seasonal variation effects on the hormones all main trials and resting trial took place at the same time of day and during the UK summer months of May to August. For each main trial the subjects consumed a standard breakfast 3 h before testing began. Subjects remained fasted until the end of each main trial but drank water *ad libitum* during this time. The subjects abstained from exercise, caffeine and alcohol intake 24 h before each main trial. All subjects were given instructions on measuring, weighing and recording food intake and were asked to complete a food record diary 24 h before each main trial and were instructed to consume a diet as similar as possible 24 h before each main trial. Total energy and macronutrient intake was determined by use of CompEat version 5.8 software (Nutrition Systems, Oxford, UK). Mean energy intake 24 h prior to each trial was 8.6 ± 2.5 MJ with 50 ± 15% from carbohydrate, 30 ± 14 % from fat and 20 ± 4 % from protein. Body mass was measured in shorts and socks before all trials.

Training days

Each participant completed an 11-day training period. Training in the laboratory was completed on 9 of the 11 days of the training period. 5 laboratory training sessions were completed on 5 consecutive days and were followed by 2 recovery days. The remaining 4 laboratory training sessions were completed on 4 days consecutively thereafter. The training sessions took place between 07:00 and

16:00. In order for the participant to be fully recovered for the post-training exercise trial the final training day was completed at least 24 h before the start of the post-training exercise trial. Each training day consisted of 1.5 h cycling at 75% $\dot{V}O_{2peak}$. Gas samples, HR and RPE measurements were collected every 10 min for the first 30 min and then every 15 min to ensure the participants were exercising at the appropriate intensity (Figure 2). If appropriate intensity was not achieved the resistance on the ergometer was amended accordingly to achieve an average of 75% $\dot{V}O_{2peak}$ over the 1.5 h cycle.

********Place Figure 2. Here********

Training measures outside laboratory

In addition to the daily 1.5 h cycling exercise in the laboratory the participants were free to undertake further training outside the laboratory. The participants were asked to keep the additional training similar to that they would normally complete in a day. The majority of training outside of the laboratory was completed in the 2 recovery days between training day 5 and 6. Training diaries were completed and HR measurements were recorded for every extra session to confirm what exercise was completed outside of the lab. This HR data was also used to calculate training impulse scores to record the intensity of training completed by the participants outside the lab. Training impulse scores are a way to quantify intensity of training by using the duration of training and the fraction of heart rate reserve (HRR) completed during the training bout. Training impulse scores were calculated as detailed in Jobson *et al.* (2009). The equation used was Training impulse = exercise duration X fraction of HR reserve X e (fraction of HR reserve X b), where e is Euler's number 2.718 and b is a constant which is equal to 1.92 in males. Prior to beginning the study each participant reported their normal training activity (duration and mode) over a 7 day period.

Salivary handling and analysis

The participants drank water *ad libitum* during the main trials; however, to avoid the possibility of diluting the saliva sample they were not permitted to drink in the 10 min before saliva sampling. Participants were seated throughout and provided

an unstimulated saliva sample by passive dribble into a 7 ml sterile vial (Sterilin, UK) with eyes open, head tilted slightly forward and making minimal orofacial movement. Minimum collection time was 2 min for each subject to allow for collection of sufficient sample volume. All saliva samples were immediately divided into aliquots and stored at –20ºC until further analysis. The salivary cortisol and testosterone concentrations were determined using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits (Salimetrics, PA 16803, USA). The mean inter-assay coefficients of variation were 3.2% and 2.5% for cortisol and testosterone, respectively. The mean intra-assay coefficients of variation were 3.2 % and 2.6% for cortisol and testosterone, respectively.

Statistical analysis

All data in the text and tables are presented as mean values and standard deviations (*s*). Data were checked for normality, homogeneity of variance and sphericity before statistical analysis. If a data set was not normally distributed, logarithmic transformation was performed on the data. If the data remained not normally distributed following logarithmic transformation non-parametric analysis was completed on the data set. RPE scores recorded during the main trials were analysed using non-parametric tests. When the data sets were parametric a two-way (trial x time) repeated measures analysis of variance (ANOVA) was completed. Significant differences were assessed using Student's paired samples t-tests with Holm-Bonferroni adjustments for multiple comparisons. Statistical significance was set at $P < 0.05$.

Results

All twelve subjects completed all laboratory training sessions except one participant completed only 80 min of his first laboratory training session due to cramp; this participant completed all other training sessions. Each participant completed 13.5 h (1.5 h per day) of cycling in the laboratory at an average intensity of 74 ± 1 % of $\dot{V}O_{2\,peak}$ over the 11-day training period. 9 of the participants completed an average of 3 h of additional training outside of the laboratory over the 11-day period. The average training impulse score for the exercise that was completed outside the lab for all participants was 101. As a reference the average training impulse score for each 1.5 h cycling training bouts in the lab was 119. This training consisted of a mixture of intermittent, team sports (hockey and football) and resistance type exercise. When compared to the participant's normal training activity the total training duration increased by 143% (7 h to 17 h) during this period.

Recovery-Stress questionnaire

Analysis of the Recovery-Stress questionnaire scores showed that Fatigue and Burnout scores were higher after the 11-day training period compared with before the training period (Figure 3)($P < 0.05$). The Fatigue scale was calculated from the answers to 2 statements "I was dead tired after work" and "I was overtired". The Burnout scale was calculated from the answers to 4 statements "I was burned out by my sport"; "I felt emotionally drained from performance"; "I felt that I wanted to quit my sport"; "I felt frustrated by my sport".

******Place Figure 3 here********

Physiological responses to exercise and time to fatigue

No differences in HR or RPE ($P > 0.05$) responses to the 55/80 and 70 bouts were found. The cycling times to complete the 70 bout were unaltered comparing pre- and post-training trials ($P > 0.05$). The average completion times for the 70 bouts were 29:17 ± 01:47 (pre-training) and 29:35 ± 01:00 (post-training) min:s.

Hormonal measurements

The average ± *s* salivary cortisol and testosterone concentrations during the REST trial were 3.5 ± 1.8 nmol·L^{-1} and 690 ± 202 pmol·L^{-1}, respectively (Figure 3 & Figure 4). *t*-test analysis indicated that salivary cortisol and testosterone concentrations were not different at post-exercise and 30 min post-exercise compared with the pre-exercise values (either Pre 55/80 or Pre 70 where appropriate) ($P > 0.05$ for all).

Compared with pre-training blunted salivary cortisol and testosterone exercise-induced (55/80 and 70) responses occurred post-training ($P < 0.05$) (Figure 4 & Figure 5).

********Place Figure 4. and Figure 5. here********

For the 55/80 bout, the post-exercise salivary cortisol peak increase above the pre-exercise level was 11 nmol.L^{-1} (210%) (pre-training) and 3 nmol.L^{-1} (44%) (post-training). In response to the 70 bout peak increases of 7 nmol.L^{-1} (117%) and 4 nmol.L^{-1} (117%) occurred pre- and post-training, respectively.

For the 55/80 bout, the post-exercise salivary testosterone peak increase above the pre-exercise level was 407 pmol.L^{-1} (58%) (pre-training) and 258 pmol.L^{-1} (37%) (post-training). In response to the 70 bout peak increases of 473 pmol.L^{-1} (83%) and 274 pmol.L^{-1} (45%) occurred pre- and post-training, respectively.

Examined as a ratio (cortisol/testosterone), values were also blunted after the 11-day training period compared with before ($P < 0.05$). Increases of 12 (152%) and 4 (40%) in response to the 55/80 bout were found before and after the training period, respectively. In response to the 70 bout of exercise 7 (65%) and 5 (67%) increases were found before and after the training period, respectively (Figure 6).

********Place Figure 6. here********

Discussion

This present study aimed to determine the salivary cortisol and testostone responses to high-intensity cycling exercise (55/80 and 70) before and after an intensified training period. More specifically, it set out to establish if the 55/80 cycle bout can highlight alterations in the hormonal responses that occur due to an intensified training period. The 55/80 bout has previously been shown to induce robust elevations in salivary cortisol and testosterone concentrations when not in a state of overreaching or the overtraining syndrome (Hough *et al.*, 2011) and it was hypothesized that this bout would be able to highlight alterations in the cortisol and testosterone responses following a period of intensified training. This intensified training intended to overreach the participants. The observations in this current study established that ~30 min, high-intensity cycle bouts (55/80 and 70) are sensitive enough to highlight reductions in the exercise-induced salivary cortisol, testosterone concentrations and cortisol/testosterone ratio responses following an 11-day endurance training period that occurred when compared to pre-training. The magnitude of the changes from pre- to post-training in the peak salivary hormonal responses to the 55/80 and 70 bouts were reductions in the order of 166% (cortisol) and 21% (testosterone) and 112% (cortisol/testosterone) (55/80) and 0% (cortisol) and 38% (testosterone) and an increase of 2% in cortisol/testosterone ratio. In addition the 11-day training period was sufficient to induce psychological fatigue in the participants as highlighted by the increases in the Recovery-Stress questionnaire stress scores over the course of the training period.

The blunting of the exercise-induced salivary cortisol responses post-training is in agreement with Urhausen *et al.* (1998). They reported blunted exercise-induced ACTH and a trend for lower exercise-induced cortisol responses in athletes suffering from the overtraining syndrome compared with normally trained athletes. This finding was suggested to be due to a suppression of the hypothalamus-pituitary axis causing a reduced ACTH response and consequently a reduction in the cortisol response to exercise. This suggestion seems plausible as Barron *et al.* (1985) reported decreased basal cortisol levels in marathon runners suffering from the overtraining syndrome. This decrease was linked to a dysfunction in the hypothalamus which was highlighted by a reduction in ACTH

secretion in response to an insulin-induced hypoglycaemia in the athletes diagnosed with the overtraining syndrome. Also as reported earlier in this current paper Meeusen *et al.* (2004) reported blunted plasma ACTH and cortisol responses to the second of a double cycle to fatigue protocol when comparing overreached athletes with those that are not in a state of overreaching or diagnosed with the overtraining syndrome. Unfortunately we are unable to confirm if any adaptations occurred in the exercise-induced ACTH over the course of this current study. So it can only be speculated that the blunted salivary cortisol response post-training may be due to a dysfunction of the hypothalamus leading to a reduction in ACTH and therefore causing a reduction in the cortisol response.

Alternatively Wittert *et al.* (1996) suggested that a desensitization of the adrenal gland could be the cause of no changes in resting plasma cortisol concentrations (03:00 – 09:00 serial sampling) that they observed in ultramarathon athletes compared to controls despite higher plasma ACTH concentrations in the athletes compared with controls. The desensitization of the adrenal gland could be a protective mechanism as constant high cortisol levels would be detrimental to the body as it would likely cause high levels of muscle protein degradation. It is unfortunate that this present study did not measure ACTH and cannot confirm if the 11-day training period had an effect on hypothalamic-pituitary function. However, based on the findings of the previous studies it seems likely that the blunted salivary cortisol response to exercise found in this present study is caused by either desensitization of the adrenal glands or by a dysfunction in the hypothalamus or pituitary gland.

The reduction in the salivary testosterone levels found in this study could be due to an alteration in the synthesis of testosterone and/or secretion in the testes. Hackney *et al.* (2003) reported reduced testosterone synthesis in the testes in endurance trained males compared with age-matched non-active controls. Testosterone production was measured by the infusion of gonadotropin-releasing hormone in a non-active group and trained runner group and found that the trained runner group had a lower testosterone response to the gonadotropin-releasing hormone than the non-active group. In the present study, the increase in endurance training over the 11-day period could have caused a reduction in testicular

production rate of testosterone. Furthermore Cumming *et al.* (1983) reported that a dysfunction in testosterone production in males could be linked to an increase in circulating cortisol levels. Acute hypercortisolism was induced in their participants by insulin or hydrocortisone administration and acute increases of cortisol occurred at the same time that a rapid decrease in circulation testosterone concentrations was seen. These authors suggested an inhibitory effect of cortisol on the luteinising hormone receptors on the Leydig cells leading to a reduction in testosterone production and therefore secretion by the testes. The 11-day training period would have exposed all participants to repeated acute cortisol increases. It is possible that the repeated elevations of cortisol levels experienced over the intensified training period had an inhibitory effect on the luteinising hormone receptor expression on the Leydig cells. This would lead to a reduction in the luteinising hormone induced testosterone production and secretion.

The physiological responses (HR and RPE) to the 55/80 and 70 bouts did not differ pre- to post-training. In addition there was no significant difference in the time to fatigue in the 70 bouts. Hormonal alterations have often been linked to overreaching and the overtraining syndrome (Barron *et al.*, 1985 and Urhausen *et al.*, 1995) which are linked to a deterioration of physical performance. Therefore, it was expected that with this alteration in cortisol and testosterone there would be a reduction in physical performance. One of the purposes of the 70 bout was to measure physical performance before and after the intensified training period. It needs to be recognized that the 70 bout did not give an ideal measure of performance as it was a cycle to fatigue or until 30 min whichever was reached first. This was designed like this as it was hypothesized that the cycle to fatigue time would be less than 30-min for most individuals looking at a previous cycle to fatigue protocol used in our lab of similar intensity (Hough *et al*., 2011). The cycle to fatigue needed to be long enough to induce a response in cortisol (~20 min) but not too long to have a large variation, comparing pre- with post-training, in the hormone responses to the cycle to fatigue due to the duration of cycle. Unfortunately, in this current study 10 out of 12 of the participants reached 30 min and therefore it is not a true reflection on performance. The purpose of the cycle to fatigue was twofold. Firstly as a performance measure but also to examine the hormonal response to a second high-intensity cycle bout.

The novel finding of this current study is the establishment that the 55/80 exercise protocol is sensitive enough to highlight adaptations in salivary cortisol and testosterone caused by an intensified endurance training period. Unlike Meeusen *et al.* (2004 & 2010) who reported hormonal reductions following an intensified training period to the second exercise bout only of their double exercise protocol, this current study reported hormonal alterations in response to both exercise bouts (55/80 & 70) post-training. Perhaps this contrast in results was due to the fact that the cycle to fatigue used by Meeusen *et al.* (2004) did not induce an increase in cortisol when the participants were not overreached or overtrained (i.e. in response to the 1st cycle to fatigue before their 10-day training camp) therefore making it difficult to highlight any alterations that occurred when overreached or overtrained. The 55/80 protocol has been shown to induce robust elevations in salivary cortisol and testosterone concentrations in a normal trained state (Hough *et al.*, 2011). This makes it easy to highlight the hormonal alterations that occurred after the period of intensified training. It should also be noted that no changes were found in the resting (i.e. pre-exercise) salivary cortisol and testosterone concentrations pre- and post-training. This suggests that the exercise-induced adaptations in the salivary hormones cortisol and testosterone reported in this current study occur prior to changes in basal measures of these salivary hormones. The fact that the resting cortisol values have not altered after the intensified training period does not agree with some of the studies mentioned previously in this discussion (Barron *et al.*, 1985) but does with others (Wittert *et al.*, 1996). These contrasting findings can be explained to be due to the different states of training the participants were in during these studies. Wittert *et al.* (1996) examined ultramarathon runners with no symptoms of suffering from overreaching or the overtraining syndrome but the participants in Barron *et al.* (1985) were diagnosed with the overtraining syndrome.

The blunting of the cortisol and testosterone responses to the 55/80 and 70 bouts following an intensified training period coupled with an increase in stress scores in a Recovery-Stress questionnaire suggests that to measure training stress with different methods (questionnaires, hormone response to a stress test) may be useful in order to reduce the incidence of unplanned overreaching or the

overtraining syndrome. This has been suggested previously by Nederhof *et al.* (2008) who in a small group (n = 3) of speed skaters examined their responses to different diagnostic tools for overreaching or the overtraining syndrome (Recovery-Stress questionnaire, profile of mood state; reaction time task; hormonal response to double cycle to fatigue protocol) while in different training states (1) not overreaching or overtraining, 2) diagnosed with non-functional overreaching and 3) recovering from non-functional overreaching). They reported a relationship between alterations in exercise-induced cortisol and ACTH concentrations and Recovery-Stress questionnaire scores. Rietjens *et al.* (2005) also examined if severe fatigue could be diagnosed by a combination of parameters (profile of mood state; resting hormone testing; cognitive reaction test). They suggested both the profile of mood state and reaction time performance were sensitive parameters for the detection of overreaching. These studies and this current study give strength to the suggestion that a multi mode approach to measuring of markers of overreaching and/or the overtraining syndrome may be useful.

Limitations

The performance measure used in this study (70) needs to be recognized as a limitation. A better performance test such as a time trial or a complete cycle to fatigue would have provided a better indication of the influence the training period had on performance levels in our participants. This study cannot claim to have measured this accurately. In addition the reproducibility of the cortisol and testosterone responses to the 55/80 bout needs to be measured. This will confirm that the hormonal alterations reported in this current study are due to the intensified training period and not just a normal variation in the hormonal response to the exercise. This warrants further investigation. It would also be of interest to examine the hormone response to the high-intensity exercise over a normal training period of similar duration to the intensified training period used in this current study. A $\dot{V}O_{2\,peak}$ test could also have been useful at the end of the intensified training period to examine if the fitness level of the participants had altered over this period. However, it must be noted that the RPE and HR responses to the exercise bouts did not alter pre- to post-training which would suggest that the fitness level of the participants had not altered.

In conclusion, the 11-day training period increased the participants' Fatigue and Burnout scores in Recovery-Stress questionnaires. Coupled with this, compared with pre-training, blunted exercise-induced salivary cortisol and testosterone responses to high-intensity, 30-min cycling bouts were found at the end of the 11-day training period. Importantly unlike similar studies completed by Meeusen *et al.* (2004 & 2010) post-training altered exercise-induced cortisol and testosterone responses were found to the first of two 30-min cycling bouts completed (55/80). A desensitization of the adrenal glands or a dysfunction in the hypothalamus or pituitary gland are the likely causes for the blunted exercise-induced salivary cortisol response following the 11-day training period. A reduction in testosterone synthesis and/or secretion in the testes is the possible cause for the salivary testosterone response to the high-intensity exercise that was observed post-training. The reduced testosterone production and secretion level might be due to an inhibitory effect of high levels of circulating cortisol on the luteinising hormone receptor expression on the Leydig cells in the testes. This study indicates that the 55/80 cycle bout can highlight the exercise-induced salivary cortisol and testosterone changes that occur due to an intensified training period. This test would be a useful assessment of an athlete's hormonal status as this status may change in response to increased training stress as found in this present study. Regular assessment of the salivary cortisol and testosterone responses to the 55/80 bout in unison with other training stress measures, for example Recovery-Stress questionnaires and performance measures, might help to reduce the occurrences of unplanned overreaching or the occurrence of the overtraining syndrome.

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Figure captions

Table 1 Participant physical and physiological characteristics (mean values with standard deviations in parentheses).

Table 2 Average HR and RPE responses to the pre- and post-training 55/80 and 70 bouts (mean values with standard deviations in parentheses).

Figure 1. Schema for the resting and exercise trials.
* Resting trial does not contain exercise bouts

Figure 2. Schema for each laboratory training session on days 1 to 5 and 8 to 11.

Figure 3. The Recovery-Stress questionnaire Fatigue and Burnout scores pre- and post-training.
Values are means.
*- Different than Pre-training ($P < 0.05$).

Figure 4. Salivary cortisol ($nmol.L^{-1}$) responses to the 55/80 and 70 cycle bouts in the REST (○) pre- (■) and post-(△) training exercise trials.
* - Main time effect vs. Pre 55/80 ($P < 0.01$) ** - Main time effect vs. Pre 70 ($P < 0.01$) †- Main effect of trial pre-training greater than post-training ($P < 0.01$).

Figure 5. Salivary testosterone ($pmol.L^{-1}$) responses to the 55/80 and 70 cycle bouts in the REST (○) pre- (■) and post-(△) training exercise trials.
* - Main time effect vs. Pre 55/80 ($P < 0.05$); ** -Main time effect vs. Pre 70 ($P < 0.05$); †- Main effect of trial pre-training greater than post-training ($P < 0.05$)

Figure 6. Salivary C/T ratio responses to the 55/80 and 70 cycle bouts in the REST (○) pre- (■) and post- (△) training exercise trials.
* - Main time effect vs. Pre 55/80 ($P < 0.01$); ** -Main time effect vs. Pre 70 ($P < 0.01$); †- Main effect of trial pre-training greater than post-training ($P < 0.05$)