Title: Reproducibility of Acute Steroid Hormone Responses in Men to Short-Duration Running

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Abstract

Purpose: Progressively overloading the body to improve physical performance may lead to detrimental states of overreaching/overtraining syndrome (OTS). Exercise-induced cortisol and testosterone have been suggested as overreaching markers with blunted cycle-induced concentrations found following an intensified-training period. To be inclusive for a running population, this study develops two 30-min running bouts: the 50/70 (based on individualized velocity at maximal oxygen uptake) and the RPE_{TP} (self-paced bout) and examines the reproducibility of plasma cortisol and testosterone responses to these bouts. **Methods:** Thirteen recreationally active, healthy males completed each running bout on three occasions, respecting time of day and blood was drawn Pre-, Post- and 30 min Post-Exercise. **Results:** Cortisol did not change in response to 50/70 or RPE_{TP} ($p > 0.05$, $\eta^2 = 0.090$ and $\eta^2 = 0.247$, respectively). Elevated (both $p < 0.01$) testosterone (50/70: 35%, $\eta^2 = 0.790$; RPE_{TP}: 42%, $\eta^2 = 0.876$) was observed, with good intra-individual coefficients of variation (CV_i) as mean ± standard deviation for cortisol (50/70: 13 ± 10%; RPE_{TP}: 12 ± 7%) and testosterone (50/70: 7 ± 5%; RPE_{TP}: 12 ± 9%). Heart rate and rating of perceived exertion were unchanged across trials (all $CV_i < 5\%$, $p < 0.05$). **Conclusions:** Both tests elicited reproducible physiological and hormonal responses. Advantageously for the practitioner, RPE_{TP} does not require *a priori* determination of exercise intensities, unlike the 50/70, enhancing its potential integration into practice. Additionally, RPE_{TP} induces greater disturbances to OTS-implicated hormones compared to 50/70 and may therefore provide a more sensitive tool to highlight NFOR/OTS.

Keywords: Performance, running test, stress, overreaching, prevention.

Introduction

Successful athletic training requires balanced overload and recovery, without which short-term performance decrements can occur (e.g. overreaching) in as little as 7 days.[1] Importantly, whilst overreached athletes can experience performance decrements in the short-term, sufficient recovery (days to weeks) facilitates a "supercompensatory" performance enhancing effect [e.g. functional overreaching (FOR)[2]][3]. Without sufficient recovery from periods of overload, "non-functional overreaching" (NFOR) can occur (requiring weeks/months to recover from fully) with NFOR complicit in the more protracted overtraining syndrome (OTS; requiring several months or even years to recover from fully).[2]

Resting concentrations of cortisol and testosterone were suggested as markers of overreaching/NFOR/OTS yet their efficacy in these regards is inconclusive with increases, decreases and no changes in concentrations under examination before to after intensified-training periods.[4–6] Exercise-induced responses appear to have greater utility, with blunted ACTH and cortisol responses to 2 consecutive continual incremental cycles to fatigue identified following a 10-day intensified-training period, compared with pre-training.[7] Following on from these findings, robust elevations of salivary cortisol (~120%) and testosterone (~33%) to a continuous, 30-min cycle bout, consisting of alternating blocks of 1 min at 55% maximal workload ($\dot{W}_{max}$) and 4 min at 80% $\dot{W}_{max}$ (i.e. the 55/80) were reported,[8] with blunted exercise-induced salivary cortisol and testosterone in response to the 55/80 shown following an 11-day[9] and salivary testosterone after a 10-day[10] intensified-training period.

However, despite some utility for the 55/80 to highlight exercise-induced overreaching-related hormonal imbalances in cyclists, its application within other athletes (e.g. runners) is evidently lacking. Given a 30-min running bout at 80% of maximal oxygen uptake ($\dot{V}O_{2max}$) has been reported to elevate plasma cortisol by ~20%,[11] and a running test to exhaustion at 100% ventilatory threshold increased plasma cortisol (~97%) and total testosterone (31%),[12] it was hypothesized that a short duration running protocol variant of the cycling 55/80 may be viable. This running variant, theoretically, could induce an acute elevation in plasma cortisol and testosterone when in a healthy state and also detect alterations in the exercise-induced responses of these hormones as a consequence of intensified-training period. To be of value in practice, this variant protocol must demonstrate reproducible hormone and physiological responses when participants are in a rested healthy state.

The aim of this study is to therefore examine whether the acute plasma cortisol and testosterone responses to two novel, continuous, 30-min treadmill-run protocols are reproducible, within rested yet active healthy participants, aiming to design a short-duration running bout that could be practically used to prevent the incidence of NFOR/OTS.

Methods

Subjects

In a randomized crossover design, 13 recreationally active males[13] volunteered to participate (Table 1). This study was granted ethical approval by the University of Bedfordshire Research Ethics Committee (2014ISPAR003) in accordance with the 2013 Declaration of Helsinki. After comprehensive verbal and written descriptions of the study, written informed consent was provided by participants.

(* Insert Table 1 near here ***)**

Design

On the first visit to the laboratories a submaximal and a $\dot{V}O_{2max}$ tests were completed on a motorised treadmill (PPS55 Med-i, Woodway, Weil am Rhein, Germany). On the following visits, 7 separate trials were completed – 6 main experimental trials and one control, resting trial (CTL). All trials were completed at 12:00 to avoid the influence of diurnal variation of the hormones being examined (Figure 1). To avoid baseline peak circulating cortisol levels due to circadian rhythm, all participants were asked to wake up no later than 08:00 on the morning of the trial. A standard breakfast chosen by the participant was consumed before 09:00 and was replicated before each main trial. Participants were requested to drink ~500 mL of water in the morning of the trial and euhydration was confirmed by a urine osmolality of ≤ 700 $mOsm \cdot kg \cdot H_20^{-1}$.[14] All participants reported to the laboratory at ~11:30 and completed a 76-statement recovery-stress questionnaire (RESTQ-76). The RESTQ-76 discriminates 48 nonspecific and 28 sport-specific areas of stress and recovery, consisting of 19 main scales in total.[15] Each of these subscales includes specific statements. The sum of scores (answer to each statement) in each of the subscales is used to examine the overall responses to the questionnaire. Each answer ranges from never (0) to always (6) and covers the participants' past 3 days. Participants did not consume any food until the end of each main experimental trial but were allowed to drink water *ad libitum* throughout the exercise bouts. Body mass was measured pre- and post-exercise and heart rate (HR) and rating of perceived

exertion (RPE) were measured in the last 15 s of each stage during the exercise bouts via short-range radio telemetry (Polar FT1, Polar Electro Oy, Kempele, Finland) and the 6-20 Borg scale, respectively.

A similar diet was consumed during the 24 hours preceding each trial and measured via a weighed food diary. A nutrition analysis software (Dietplan, Version 6.70.74, Forestfield, West Sussex, UK) was used to determine mean energy (9439 ± 3954 kJ), carbohydrate (58% ± 12%), fat (27 % ± 13%), and protein (14% ± 2%) intake.

Methodology

A 3-min warm-up run at 7 $km \cdot h^{-1}$ and 1% gradient was undertaken prior to the submaximal test. A 4-stage, 16-min, incremental treadmill-run test was then completed in order to determine the running speed/oxygen consumption ($\dot{V}O_2$) relationship.[16] The initial speed was self-selected between 6.5 – 12.0 $km \cdot h^{-1}$. Speed was then increased by 1 $km \cdot h^{-1}$ every stage. A 20-min resting recovery was then undertaken. $\dot{V}O_{2max}$ was assessed using an incremental incline-ramped test.[16] The gradient was increased by 1% every minute until volitional exhaustion. The initial speed was set at the speed corresponding to a HR of ~150 $beats \cdot min^{-1}$ (range: 9.5 – 13.0 $km \cdot h^{-1}$) on the submaximal test and remained constant throughout. Expired gas was analysed by using a breath-by-breath ergospirometry system (MetaLyzer 3B, Cortex, Leipzig, Germany). The $v\dot{V}O_{2max}$ was determined by regressing $\dot{V}O_2$ exercise intensity for submaximal exercise and extrapolating this relationship to $\dot{V}O_{2max}$.[17]

(* Insert Figure 1 near here ***)**

In the 6 main exercise trials the participants completed each of the 2 designed running bouts on 3 separate occasions - 1 familiarisation (FAM) and 2 main trials (T1 and T2), to avoid any learning effects. All trials were randomly assigned. Participants abstained from exercise, caffeine and alcohol intake 24 hours before each main trial. Blood samples were drawn Pre-, Post-, and 30 min Post-Exercise in T1 and T2. The tests were both 30-min, continuous treadmill-running and were designed as follows: (a) alternating blocks of 1 min at 50% $v\dot{V}O_{2max}$ and 4 min at 70% $v\dot{V}O_{2max}$ (50/70); (b) alternating 1 min at an RPE of 11 (fairly light) and 4 min at 15 (hard) on the 6-20 Borg scale (RPE_{TP}), where the treadmill speed could be adjusted but not seen by the participant to maintain the RPE in the target range; (c) a 30-min no exercise, control trial (CTL) (Figure 1). In all exercise trials, the treadmill slope was set at 1% gradient.

Analytical Procedures: Whole blood samples were collected by venepuncture from an antecubital vein into 5 mL tri-potassium ethylenediaminetetraacetic acid (K_3EDTA) vacutainers (Vacuette, Greiner Bio-One, Stonehouse, UK). Blood was centrifuged at 1500 *g* for 10 min at 4°C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK) and plasma was transferred into 1.5 mL aliquots (Eppendorf, Hamburg, Germany) to be stored at -80°C. Plasma cortisol and testosterone concentrations were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (IBL International, Hamburg, Germany). All samples were analysed in duplicate and average concentrations were used. The sensitivity of the plasma cortisol and testosterone kits is 6.8 nmol.L^{-1} and 0.29 nmol.L^{-1}, respectively and the mean intra-assay CV were 3.0% (cortisol) and 4.6% (testosterone), according to the manufacturers specifications. The mean inter-assay CV were 3.5% and 5.7% for cortisol and testosterone, respectively.

Statistical Analysis

Statistical analyses were accomplished by using the IBM Statistical Package for Social Sciences® (SPSS) Statistics version 23.0 (SPSS Inc., Chicago, IL). Raw data were checked for normality and homoscedasticity, using the Shapiro-Wilk test and scatter plots, respectively. Non-normally distributed data sets were log transformed (to base 10) and rechecked for normality. Normally distributed data sets (plasma cortisol and testosterone) were analysed using a two-way repeated measures analysis of variance (ANOVA). On finding an effect, paired sample t-tests were used with Bonferroni adjustments. Partial eta squared (η^2) values were used to examine the size of the effect when examining the exercise-induced response of plasma cortisol and testosterone. A one-way repeated measures ANOVA with paired-sample t-test with Bonferroni corrections was used to examine HR and speed in CTL and exercise trials, and hormonal responses during CTL. Reproducibility analysis was accomplished by determining the CV_i of all physiological and hormonal measurements. The CV_i were presented as a percentage and were calculated by hand using the equation $CV_i = (SD_t/\bar{X}_t)*100$, where SD_t is the standard deviation of the hormone responses to the main experimental trials averaged, and $\bar{X}_t$ is the average of the hormone concentrations at Pre-, Post- and 30 min Post-Exercise averaged[18]. The ICC used was a two-way model, based on the examination of single measures, i.e. ICC (2,1). Cohen's *d* effect sizes (ES) were used to examine the magnitude of hormonal change between trials,[19] were calculated by hand as detailed in Vincent and Weir,[20] and were categorized using standardized thresholds of < 0.2 trivial, 0.21 – 0.60 small, 0.61 – 1.20 moderate, 1.21 – 2.0 large, and > 2.0 very large.[19] The alpha level of significance was set as $p <$

0.05. Data is reported as mean ± SD. All results were presented as raw data to facilitate its comprehension.

Results

Hydration status: Urine osmolality did not differ across all trials and was 348 ± 204 mOsmol·kg^{-1} H_2O in T1, 351 ± 200 mOsmol·kg^{-1} in T2 (50/70), 345 ± 198 mOsmol·kg^{-1} H_2O in T1, 310 ± 168 mOsmol·kg^{-1} in T2 (RPE_{TP}) and 301 ± 166 mOsmol·kg^{-1} H_2O in CTL ($p > 0.05$).

Recovery-Stress Questionnaires: No changes in the RESTQ-76 Sport scores were found in any of the stress or recovery scales across all trials ($p > 0.05$).

Physiological Responses to Exercise: No differences in HR or speed were found when comparing FAM, T1 and T2 in any of the exercise bouts ($p < 0.05$). When comparing both exercise bouts, a significant trial effect for speed, HR and RPE was found ($p < 0.01$). Average speed and HR were 21% and 9% higher in the RPE_{TP} compared with the 50/70, respectively. The RPE scores in the RPE_{TP} were ~17% higher than in the 50/70. Reproducibility data for speed, HR and RPE and average HR and speed in response to the 50/70 and RPE_{TP} are presented in Table 2.

(* Insert Figure 2 near here ***)**

Hormonal Responses During CTL: Plasma cortisol decreased from Pre- to Post-CTL ($p < 0.01$) by ~18% ± 16%. Plasma testosterone did not alter over time ($p > 0.05$ for all).

Hormonal Responses to Exercise: No trial effect was observed in the 50/70 ($p = 0.65$) or the RPE_{TP} ($p = 0.72$) when examining plasma cortisol responses. A time effect was observed in the 50/70, with cortisol decreasing from Post-Exercise to 30-min Post-Exercise ($p < 0.01$, $\eta^2 = 0.090$). No time effect was found in the RPE_{TP} ($p = 0.07$, $\eta^2 = 0.247$). Cortisol levels changed from Pre- to Peak Post-Exercise by -3% and +29% (50/70), and by +34% and +47% (RPE_{TP}) in T1 and T2, respectively. Individual exercise-induced changes are presented in Figure 2. Pre-Exercise cortisol samples did not differ ($p = 0.89$) across trials. No trial effect was observed when comparing the 50/70 with the RPE_{TP} ($p = 0.35$). For plasma testosterone, no trial effect was found when comparing T1 and T2 in the 50/70 ($p = 0.51$) and the RPE_{TP} ($p = 0.49$). However, a significant time effect was shown in 50/70 ($p < 0.001$) and the RPE_{TP} ($p < 0.001$). Pairwise comparisons showed testosterone acutely elevated in all exercise trials and remained elevated at 30 min Post-Exercise in the RPE_{TP} (both $p < 0.01$, $\eta^2 = 0.790$ and $\eta^2 =$

0.876 in the 50/70 and RPE_{TP}, respectively). Testosterone levels changed from Pre- to Post-Exercise by +30% and +39% (50/70), and by +46% and +38% (RPE_{TP}) in T1 and T2, respectively. Individual exercise-induced changes are presented in Figure 2. Pre-Exercise testosterone samples did not differ (p = 0.66) across trials. No trial effect was observed when comparing the 50/70 with the RPE_{TP} (p = 0.11). All reproducibility data and average plasma cortisol and testosterone concentrations for T1 and T2 are presented in Table 2.

(* Insert Table 2 near here ***)**

Discussion

This study aimed to examine the responses of plasma cortisol and testosterone responses to 2 different continuous, 30-min, high-intensity running bouts and the reproducibility of these responses. It was hypothesized that the hormonal concentrations would acutely elevate in response to all bouts and that these responses would be reproducible. The intra-individual variability in plasma cortisol and testosterone observed in this present study are within the normal variability associated with these hormones, and therefore support the reproducibility of the hormonal responses to the 50/70 and the RPE_{TP}. In fact, the RPE_{TP} (a potentially more practically applied field test due to its self-paced design) has shown to elicit greater physiological responses than the 50/70 bout, as well as reproducible plasma cortisol and testosterone responses. However, only plasma testosterone markedly elevated in response to this running tool, suggesting testosterone may be a better indicator of an exercise-related stress reaction.

Cortisol is known to be a stress-related hormone that rises during and after psychological stress.[21] Analysis of the scores to the RESTQ-76 showed no disparities in any of the scales, detailing the participants were in a similar state of predisposition to undertake physical activity on every trial and therefore the hormonal responses reported have not been influenced by a change in wellbeing.

The reproducibility of the physiological responses to both tests was examined. Being a self-paced tool, the RPE_{TP} could provoke different HR responses if the speeds chosen by the participants were different when completing the bouts on different occasions. In this study, HR and speed did not alter across all exercise trials. These results are important, as an alteration in the speeds would be indicative of a subsequent alteration in exercise intensity, and therefore influence the response of both cortisol and testosterone. Additionally, the HR

and speed responses were shown to be reproducible to both tests with CV_i of 2.9 ± 2.1% for HR (50/70), and 1.8 ± 1.3% and 2.2 ± 1.8% for HR and speed (RPE_{TP}). These data suggest that both bouts induced a similar physiological strain, hence the similar HR, RPE and running speeds.

Similar studies to this one have reported a significant elevation of salivary cortisol and testosterone in response to a continuous 30-min, cycle bout when in a healthy state.[8–10] Duration and intensity of exercise sessions are two important factors known to cause an exercise-induced increase in plasma and salivary cortisol concentrations,[22] with exercise intensity above 60% $\dot{V}O_{2max}$ for at least 20-30 min being required for cortisol to elevate.[23] In this current study, plasma cortisol did not significantly increase to either the 50/70 or the RPE_{TP}. There was, however, a percentage-elevation from Pre- to Post-Exercise in both trials in the RPE_{TP} (34% and 47%) and in T2 in the 50/70 (29%). Individual cortisol levels show contrasting responses, ranging from moderate decreases to robust increases. As the RPE_{TP} is a self-paced bout, each participant exercised at an intensity dependant of an individual perceived exertion. Although the RPE_{TP} bout was designed to elicit an RPE of 15 (hard) for the majority of the test (24 min), it was not confirmed whether this would provoke an exercise intensity stressful enough to acutely elevate cortisol levels. However, a consistent exercise-induced elevation in plasma testosterone was seen in all exercise trials. Furthermore, testosterone levels did not change with time during CTL, whereas cortisol significantly decreased from Pre- to Post-CTL. It may be reasonable to suggest that the circadian rhythm of cortisol is likely to have led to 50/70 and RPE_{TP} being unable to induce the hypothesised acute elevation, which was not assumed due to Hough *et al.*[8] reporting no alteration in resting plasma cortisol between 12:00-13:00. Cortisol is known to have a high intra-individual variability.[24] When examining the intra-individual variation across trials this study shows an intra-individual variation of ~13% and ~12% in plasma cortisol in the 50/70 and RPE_{TP}, respectively. At first examination, these data may seem a little high, however, the within-subject variability in cortisol has been reported to be ~21.7%.[25] The CV_i for testosterone is also within the 12.6%[25] and the 11.8%[26] intra-individual variability, suggesting the variability found falls within normal biological variability values reported previously. Any shift from the reported variation may be due to the fact these studies have examined the variability of resting levels, while the present study has looked at the exercise-induced responses. ES were used to examine the magnitude of change between trials, with Cohen[27] proposing that small differences would be described if presenting an ES value of 0.21. The ES for cortisol and testosterone were 0.07 and 0.04

(50/70) and 0.03 and 0.04 (RPE_{TP}), respectively. These data support the trivial changes in the hormones examined in this study when compared across trials.

Practical applications

- Testosterone may be a better indicator of a hypothalamic-pituitary activation following short-duration, high-intensity exercise when compared to cortisol.
- Both tests elicited reproducible plasma cortisol responses but did not acutely elevate its concentration. This means it may be inappropriate to measure cortisol as a biomarker to highlight exercise-induced stress.
- Testosterone elevated in both tests and these responses were reproducible. The intra-individual variability of testosterone responses is at a level that suggests that both tests could highlight blunted acute responses following an intensified-training period, emphasising its usefulness to prevent and avoid the incidence of NFOR/OTS.
- The RPE_{TP} is a self-paced running bout, hence it does not require preliminary testing for determination of exercise intensities. Therefore, it may be more practically applied in an athletic/elite population and its short duration may be advantageous if incorporating it within a training session.

Conclusions

Hypothetically cortisol and testosterone would acutely elevate in response to both tests and these would provoke reproducible hormonal and physiological responses. We propose that cortisol is very individualised, and the exercise-induced responses may be influenced by a circadian rhythm. Additionally, using the RPE_{TP} may be more practically applied in the field as it will not require preliminary testing to determine exercise intensities.

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