Title (max 85 characters): Exercise-induced salivary hormone responses to high-intensity, self-paced running

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Abstract

Purpose: Physical overexertion can lead to detrimental overreaching states without sufficient recovery, which may be identifiable by blunted exercise-induced cortisol and testosterone responses. A running test (RPE_{TP}) elicits reproducible plasma cortisol and testosterone elevations (in a healthy state) and may detect blunted hormonal responses when overreached. This current study determines the salivary cortisol and testosterone responses reproducibility to the RPE_{TP}, to provide greater practical validity using saliva compared to the previously utilized blood sampling. Secondarily, the relationship between the salivary and plasma responses will be assessed. **Methods:** Twenty-three active, healthy males completed the RPE_{TP} on three occasions. Saliva (N=23) and plasma (N=13) were collected Pre-, Post- and 30 min Post-Exercise. **Results:** Salivary cortisol did not elevate in any RPE_{TP}-trial, and reduced concentrations occurred 30 min Post-Exercise (P = 0.029, η^2 = 0.287); trial differences were observed (P < 0.001, η^2 = 0.463). The RPE_{TP} elevated (P < 0.001, η^2 = 0.593) salivary testosterone with no effect of trial (P = 0.789, η^2 = 0.022). Intra-individual variability was 25% in cortisol and 17% in testosterone. 'Fair' ICCs of 0.46 (cortisol) and 0.40 (testosterone) were found. Salivary and plasma cortisol positively correlated (R = 0.581, P = 0.037) yet did not for testosterone (R = 0.345, P = 0.248). **Conclusions:** The reproducibility of salivary testosterone response to the RPE_{TP} is evident and supports its use as a potential tool, subject to further confirmatory work, to detect hormonal dysfunction during overreaching. Salivary cortisol responds inconsistently in a somewhat individualized manner to the RPE_{TP}.

Keywords: testosterone, cortisol, preventive measures, stress, overreaching.

Introduction

Effective physical performance adaptations require an appropriately prescribed and periodized training program.[1] When overreaching occurs, a reduced athletic capacity (transiently or otherwise) may be observed, due to imbalanced overload and recovery periodisation.[2] Appropriate recovery may elicit a "supercompensatory" performance response referred to as functional overreaching (FOR).[3] Yet, insufficient recovery from prolonged periods of intensified-training may lead to "non-functional overreaching" (NFOR) requiring weeks/months for full recovery – whilst – unchecked NFOR can progress to overtraining syndrome (OTS) which can, on occasions, demand years for full recovery to occur.[4] Prevalence of NFOR/OTS during an elite athlete's career can range from ~35%[5] to 67%[6] yet little progress has been made regarding objective biomarkers that detect the onset/magnitude of overreaching.[3]

Resting cortisol and testosterone concentrations have been proposed as overreaching/OTS markers, as they provide a ratio of catabolic to anabolic activity.[3] However, their alterations at rest are inconsistent when comparing pre to post periods of overload.[7,8] Recently, their acute responses to exercise have shown promise as an indicator of hormonal dysfunction following intensified-training periods.[9,10] Blunted exercise-induced salivary cortisol and testosterone responses were shown following a 30-min cycling bout, known as the 55/80 [1 min at 55% maximal workload ($\dot{W}_{max}$) and 4 min at 80% $\dot{W}_{max}$] following an 11-day[9] and a 10-day[10] intensified-training period, suggesting these exercise-induced salivary hormones are potentially useful biomarkers of overreaching/OTS. Recently, a treadmill-derivative [rating of perceived exertion protocol (RPE_{TP})][11] of the 55/80 cycle[12] was developed and shown to induce reproducible elevations of plasma testosterone but not cortisol.[11]

Therefore, this study primarily sought to, in attempt to increase practical validity, determine whether the same RPE_{TP} cortisol and testosterone responses in plasma[11] could be replicated in salvia, in healthy (i.e. non-overreached) adult, male individuals. If salvia was to show such validity, the RPE_{TP} could become a more practical tool to detect and subsequently inform practitioner decision-making, regarding any potential hormonal dysregulation associated with overreaching/OTS. Secondarily, this study also intended to assess the relationship between saliva and blood cortisol and testosterone responses to the RPE_{TP}, albeit in a subsection of previously measured participants. It was hypothesised that (i) salivary testosterone but not cortisol would acutely elevate in response to the RPE_{TP}; (ii) these responses would be reproducible; and (iii) the salivary hormone responses would correlate with their venous surrogates.

Methods

Participants

This study was completed to expand upon previously published data.[11] Twenty-three 'recreationally-trained' and 'trained' (categorised as per[13] and reflective of performance levels 2 and 3) males [age 21 ± 2 years; height 177 ± 6 cm; body mass 76.1 ± 13.1 kg; maximal heart rate (HR_{max}) 191 ± 9 beats·min^{-1}; maximum oxygen uptake ($\dot{V}O_{2max}$) 55 ± 6 mL·kg^{-1}·min^{-1}] volunteered to participate in this study. Partial data from thirteen participants from the previously published study[11] (physiological, plasma cortisol, plasma testosterone and anthropometric data) are included in the present study. The study was conducted in accordance with the 2013 Declaration of Helsinki under ethical approval [University of Bedfordshire Research Ethics Committee (2014ISPAR003)]. Following verbal and written study descriptions participants provided written informed consent.

Design

The original research[11] examined the reproducibility of plasma cortisol and testosterone responses to two novel running protocols. The present study extends this work[11] by examining salivary cortisol and testosterone responses to one of these running protocols (RPE_{TP}), given saliva is a more ecologically valid sample compared to plasma.

Participants undertook 5 main trials within a temperature-controlled laboratory (see Figure 1). On the first visit, a submaximal running test followed by a $\dot{V}O_{2max}$ test was undertaken to physiologically characterise participants. During the subsequent 4 visits (separated by a minimum of 4 to 7 days), 3 exercise trials (T1, T2 and T3), and one resting control trial (CTL) were completed. In each exercise trial, participants undertook the RPE_{TP} (Figure 1), which has been detailed previously.[11] CTL identified the influence of the circadian rhythm on the hormones measured.[14,15] All participants woke before 8 AM on the morning of the trial which started at 12 PM for diurnal variation control purposes.[16] A 76-statement recovery-stress questionnaire (RESTQ-76[17]) was completed before the start of each exercise bout, as used previously[11]. No differences in RESTQ-76[17] metrics were observed prior to trial completion, indicating the participants' were in a similar state of well-being and pre-disposition to exercise prior to all trials, likely not overreached and that any alterations in the hormones examined were not due to pre-trial stress and/or well-being (or variation in said measures pre-trial).

Abstinence from exercise, caffeine and alcohol intake 24 hours before each main trial was requested, and a standard breakfast chosen by the participant was consumed by 9 AM (repeated prior to each visit). The participants' pre-trial 24 h nutritional intake was determined via a weighed food diary. Nutrition analysis software (Dietplan, Version 6.70.74, Forestfield, West Sussex, UK) was used on the food diaries and mean energy (9851 ± 4182 kJ), carbohydrate (56% ± 12%), fat (25 % ± 13%), and protein (17% ± 2%) intake were determined. Euhydration was confirmed by a urine osmolality of ≤ 700 $mOsm \cdot kg \cdot H_2O^{-1}$.[18] Food consumption was not allowed until the end of each main experimental trial but water was provided *ad libitum* up to 10 min before saliva sample collection.

***** Insert Figure 1 near here *****

Methodology

Submaximal running and $\dot{V}O_{2max}$ tests. The protocols used for determination of $\dot{V}O_{2max}$ have been detailed and justified in previous research.[11,19] Briefly, a 4x4-min-stage, incremental treadmill-run submaximal test was completed, to determine the running speed/oxygen consumption ($\dot{V}O_2$) relationship.[20] The initial speed was self-selected between 6.5 – 12.0 $km \cdot h^{-1}$ and increased by 1 $km \cdot h^{-1}$ every stage. The speed corresponding to a HR of ~150 $beats \cdot min^{-1}$ (range: 8.0 – 14.0 $km \cdot h^{-1}$) on the submaximal test was noted and, after a 20-min recovery period, used on the incline-ramped $\dot{V}O_{2max}$ test.[11,20] The speed was maintained throughout with a 1% increase in gradient every minute until volitional exhaustion. Expired gas was analysed through a breath-by-breath ergospirometry system (MetaLyzer 3B, Cortex, Leipzig, Germany). This protocol determines the velocity at $\dot{V}O_{2max}$ ~~(v$\dot{V}O_{2max}$)~~, from which percentages were used in the original study[11]. Such inferences were not required for this study as only the self-paced RPE_{TP} protocol was utilized. The participants' $\dot{V}O_{2max}$ was established in accordance with the British Association of Sports and Exercise Sciences' criteria.[19]

RPE_{TP} and CTL: Briefly and as described in full previously[11], the RPE_{TP} is a self-paced, continuous, 30-min running bout, with alternating blocks of 1 min at 11 (fairly light) and 4 min at 15 (hard) on the 6-20 Borg scale[21]. Speed was self-adjusted to maintain exertion in the target range and blinded from the participant to maintain the exertion in the target range. Saliva samples were collected pre-, immediately post-, and 30 min post-exercise in all exercise trials. The CTL followed the same scheme as in Figure 1 for the RPE_{TP}, but no exercise was completed, therefore sample timepoints are referred to as pre-CTL, post-CTL and 30 min post-CTL. Blood samples were also collected in the first 13 participants immediately before saliva sampling. Heart rate (HR) and RPE were measured in the last 15s of each stage via short-range radio telemetry (Polar FT1, Polar Electro Oy, Kempele, Finland) and the 6-20 Borg scale,[21] respectively.

Saliva handling and analysis: Saliva samples were collected into 7 mL polystyrene sterile containers (Sterilin, Thermo Scientific, Loughborough, UK) by unstimulated passive drool, with eyes closed, head tilted slightly forward and avoiding any orofacial movement.[22] Water consumption was not allowed within the 10 min preceding sampling. Minimum collection time was 3 min for each participant to allow for collection of sufficient sample volume (~2 mL). Samples were then centrifuged at 14600 *g* for 10 min (Espresso Microcentrifuge, Thermo Scientific, Loughborough, UK) and the supernatant was transferred into 1.5 mL aliquots (Eppendorf, Hamburg, Germany) to be stored at -80°C until further analysis.

Salivary cortisol and testosterone concentrations were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics, PA 16803, USA). All samples were analysed in duplicate and average concentrations were used. The determined mean intra-assay CVs were 4.8% (salivary cortisol) and 4.4% (salivary testosterone). The present analyses resulted in in-lab mean inter-assay CVs of 5.1% and 6.8% for salivary cortisol and testosterone, respectively.

Venous plasma handling and analysis: All analytical procedures for blood collection, treatment and analysis have been detailed previously.[11] Briefly, whole blood samples were collected by venepuncture from the antecubital fossa into tripotassium ethylenediaminetetraacetic acid (K_3EDTA) tubes, centrifuged at 4ºC for 10 min (1500*g*) and the plasma stored at -80ºC before further analysis. The ELISA kits (IBL International, Hamburg, Germany) mean intra- and inter-assay CVs were 3.0% and 4.6%, and 3.5% and 5.7% for plasma cortisol and testosterone, respectively. The venous blood sample data was taken from previously published work[11] and has been used for correlation with the salivary data presented in this present study only.

Statistical Analysis

The IBM Statistical Package for Social Sciences® (SPSS) Statistics version 23.0 (SPSS Inc., Chicago, IL) was used for all statistical analysis. The Shapiro-Wilk test and scatter plots were used for verification of normality and homoscedasticity of raw data, respectively. When non-normally distributed (all variables), log transformation to base 10 was completed with subsequent normality rechecked. All data were then deemed normally distributed, except for speed (how this analysis was completed is detailed below). Magnitude of effect was examined using the Cohen's *d* effect sizes (ES)[23], determined by hand as described in Vincent and Weir,[24] and labeled using consistent thresholds of < 0.2 trivial, 0.21 – 0.49 small, 0.50 – 0.80 moderate, > 0.80 large.[24,25] The alpha level of significance was set as $P < 0.05$. Data is reported as mean (SD), and all results are presented as raw data to facilitate comprehension. Salivary cortisol and HR_{max} data were collapsed for all correlation analysis.

Salivary Hormone and Physiological Data Analysis: Salivary testosterone, speed and HR data sets were collapsed given there were no significant differences between any trial (excluding CTL) ($P > 0.05$). Salivary cortisol was not collapsed as a trial effect was observed. A two-way repeated measures analysis of variance (ANOVA) was used on the normalised data (salivary hormones and HR), with unchanged significant effects observed. On finding an effect, paired sample *t*-tests were used, and Bonferroni adjustments applied (also used to examine the hormonal responses during CTL), with partial eta squared (η^2) values determining the size of the effect. A non-parametric 2-related sample Wilcoxon test was used for between-trial comparisons for speed.

Reproducibility Analysis: Intra-individual coefficients of variation (CV_i) for all physiological and hormonal measurements were calculated. The intra-individual mean concentrations ($\bar{X}_t$) and SDs (SD_t) were used to calculate the CV_i using the equation $CV_i = (SD_t/\bar{X}_t)*100$. A two-way model based on the examination of single measures intraclass correlation coefficient ($ICC_{2,1}$) was also used on the collapsed data to account for the between-individual variability.[27] Guidelines on ICC models propose that values considered poor sit below 0.40, whereas fair sit within 0.40-0.59, good between 0.60-0.74, and excellent if or above 0.75.[28]

Correlation Analysis: Pearson's correlation was used to determine the correlation between the salivary and plasma cortisol and testosterone concentrations, and the individual absolute change in salivary cortisol and testosterone with HR_{max}. As there was no change in the cortisol response to exercise, these data have been collapsed. The correlation between plasma and salivary testosterone has been examined at pre-, post- and 30 min post-exercise. The level of significance was set as $P < 0.05$.

Results

Acute Hormonal Responses

All reproducibility data and average salivary cortisol/testosterone concentrations are presented in Table 1.

***** Insert Table 1 near here *****

Salivary cortisol. A trial effect was observed (P < 0.001, η^2 = 0.463), with average responses being lower in T3 compared to T2 (P = 0.002). A time effect (P = 0.029, η^2 = 0.287) was also observed. Pairwise comparisons showed cortisol did not acutely elevate in any exercise trial (ES = 0.10 in T1, ES = -0.11 in T2, ES = 0.02 in T3, all P > 0.05), but a lower concentration was observed at 30 min post-exercise when compared to post-exercise in T1 (P = 0.003, ES = 0.32) and T2 (P = 0.043, ES = 0.11). Individual acute responses are presented in Figure 2.

Salivary testosterone. There was no effect of trial (P = 0.789, η^2 = 0.022), but a significant time effect was observed (P < 0.001, η^2 = 0.593). Pairwise comparisons showed salivary testosterone acutely elevated (P < 0.001) and remained elevated at 30 min post-exercise (P < 0.05) in all exercise trials. Average acute percentage-elevations were ~23% (ES = -0.94) in T1, ~40% (ES = -1.10) in T2 and ~32% (ES = -0.87) in T3. Individual exercise-induced changes are presented in Figure 2.

Plasma cortisol and testosterone. The plasma cortisol and testosterone values can be examined in detail elsewhere.[11] Briefly, average raw data for plasma cortisol ($nmol \cdot L^{-1}$) was 259.1 ± 105.3, 313.9 ± 125.8, and 292.7 ± 123.2 at pre-, post-, and 30 min post-exercise, respectively. The average raw data for plasma testosterone ($nmol \cdot L^{-1}$) was 13.4 ± 2.6, 18.9 ± 3.7, and 15.0 ± 3.2 at pre-, post-, and 30 min post-exercise, respectively.

Plasma and Salivary Hormone Correlation. Plasma and salivary cortisol were shown to positively correlate (R = 0.581, P = 0.037). However, no correlation was observed between plasma and salivary testosterone concentration levels at pre-exercise (R = 0.430, P = 0.143), post-exercise (R = 0.250, P = 0.409), and 30 min post-exercise (R = 0.340, P = 0.256), as presented in Figure 3.

***** Insert Figure 2 near here *****

***** Insert Figure 3 near here *****

Individual Absolute Change in Salivary Hormone and Physiological Responses Correlation. Salivary cortisol and HR_{max} were shown to positively correlate (R = 0.632, $P < 0.001$). A correlation between salivary testosterone and HR_{max} was not observed (R = 0.094, $P = 0.671$), as presented in Figure 4.

***** Insert Figure 4 near here *****

Hormonal Responses During CTL

Salivary cortisol concentrations were lower at post-CTL and 30 min post-CTL than Pre-CTL by ~28% ± 17% and ~37% ± 19%, respectively (both $P < 0.001$). Pre-CTL salivary testosterone was not different from post-CTL ($P = 0.142$) but was ~12% ± 5% higher than 30 min post-CTL ($P = 0.003$) (Table 1).

***** Insert Figure 5 near here *****

Speed/HR Acute Responses and Urine Osmolality

No differences between collapsed trials were found in speed or HR ($P > 0.05$ for all) (see Figure 5). Reproducibility and average data for speed and HR in response to the RPE_{TP} trials are presented in Table 1. Urine osmolality did not differ between trials ($P > 0.05$).

Discussion

This study's primary aim was to determine whether the same RPE_{TP} cortisol and testosterone responses in plasma[11] could be replicated in saliva, in healthy (i.e. non-overreached) adult, male individuals. Indeed, the RPE_{TP} significantly and acutely elevated salivary testosterone in T1 (515 to 630 $pmol \cdot L^{-1}$, ES = -0.94), T2 (491 to 663 $pmol \cdot L^{-1}$, ES = -1.10), and T3 (523 to 661 $pmol \cdot L^{-1}$, ES = -0.87). However, salivary cortisol did not significantly elevate in any trial, as shown previously elsewhere[11], thus accepting hypothesis (i). Furthermore, the CVi in salivary testosterone observed in this present study (17 ± 7%) is similar to the exercise-induced variance observed for plasma testosterone elsewhere (12 ± 9%)[11]. This has not been observed for salivary cortisol (25 ± 15%), whose variability in plasma to the RPE_{TP} is moderately lower (12 ± 7%)[11], partially accepting hypothesis ii). The secondary aim sought to assess the relationship between saliva and blood cortisol and testosterone responses to the $RPE_{TP,}$ albeit in a subsection of previously measured participants. A correlation between salivary and plasma testosterone levels was not observed (see Figure 3). Whilst salivary cortisol and plasma surrogates did correlate, their lack of change across trials limits the utility in this cortisol specific inference. Taken together (cortisol and testosterone correlations) the data rejects hypothesis (iii).

Previous data demonstrates a correlation between plasma and salivary testosterone[29,30] – however – this is in resting samples unlike the 'exercise response' data from the present study (see Figures 2 and 3, and Table 1). Similarly, Hough et al. (2011)[12] did not observe a correlation in exercise-induced responses of plasma and salivary testosterone, although the authors suggest caution is required when interpreting their data. Specifically, they[12]: (i) missed some post-exercise blood samples; and (ii) proposed that the correlation between plasma and salivary testosterone might not have occurred as testosterone elevates to exercise stress quicker in the blood than saliva. Indeed, it has been observed elsewhere that despite parallel increases in blood and saliva testosterone after oral testosterone undecanoate administration in healthy men, the absorption curves showed a high interindividual variability in the time at which maximum concentrations were reached.[31]

Furthermore, Fiers et al. (2014)[32] have observed that salivary testosterone concentrations were not identical to comparable serum free testosterone due to testosterone binding with salivary proteins. In this present study, we speculate that the correlation may not have been observed due to timing of the testosterone entering the saliva. Supporting our speculation, we have observed that post-exercise plasma testosterone correlates only with the 30 min post-exercise salivary testosterone in T1 (data not presented). However, it should be noted that salivary testosterone significantly increased in response to the RPE_{TP} in all trials, and that the intra-individual variability in the present study was 17 ± 7%. Importantly, this present exercise-induced variability in salivary testosterone (17 ± 7%) is noticeably lower than the 37% blunted elevation in cycling-induced salivary testosterone responses after an 11-day period of intensified training (compared with a mean ~58% elevation pre-training) in active males suspected to be overreached.[9] Suggesting the RPE_{TP} may be useful when measuring testosterone responses in the saliva of healthy male individuals.

A mean increase in salivary cortisol was not present, yet some participants demonstrated an increase at the individual level (see Figure 2 and Table 1; although responses are not replicated across trials at the level of the individual), likely due to exercise intensity variability between participants. Indeed, positive correlations between HR_{max} observed during the RPE_{TP} and absolute change values in salivary cortisol were observed (see Figure 4). Cortisol has been reported to acutely elevate in response to exercise.[12,33] Although it has been proposed that exercise must be at an intensity above 60% $\dot{V}O_{2max}$ for at least

20-30 min to induce an elevation in cortisol levels,[34] this is not always observed.[35] As no acute elevation and a between-trial difference was observed, the RPE_{TP} may not have provided a sufficient physiological strain to activate an exercise-induced salivary cortisol response in all participants; as also observed in its plasma surrogate.[11] These data may suggest that the variability observed in the salivary cortisol sensitivity to exercise may be driven by exercise intensity. However, no correlation was present between salivary testosterone and HR_{max} (see Figure 4) despite a consistent elevation in this hormone in response to the RPE_{TP} and low inter- (see Figure 2, row D) and intra-individual (Table 1) variability. The data reinforces the highly-sensitive nature of salivary testosterone to exercise (certainly in response to the RPE_{TP}, as observed elsewhere in its plasma surrogate[11]), highlighting its potential utility within hypothalamic-pituitary-gonadal dysregulation associated with NFOR/OTS.

Practical Applications

- Salivary testosterone is sensitive and a reproducible biomarker to the RPE_{TP} indicating a triggered activation of the hypothalamic-pituitary-gonadal complex after short-duration, high-intensity running exercise.
- Salivary cortisol demonstrates a somewhat individualized yet non-sensitive response to the RPE_{TP} and from the present and related experimental designs, is currently an unproven biomarker-related exercise-induced stress responsiveness.
- The RPE_{TP} elicits reproducible physiological and salivary testosterone hormone responses with greater practical application/integration than previous methods (e.g. saliva and not plasma samples); with further proof of concept (i.e. analysis of the effects of a period of intensified training on the RPE_{TP}-induced responsiveness of salivary testosterone) it may be a useful potential tool in NFOR/OTS paradigms.

Conclusions

Physiological responses (HR and speed at prescribed RPE) were shown reproducible across all RPE_{TP} trials within the present study; within a larger sample than used previously elsewhere.[11] Salivary cortisol was not sensitive to the RPE_{TP} (as shown elsewhere albeit in plasma surrogates[11]). Despite cortisol salivary levels correlating with its plasma surrogate as hypothesised, acute cortisol responses may be influenced by diurnal variation and an individualised response to RPE_{TP} (likely exercise intensity driven), rendering it an unreliable biomarker to highlight exercise responsiveness, within the present design. The present RPE_{TP} data suggests salivary testosterone to be a more robust marker of a triggered endocrine mobilization during exercise. Yet, and despite no strong correlation observed in the exercise-induced salivary and plasma testosterone levels, the variability between the acute responses of plasma and salivary testosterone to the RPE_{TP} are relatively similar. Therefore, the consistent and sensitive exercise responsiveness of salivary testosterone to the RPE_{TP} suggest: (i) greater utility than cortisol; (ii) a more practically compatible bio-sample than plasma; and (iii) changes in salivary testosterone were no due to inconsistent physiological strain. Nevertheless, future work is required to detail proof of concept regarding the salivary testosterone sensitivity to the RPE_{TP} in an active population following a period of intensified training. Such data would demonstrate quantitively whether RPE_{TP}-induced salivary (and plasma) testosterone responses and their blunting, are a suitable tool to highlight the incidence of NFOR/OTS.

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Figure Captions

Figure 1 Schematic presentation of (A) the experimental trial day protocol and procedures, (B) the study design, and (C) the RPE_{TP} design; Submax, submaximal treadmill-run test; $\dot{V}O_{2max}$, maximal oxygen uptake test; CTL, control resting trial.

Figure 2 Salivary hormone responses to the RPE_{TP} and CTL protocols at Pre-Exercise (Pre-CTL), Post-Exercise (Post-CTL) and 30 min Post-Exercise (30 min Post-CTL): (A) Salivary cortisol; (B) Salivary testosterone; (C) Individual absolute changes in salivary cortisol; (D) Individual absolute changes in salivary testosterone.
#Trial difference (T3 different than T2). *Different than Pre-exercise values ($P < 0.01$). **Different than Pre-exercise values ($P < 0.05$). ‡Different than Post-exercise values ($P < 0.05$). †Different than CTL ($P < 0.01$). δ - small effect size for trial; ✣ - trivial effect size for trial.

Figure 3 Collapsed salivary and plasma cortisol correlation (A), and salivary and plasma testosterone correlation analysis at pre-exercise (B), post-exercise (C), and 30 min post-exercise (D).

Figure 4 Correlation analysis between HR_{max} observed during RPE_{TP} and collapsed individual absolute changes in salivary cortisol (top) and salivary testosterone (bottom).

Figure 5 Heart rate and speed responses to the RPE_{TP} on each separate experimental trial (all $P > 0.05$).

Table Captions

Table 1 Average raw data for urine osmolality, the physiological and hormone responses in the CTL, T1, T2 and T3 bouts and reproducibility data (when applicable) data for T1, T2 and T3 only.