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 (C) and testosterone (T) responses to two, different ~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 C and T concentrations assessed. Compared with pre-training blunted exercise-induced salivary C, T and C/T responses to both bouts post-training were observed ($p < 0.05$ for all). Comparing pre- with post-training the absolute exercise-induced salivary C,T and C/T decreased from 11.1 to 3.1 and 7.0 to 4.4 $nmol \cdot L^{-1}$ (C), from 407 to 258 and from 473 to 274 $pmol \cdot L^{-1}$ (T) and from 12 to 4 and 7 to 5 (C/T) 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 (29:17 (pre-training) 29:35 (post-training) min:s) 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 C and T 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 intensify their physical training (by elevating volume, duration and/or intensity of training) over a short term e.g. a training camp. This intensification of training can lead 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" (FOR) (Meeusen *et al.*, 2006 & 2012). If this intensified training continues the athlete can move into a state of "non-functional overreaching" (NFOR) that will lead to a reduction in physical performance that may not resume for several weeks or months. Despite the benefits of overreaching (OR) it is possible to develop the Overtraining Syndrome (OTS) 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). Therefore, identifying a reliable biological marker to monitor training stress would be beneficial to highlight the incidence of OR and aid in reducing the risk of developing OTS.

Resting circulating cortisol (C) and testosterone (T) concentrations have been examined in athletes as possible biological markers of OR and the OTS (for review see Urhausen, Gabriel & Kindermann, 1995). C and T 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 C and T concentrations and the C/T ratio are reliable markers of OR/the OTS.

Perhaps instead of examining the resting levels of these hormones during normal training, OR and OT 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 C,adrenocorticotrophic hormone (ACTH), prolactin and growth hormone (GH) to short duration, high-intensity exercise could distinguish between normally trained and OR athletes and athletes in a state of NFO and OTS. 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 C and ACTH concentrations to the second exercise bout of a double incremental cycle to fatigue protocol decreased by ~118% (C) and ~73% (ACTH) following a 10-day training period compared with before the training period. The training volume was increased by 58% over this 10-day training period and the athletes were classed as OR at the end of this training period 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 C and ACTH concentrations to short duration, high-intensity exercise are altered and more specifically blunted following a period of intensified training. Moreover it suggests that the double incremental cycle to fatigue protocol may be a useful tool to measure the endocrine adaptations that are reported to occur while OR. 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 NFO and OTS. Athletes in a state of the OTS showed little or no exercise-induced increase in both hormones in response to the second maximal exercise bout whereas NFO athletes showed large exercise-induced increases in both hormones (~300% (PRL) 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 OR and OT. In addition these alterations may be able to distinguish between states of NFO

and the OTS. These findings are positive conclusions in the examination of the endocrine alterations in OR and OT. 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 OR 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 C and T 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 to the 55/80 bout when not OR or OT should make it easier for any alterations in these hormones when OR to be highlighted. Therefore the aim of this present study was to examine the responses of salivary C and T 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 exercise (~75% peak oxygen uptake ($\dot{V}O_{2\,peak}$)). This duration of the intensified training period should be sufficient to induce an OR/OT 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) will also be completed 2 h after completion of the 55/80 bout. 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 OR in the participants in unison with a deterioration of performance levels in the 70 exercise bout. In addition the C and T 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 OR and or OTS 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 main trials. The work rate equivalent to 75% $\dot{V}O_{2peak}$ was interpolated from the relationship between $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$) 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% $\dot{V}O_{2peak}$ (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 main 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. 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 (REST-Q) was completed at the beginning of each main trial. The REST-Q 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 REST-Q 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 trial the final training day was completed at least 24 h before the start of the post-training trial. Each training day consisted of 1.5 h cycling at 75% $\dot{V}O_{2\,peak}$. 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% $\dot{V}O_{2\,peak}$ 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 (TRIMP) scores to record the intensity of training completed by the participants outside the lab. TRIMP 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. TRIMP scores were calculated as detailed in Jobson *et al.* (2009). The equation used was TRIMP = 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 TRIMP score for the exercise that was completed outside the lab for all participants was 101. As a reference the average TRIMP 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.

REST questionnaire

Analysis of the REST-Q 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. Time to fatigue on the 70 bout were not different before and after training ($P > 0.05$) (Table 2). The average times to fatigue for the 70 bouts 29:17 ± 01:47 (pre-training) and 29:35 ± 01:00 (post-training) min:s.

Hormonal measurements

The average ± *s* salivary C and T concentrations during the REST trail 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 C and T 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 (C/T), 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 C and T 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 C and T concentrations when not in a state of OR or OTS (Hough *et al.*, 2011) and it was hypothesized that this bout would be able to highlight alterations in the C and T responses following a period of intensified training. This intensified training intended to OR 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 C, T concentrations and C/T 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% (C) and 21% (T) and 112% (C/T) (55/80) and 0% (C) and 38% (T) and an increase of 2% in C/T ratio. In addition the 11-day training period was sufficient to induce psychological fatigue in the participants as highlighted by the increases in the REST-Q stress scores over the course of the training period.

The blunting of the exercise-induced salivary C responses post-training is in agreement with Urhausen *et al.* (1998). They reported blunted exercise-induced ACTH and a trend for lower exercise-induced C responses in athletes suffering from OTS 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 C response to exercise. This suggestion seems plausible as Barron *et al.* (1985) reported decreased basal C levels in marathon runners suffering from OTS. 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 OTS. Also as reported earlier in this current paper Meeusen *et al.* (2004) reported blunted plasma ACTH and C responses to the second of a double cycle to fatigue protocol when comparing OR athletes with those that are not in a

state of OR or diagnosed with OTS. 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 C 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 C response.

Alternatively Wittert *et al.* (1996) suggested that a desensitization of the adrenal gland could be the cause of no changes in resting plasma C 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 C 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 C 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 T levels found in this study could be due to an alteration in the synthesis of T and/or secretion in the testes. Hackney *et al.* (2003) reported reduced T synthesis in the testes in endurance trained males compared with age-matched non-active controls. T production was measured by the infusion of gonadotropin-releasing hormone (GnRH) in a non-active group and trained runner group and found that the trained runner group had a lower T response to the GnRH 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 T. Furthermore Cumming *et al.* (1983) reported that a dysfunction in T production in males could be linked to an increase in circulating C levels. Acute hypercortisolism was induced in their participants by insulin or hydrocortisone administration and acute increases of C occurred at the same time that a rapid decrease in circulation T concentrations was seen. These authors suggested an inhibitory effect of C on the LH receptors on the Leydig cells leading to a reduction in T production and therefore secretion by the testes. The

11-day training period would have exposed all participants to repeated acute C increases. It is possible that the repeated elevations of C levels experienced over the intensified training period had an inhibitory effect on the LH receptor expression on the Leydig cells. This would lead to a reduction in the LH induced T 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 OR and the OTS (Barron *et al.,* 1985 and Urhausen *et al.,* 1995) and OR and the OTS are linked to a deterioration of physical performance. Therefore, it was expected that with this alteration in C and T 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 C and T caused by an intensified endurance training period. What is also novel is that unlike Meeusen *et al.* (2004 & 2010) this current study reported alterations in the C and T responses to both exercise bouts (55/80 & 70) post-training although the greater percentage reductions in hormones were in response to the 55/80 bout. Meeusen *et al* (2004 & 2010) reported reductions in the hormone response following an

intensified training period only to the second exercise bout of a double cycle to fatigue protocol. 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 C when the participants were not OR or OT (i.e. in response to the 1st cycle to fatigue before their 10-day training camp). As there was no elevation of C in response to this exercise when normally trained it means that any alteration in the exercise-induced hormone responses may be difficult to highlight. As the 55/80 protocol has been shown to induce robust elevations in salivary C and T concentrations when in a normal trained state as reported by Hough *et al.* (2011) this may have made it easier to highlight hormonal alterations that occurred after a period of intensified training. It should also be noted that no changes were found in the resting (i.e. pre-exercise) salivary C and T concentrations pre- and post-training. This suggests that it is possible that the exercise-induced adaptations in the salivary hormones C and T reported in this current study occur prior to changes in basal measures of these salivary hormones. The fact that the resting C 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. The participants in Wittert *et al.* (1996) were ultramarathon runners and had no symptoms of suffering from OR or OTS but the participants in Barron *et al.* (1985) were suffering from OTS which had been diagnosed by physicians.

The blunting of the C and T responses to the 55/80 and 70 bouts following an intensified training period coupled with an increase in stress scores in a stress/recovery 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 OR or OTS. 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 OR or OTS (RESTQ, Profile of mood state (POMS); reaction time task; hormonal response to double cycle to fatigue protocol). One of the skaters was neither OR or OT, one was diagnosed with NFO and the other recovering from NFO. They reported large exercise induced increases in C and ACTH concentrations in

response to the 2nd cycle to fatigue exercise bout when NFO compared to when they were recovering from NFO. In addition to the hormonal differences when in different stages of OR they reported higher stress scores on the RESTQ compared with when recovering form NFO. Rietjens *et al* (2005) also examined if severe fatigue could be diagnosed by a combination of parameters (POMS; resting hormone testing; cognitive reaction test). They suggested both the POMS and reaction time performance were sensitive parameters for the detection of OR.

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 C and T 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 confirm 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 REST-Q questionnaires. Coupled with this, compared with pre-training, blunted exercise-induced salivary C and T responses to high-intensity, 30-min cycling bouts were found at the end of the 11-day training period. Importantly unlike a similar study completed by Meeusen *et al.* (2004 & 2010) post-training altered exercise-induced C and T 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 C response following the 11-day training period. A reduction in T synthesis and/or secretion in the testes is the possible cause for the salivary T synthesis in response to the high-intensity exercise that was observed. The reduced T production and secretion level might be due to a inhibitory effect of high levels of circulating C on the LH receptor expression on the Leydig cells in the testes. This study indicates that the 55/80 cycle bout can highlight the exercise-induced salivary C and T 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 C and T responses to the 55/80 bout in unison with other training stress measures, for example stress-recovery questionnaires and performance measures, might help to reduce the occurrences of unplanned OR or the occurrence of OTS.

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Figure captions

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

Figure 1. Schema for the resting and.
*Resting trial contains no exercise.

Figure 2. Schema for the training days.

Figure 3. Salivary cortisol (nmol.L^{-1}) response to the 55/80 and 70 cycle bouts in the resting (○) pre- (■) and post-(△) training.
* - Different than Pre 55/80 ** - Different than Pre 70. †- Different than Pre-training

Figure 4. Salivary testosterone (pmol.L^{-1}) response to the 55/80 and 70 cycle bouts in the resting (○)pre- (■) and post- (△) training.
* - Different from Pre 55/80; ** -Different from Pre 70; †- Different than Pre-training

Figure 5. Salivary C/T ratio response to the 55/80 and 70 cycle bouts in the resting (○)pre- (■) and post- (△) training.
* - Different from Pre 55/80; †- Different than Pre-training