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Salivary and plasma cortisol and testosterone responses to interval and tempo running and bodyweight-only circuit training in endurance trained men

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Salivary and plasma cortisol and testosterone responses to interval and tempo running and
bodyweight-only circuit training in endurance trained men

Running title

Steroid hormone response to endurance training

Keywords

cortisol, testosterone, acute exercise, endurance, hormones

For Peer Review Only

Abstract

The aim of this study was to examine the acute response of plasma and salivary cortisol and testosterone to three training protocols. 10 trained endurance athletes participated in three experimental trials; interval training (INT), tempo run (TEMP) and bodyweight only circuit training (CIR), on separate days. Blood and saliva samples were collected pre and 0, 15, 30 and 60 minutes post-exercise. Peak post-exercise salivary cortisol was higher than pre-exercise in all trials ($p < 0.01$). After INT salivary cortisol remained elevated above pre-exercise 60 minutes post-exercise. Salivary testosterone also increased post-exercise in all trials ($p < 0.05$). Plasma and salivary cortisol were correlated between individuals ($r = 0.81, 0.73-0.88$) and within individuals ($r = 0.81, 0.73-0.87$) ($p < 0.01$). Plasma and salivary testosterone was also correlated between ($r = 0.57, 0.43-0.69$) and within individuals ($r = 0.60, 0.45-0.72$), ($p < 0.01$). Peak cortisol and testosterone levels occurred simultaneously in plasma and saliva, but timing of post-exercise hormone peaks differed between trials and individuals. Further investigation is required to identify the mechanisms eliciting an increase in hormones in response to a bodyweight-only aerobic circuit session. Furthermore, saliva is a valid alternative sampling technique for measurement of cortisol, although the complex, individual and situation dependant nature of the hormone response to acute exercise should be considered.

Keywords

cortisol, testosterone, acute exercise, endurance, hormones

Introduction

Stress is a widely researched topic, and it is evident that there is no single response, with different types of stress; for example acute or chronic, physical, psychological or immunological having their own distinctive neurochemical identity (Jessop, 1999).

Therefore, biochemical pathways exist which are specific for different types of stressor, acute physical stress such as exercise is known to mobilise glucocorticoid and catecholamine

1
2
3 biochemical pathways. Cortisol plays a role in stimulation of gluconeogenesis and
4 mobilisation of free fatty acids to initiate glucose maintenance (Salway, 2006), and this is
5 particularly important in response to exercise. Furthermore, acute stress has also been shown
6 to increase circulating levels of testosterone (Sutton, Coleman, Casey, & Lazarus, 1973).
7
8 Proposed mechanisms for release include lactate stimulated secretion (Farrell, Garthwaite, &
9 Gustafson, 1983; Lin, Wang, Wang, & Wang, 2001; Lu et al., 1997; Port, 1991) and an
10 increase in circulating catecholamines (Chrousos, 1998; Jezova & Viggas, 1981). However,
11 the glucocorticoid and catecholamine responses to stress appear to interact in complex and
12 opposing ways (Komesaroff & Funder, 1994).
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23 Many studies have investigated the effect of acute continuous exercise on cortisol and
24 testosterone levels; with most reporting increases post-exercise (Allgrove, Gomes, Hough, &
25 Gleeson, 2008; Budde et al., 2010; Jacks, Sowash, Anning, McGloughlin, & Andres, 2002;
26 Kokalas, Tsalis, Tsigilis, & Mougios, 2004; O'Connor & Corrigan, 1987; Rudolph &
27 McAuley, 1998). An increase in cortisol has also been observed after intermittent exercise
28 (Dimitriou, Sharp, & Doherty, 2002; Hough, Papacosta, Wraith, & Gleeson, 2011;
29 Vuorimaa, Ahotupa, Hakkinen, & Vasankari, 2008). However, some studies have shown no
30 change in cortisol levels (Eliakim et al., 2009; Moreira, Arsati, de Oliveira Lima Arsati, da
31 Silva, & de Araujo, 2009), suggesting athletes may become accustomed a certain type and
32 intensity of exercise and require extra stress to elicit a hormone response (Vuorimaa et al.,
33 2008). A threshold of exercise at 60% VO_{2max} for >20 minutes has been proposed to elicit
34 an increase in cortisol levels; however, this is contentious, with observation that exercise
35 above 60% VO_{2max} for 30 minutes failed to elicit an increase in cortisol levels (VanBruggen,
36 Hackney, McMurray, & Ondrak, 2011) and an increase seen after shorter duration exercise,
37 such as a 30 second Wingate test (Crewther, Lowe, Ingram, & Weatherby, 2010). However,
38 is it very difficult to compare studies, given the range of sample timing and examination of
39 different exercise intensities and modes of exercise, consequently negating any large general
40 meta-analyses.
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5 Much research has focused on the hormonal response to resistance training, particularly
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7 protocols to increase strength and hypertrophy. The protocol design, including intensity and
8
9 volume of training, appears to underpin the hormonal response (Crewther, Keogh, Cronin, &
10
11 Cook, 2006). Schemes designed to induce hypertrophy have been shown to result in a larger
12
13 increase in cortisol and testosterone than those designed to elicit neural or strength
14
15 adaptations (Hakkinen & Pakarinen, 1993; Kraemer et al., 1991; Kraemer et al., 1990;
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17 Linnamo, Pakarinen, Komi, Kraemer, & Hakkinen, 2005; McCaulley et al., 2009; Raastad,
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19 Bjoro, & Hallen, 2000; Smilios, Piliandis, Karamouzis, & Tokmakidis, 2003). The
20
21 intensity of the exercise must be sufficient to elicit a significant hormonal response (Beaven,
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23 Gill, & Cook, 2008b; Cadore et al., 2009; Fry & Lohnes, 2010; Smilios et al., 2003) and
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25 training status may also be important (Ahtiainen, Pakarinen, Kraemer, & Hakkinen, 2004;
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27 Kraemer et al., 1999). However, despite the number of studies concerned with resistance
28
29 weight training protocols; to date, no studies have examined the response to bodyweight-
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31 only circuit training; a common training method devised in the 1960's that is employed by
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33 endurance athletes (Morgan & Adamson, 1965).
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38 Previous research has established that many hormones can be measured from both blood and
39
40 saliva, with strong correlations between salivary and total blood measures of cortisol ($r=0.81$
41
42 to 0.86) and weak to moderate correlations for testosterone ($r=0.57$ to 0.87) (Aardal &
43
44 Holm, 1995; Crewther et al., 2010; O'Connor & Corrigan, 1987; Vittek, L'Homedieu,
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46 Gordon, Rappaport, & Southren, 1985). Acute levels of free cortisol in saliva have been
47
48 shown to be 70% of the free cortisol in the serum, due to a relative abundance of the cortisol-
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50 metabolising enzyme 11- β hydroxysteroid dehydrogenase in the salivary gland (Kirschbaum
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52 & Hellhammer, 2000; Obmiński, 1998). Serum and salivary cortisol have also shown a
53
54 stronger correlation at lower serum levels (Aardal & Holm, 1995; Obmiński & Stupnicki,
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56 1997; VanBruggen et al., 2011). It is suggested that an exponential relationship may be
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58 appropriate, as the free cortisol levels increase more rapidly once the binding capacity of
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3 cortisol binding globulin (CBG) is exceeded (Gozansky, Lynn, Laudenslage, & Kohurt,
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5 2005; Port, 1991).
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9 Furthermore, studies examining the hormonal response to exercise have reported delays of
10
11 five to 30 minutes for peak cortisol and testosterone levels in saliva after exercise (Crewther,
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13 Cronin, Keogh, & Cook, 2008; Crewther et al., 2010; Hough et al., 2011; O'Connor &
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15 Corrigan, 1987; VanBruggen et al., 2011). It is important to realise that measurement of
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17 hormones in saliva is complex, individual and situation dependent; therefore, clarification of
18
19 optimum post-exercise sampling time can be difficult. However, there is evidence that
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21 salivary measures are a more sensitive marker of the hormonal response to acute exercise
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23 than blood (Crewther et al., 2010; Gozansky et al., 2005; Obmiński & Stupnicki, 1997) as
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25 salivary measures are often indicative of the free or 'biologically active' biomarkers as they
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27 diffuse from the blood into the oral cavity and are not bound to albumin (Humphrey &
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29 Williamson, 2001).
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33 The aims of the present study were; firstly, to examine the salivary cortisol and testosterone
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35 response to three different training sessions in runners, notably the effect of bodyweight
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37 circuit exercise, chosen as they are common sessions undertaken by endurance athletes.
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39 Secondly, to investigate the correlation between blood and salivary hormone measures and
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41 timing of post-exercise peak hormone levels in both media.
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48 **Methods**

49 **Participants**

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52 Ten healthy male runners participated in the study. All competed regularly in running,
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54 triathlon and ironman competitions and trained 4-8 times per week. The main participant
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56 characteristics are presented in table 1. The study was approved by the University of
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3 Greenwich ethics committee and participants received written and verbal instructions and
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5 gave their written informed consent.
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8 9 **Procedures**

10 On five separate occasions separated by at least three days participants reported to the
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12 laboratory between 3pm and 8pm, as cortisol and testosterone levels show diurnal stability at
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14 this time (Rose, Kreuz, Holaday, Sulak, & Johnson, 1972). Participants continued their
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16 habitual training during the study period, however they were asked to refrain from eating
17
18 three hours prior to all trials, and from strenuous exercise, caffeine and alcohol consumption
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20 in the 24 hours before each trial.
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23 24 25 **Preliminary measures**

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27 On the first visit participants provided a fingertip capillary blood sample for
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29 measurement of blood lactate before they undertook an incremental maximal oxygen uptake
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31 (VO_{2max}) test on a pre-programmed treadmill (Woodway ELG55, Weil am Rhein, Germany).
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33 In order to establish each participant's lactate threshold, during the VO_{2max} test an interrupted
34
35 incremental speed protocol was employed similar to that described by others (Vuorimaa et
36
37 al., 2008). Following a five minute warm up ($5-10 \text{ km}\cdot\text{hr}^{-1}$), participants commenced
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39 running at $2 \text{ km}\cdot\text{hr}^{-1}$ below predicted 10 mile pace. Each stage was two minutes in duration
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41 and the treadmill speed increased $1 \text{ km}\cdot\text{hr}^{-1}$ per stage and incline remained 1% throughout.
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43 After each stage participants stopped for 45 seconds for a fingertip capillary blood sample to
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45 be collected. Expired gas was analysed with a calibrated automatic gas analyser to
46
47 determine oxygen consumption (VO_2). Heart rate (HR) was measured with a Polar HR
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49 monitor (Polar Electro Oy, Kempele, Finland) and rating of perceived exertion (RPE) on a 6-
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51 20 Borg scale (Borg, 1982), both recorded in the final 15 seconds of each stage, participants
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53 continued running until volitional exhaustion.
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5 Blood lactate measures were analysed with a BioSen C line machine (EFK Diagnostic,
6 Barleben, Germany). Lactate threshold was deemed to be 1 mmol.L⁻¹ above the resting value
7 (Yoshida, Chida, Ichioka, & Suda, 1987). Speed and percentage VO_{2max} at lactate threshold
8
9 were then calculated.
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12 13 14 15 **Main trials**

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17 On visits 2-5, participants undertook three main training protocols and a rest trial, in a
18 randomised order, separated by at least three days. During all trials heart rate was recorded
19 every 1.5 minutes and RPE every three minutes during the exercise trials. The trials were as
20 follows:
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27 A. Circuit training consisting of three sets of ten exercises with 30 minutes total session
28 duration. The exercises were: sit ups, press ups, squat jumps, back raises, burpees, plank,
29 bicycle exercise, stationary running, tricep dips and step ups. Exercises did not involve any
30 external weights and were performed on a mat where necessary or while standing on the
31 floor. Tricep dips were performed on a box (30 cm) and the step ups on a bench (35 cm).
32 Each exercise was performed for 30 seconds with 30 seconds recovery between exercises
33 and began with sit-ups. Participants were told to perform as many repetitions as possible in
34 each 30 second period.
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40 B A tempo run performed for 30 minutes at a constant speed which coincided with lactate
41 threshold established during the VO_{2max} test.
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47 C An interval session (31 minutes), consisting of six intervals of 3.5 minutes duration at the
48 treadmill speed equivalent to 90% VO_{2max}, interspersed by recovery periods of two minutes
49 duration at the speed equivalent to 30% VO_{2max}. Mean heart rate was adjusted for the
50 proportion of time spent during recovery (32.3%) and repetitions (67.7%).
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5 D Participants sat and rested for the 30 minutes duration of this trial.
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9 **Saliva collection and analysis**

10 Stimulated saliva samples were collected pre-exercise, and 0, 15, 30 and 60 minutes post-
11 exercise. Participants drank water ad libitum during all the trials but were required to stop
12 drinking five minutes before each sample collection to avoid dilution. Participants provided
13 a stimulated saliva sample into a sterile container, with Parafilm to chew on to increase flow,
14 since cortisol and testosterone are unaffected by saliva flow rate (Granger, Schwartz, Booth,
15 & Arentz, 1999; Kirschbaum & Hellhammer, 1994). Prior to collection participants were
16 instructed to chew for one minute before swallowing any saliva in the oral cavity. The
17 sampling time was three minutes to allow collection of a sufficient saliva volume. Samples
18 were refrigerated at 4°C until the end of the recovery period and were divided into four
19 aliquots and stored at -80°C. Saliva was analysed for cortisol and testosterone with
20 commercially available ELISA kits (Salimetrics, State College, PA, USA). The sensitivity of
21 the kits were 0.029 ng.mL⁻¹ for cortisol and 1 pg.mL⁻¹ for testosterone. The mean intra assay
22 coefficients of variation were 8.0% for cortisol and 9.1% testosterone for duplicate samples.
23 The mean inter assay coefficients of variation were 7.4% and 5.2 % for cortisol and
24 testosterone, respectively.
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44 **Blood collection and analysis**

45 Prior to the interval session, tempo run and rest trial participants were fitted with a cannula in
46 the forearm (21G Venflon, Becton, Dickinson and Co., Oxford, United Kingdom). During
47 and after the circuit session all blood samples were taken by venepuncture (21G BD
48 Vacutainer Safety-Lok blood collection set; Becton, Dickinson and Co.) from an antecubital
49 vein as there was a risk the cannula could be dislodged during the activities. Blood samples
50 were collected into 6 mL tripotassium ethylenediaminetetraacetic acid (K₃EDTA)
51 Vacutainers (Becton, Dickinson and Co.), pre-exercise and 0, 15, 30 and 60 minutes post-
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3 exercise. Blood samples were refrigerated at 4°C until the end of each trial (for no longer
4 than two hours). Samples were identified as being stable for up to four hours at 4°C prior to
5 centrifugation and freezing (Tuck et al., 2008). After each trial blood samples were
6
7 centrifuged at 1,500g for 10 minutes, and the plasma was divided into aliquots and stored at -
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9 80 °C until analysis. Plasma cortisol and testosterone concentrations were determined using
10
11 commercially available ELISA kits (DRG Instruments, Germany). The sensitivity of the kits
12
13 was 2.5 ng.mL⁻¹ (plasma cortisol) and 0.083 ng.mL⁻¹ (plasma testosterone). The mean intra
14
15 assay coefficients of variation were 9.3% and 6.1% for cortisol testosterone, respectively.
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17 The mean inter assay coefficients of variation were 6.2% and 7.5% for cortisol and
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19 testosterone, respectively.
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25 **Statistical analysis**

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27 Mean hormone levels are presented with the standard deviation. All data in figures are
28
29 presented as mean values and SEM for clarity. Data were checked for normality,
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31 homogeneity of variance, and sphericity before statistical analysis. A one way repeated
32
33 measures analysis of variance (ANOVA) was used to examine mean and peak HR and RPE
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35 data. A two way repeated measures ANOVA was used to examine the salivary and plasma
36
37 hormone data. Significant differences were assessed with Student's paired t-test with
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39 Bonferoni post hoc adjustments for multiple comparisons. Pearson's product moment
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41 correlation coefficient and agreement analysis were used to assess correlations between
42
43 salivary and plasma measures, the saliva-plasma relationship was assessed between (pooled
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45 data) and within individuals after Fisher transformation (\pm 95% confidence intervals).
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47 Statistical significance was accepted at $P < 0.05$.
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54 **Results**

Trial characteristics

The characteristics in terms of running speed, percentage $\text{VO}_{2\text{max}}$ and percentage maximal HR for each trial are presented in table 2. Mean session maximum HR (%) was higher in INT and TEMP compared to CIR ($p<0.001$) but did not differ between INT and TEMP trials.

HR and RPE

The mean HR response was greater in INT and TEMP compared to CIR ($p<0.01$), no difference was observed between INT and TEMP. There was a significant effect of trial for peak HR ($p<0.01$), higher in INT compared to CIR and TEMP ($p<0.05$) (table 3). TEMP showed a gradual significant rise in HR throughout the duration of the trial (135 ± 15 rising to 159 ± 11 bpm, $p<0.01$). However, in INT there was a significant increase in HR during the 3.5 minute interval compared to the recovery periods between repetitions (160 ± 11 bpm versus 114 ± 12 bpm, $p<0.01$). CIR showed intermittent changes in HR during the trial; however HR remained below that observed during TEMP for the entire session (figure 1). Mean RPE was higher for INT compared to TEMP and CIR ($p<0.01$). A significant correlation was revealed between session maximum HR (%) and RPE ($r=0.52$, $0.23-0.74$, $p<0.01$).

Salivary cortisol response

The salivary cortisol response for the four trials is presented in figure 2. There was no change in cortisol levels for the duration of the resting trial. Salivary cortisol levels increased from pre-exercise to post-exercise in INT and remained elevated throughout the 60 minutes post-exercise period. Pre to post-exercise peak values significantly increased after all trials ($p<0.05$), by $288\% \pm 220\%$ after INT, $106 \pm 156\%$ after TEMP and $82 \pm 39\%$ after CIR. Increases in salivary cortisol were not significantly correlated with maximum HR (%) or RPE.

Salivary testosterone response

There was no change in salivary testosterone levels during the resting trial. Salivary testosterone increased immediately post-exercise in all exercise trials ($p < 0.03$) (figure 3). Furthermore, pre to post-exercise peak values showed a significant increase after all trials ($p < 0.002$), by $53\% \pm 39\%$ after INT, $63 \pm 40\%$ after TEMP and $30 \pm 13\%$ after CIR. Salivary testosterone returned to pre-exercise values within 60 minutes of recovery in all trials.

Plasma and salivary hormone correlations

As expected, plasma showed a higher mean cortisol concentration compared to saliva ($145.3 \pm 68.0 \text{ ng.mL}^{-1}$ vs. $2.41 \pm 1.89 \text{ ng.mL}^{-1}$ respectively). This trend was mirrored in salivary ($145.7 \pm 48.1 \text{ pg.mL}^{-1}$) and plasma ($5518.9 \pm 1873.0 \text{ pg.mL}^{-1}$) testosterone ($p < 0.0001$). Overall there was a correlation between saliva and plasma cortisol ($r = 0.81$, $0.73-0.88$, $p < 0.01$) (figure 4) and testosterone levels ($r = 0.57$, $0.43-0.69$, $p < 0.01$) when comparing between individuals (pooled data) (figure 5). Significant within individual correlations (average data) were also revealed between salivary and plasma cortisol ($r = 0.81$, $0.73-0.87$, $p < 0.0001$) and similarly for testosterone ($r = 0.60$, $0.45-0.72$, $p < 0.0001$). For cortisol a stronger correlation was revealed for plasma values $> 145 \text{ ng/mL}$ (CBG binding limit) ($r = 0.46$, $0.25-0.62$) than below (0.69 , $0.48-0.87$) ($p < 0.001$).

Peak hormonal measures

Comparison of peak hormonal measures revealed post TEMP salivary cortisol peaked at ~ 0 minutes post-exercise and plasma levels ~ 15 minutes post-exercise. Salivary and plasma testosterone measures both peaked ~ 0 minutes post TEMP. After INT both plasma and salivary cortisol levels peaked at ~ 15 minutes post-exercise and testosterone levels ~ 0 minutes post-exercise. After CIR salivary cortisol and testosterone peaked at ~ 0 minutes post exercise. However, for both INT and CIR there were large inter-individual differences

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3 within this trial, as peak values ranging from 0 to 30 minutes post exercise for cortisol and
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5 testosterone.

6 7 8 9 **Discussion**

10 The aims of the present study were firstly, to investigate the salivary cortisol and testosterone
11 response to three different training protocols in runners, particularly a bodyweight only
12 circuit session and secondly, to assess the correlation of plasma and salivary hormone
13 measures and timing of post-exercise peak hormone concentrations.
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21 A significant increase in salivary cortisol concentration was observed after the INT session
22 compared to rest. Participants reported the INT session to be more strenuous overall with a
23 significantly higher mean RPE and the intermittent nature of the INT trial, with periods at
24 90% VO_{2max} , may have contributed to the higher perceived exertion. However, the INT and
25 TEMP trials showed no difference in mean maximum HR (%), but a significant weak to
26 moderate correlation was revealed between mean maximum HR (%) and mean RPE
27 ($r=0.52$), suggesting HR (%) was linked with perceived exertion. The prolonged increase in
28 salivary cortisol levels seen after INT, are supported by other researchers (Elloumi, Maso,
29 Michaux, Robert, & Lac, 2003; Hough et al., 2011) and may reflect the high metabolic
30 demand of the trial, which in turn led to activation of the HPA axis and an increase in
31 cortisol secretion. There is evidence that cortisol levels are correlated with blood lactate
32 (Farrell et al., 1983; Port, 1991) and suggestion that lactate may activate chemoreceptors in
33 the working muscle and stimulate the HPA axis (Farrell et al., 1983). Although highly
34 speculative, given the lack of blood lactate measurements in the study, the periods of
35 exercise above 90% VO_{2max} during INT may have contributed to a higher level of blood
36 lactate accumulation than TEMP or CIR. In all exercise trials there was a significant
37 increase in peak post-exercise salivary cortisol, compared to pre-exercise values, with the
38 largest increase seen after INT; this suggests all trials were of sufficient intensity to elicit a
39 hormonal response. These findings reflect those reported by another group who examined
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3 athletes completing a 40 minute tempo run at 80% velocity of VO_{2max} (vVO_{2max}) and a 40
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5 minute repetition session, which consisted of two minutes run ($100\%vVO_{2max}$) and two
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7 minutes recovery (slow walk) (Vuorimaa et al., 2008). Both trials showed a significant
8
9 increase in serum cortisol concentration post-exercise. However, the authors postulated that
10
11 if the total work output of tempo and intervals runs were equated, the serum cortisol
12
13 response to the interval session may have been greater than tempo; therefore, the closely
14
15 matched work outputs of TEMP and INT in the present study reflect this suggestion.
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19 There was an increase in salivary testosterone post-exercise in both running based exercise
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21 trials, with post-exercise peak testosterone over 50% higher than pre-exercise. These
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23 findings are supported by studies in runners (Vuorimaa et al., 2008), cyclists (Hough et al.,
24
25 2011) and rowers (Kokalas et al., 2004). Possible mechanisms for this change include
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27 increased production of testosterone by sympathetic stimulation of the testes (Fahrner &
28
29 Hackney, 1998). Furthermore, activation of the sympathetic nervous system and increased
30
31 lactate accumulation may have contributed to the increase in testosterone concentration,
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33 although supporting evidence is limited to rats (Lu et al., 1997). There is speculation that
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35 protein binding affinity can be affected by changes in pH and temperature elicited by
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37 exercise, this in turn may lead to a higher free proportion of cortisol and testosterone in the
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39 blood and increased levels in saliva (Obminski & Stupnicki, 1996; Rosner, 1990). However,
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41 a more recent study showed no binding affinity changes after endurance exercise (Fahrner &
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43 Hackney, 1998) and further research is required to support this mechanism. Given evidence
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45 of reduced blood flow to the liver during exercise (Rowell, Blackmon, & Bruce, 1964);
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47 reduced hepatic clearance of testosterone is another possible reason for the increase, rather
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49 than a higher secretion rate (Cadoux-Hudson, Few, & Imms, 1985; Sutton et al., 1973).
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51 Dissimilarly to cortisol, salivary testosterone returned to baseline 60 minutes post-exercise in
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53 all trials, which suggests different mechanisms of release or clearance of these hormones
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55 occurring in response to exercise stress (Jessop, 1999).
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3 In the present study, novelty exists with investigation of the acute cortisol and testosterone
4 response to bodyweight-only circuit training. There was a significant increase in post-
5 exercise peak salivary cortisol (82%) and testosterone (31%) in response to CIR. The
6 presence of a concomitant increase in cortisol and testosterone mimics the hormonal
7 response to hypertrophy based resistance training protocols, with those designed to increase
8 strength showing little or no change (Crewther et al., 2008; Hakkinen & Pakarinen, 1993;
9 Linnamo et al., 2005; McCaulley et al., 2009; Smilios et al., 2003). It has also been
10 suggested that the absolute workload is important to elicit an increase in cortisol and
11 testosterone, but the design of the session including recovery time should also be considered
12 (Cadore et al., 2009; Hickson, Hidaka, Foster, Falduto, & Chatterton, 1994; Kraemer et al.,
13 1993; Kraemer et al., 1991; Smilios et al., 2003). The increase in cortisol and testosterone is
14 thought to represent a catabolic and anabolic state, which is essential to initiate an increase in
15 muscle growth (Crewther et al., 2006).

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31 Despite the post-exercise increase in testosterone, this ranged from 13% to 62%,
32 demonstrating a large individual variation in response. This finding could be explained by
33 evidence that individuals elicit different testosterone responses to strength training protocols
34 (Beaven, Gill, & Cook, 2008a). Beaven and colleagues also reported large standard error
35 measurements for testosterone concentrations between individuals in response to the
36 different training protocols, suggesting that pooled data may impact the validity and
37 interpretation of study findings.

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48 Another aspect worthy of consideration is that the participants in the study were endurance
49 trained, and undertook little or no previous strength training, as a less pronounced hormonal
50 response has been seen in endurance trained compared to resistance trained athletes
51 (Tremblay, Copeland, & Van Helder, 2004). Furthermore, others have suggested that
52 elevated hormones do not necessarily enhance muscle growth or strength in un-trained
53 participants (West et al., 2010b; Wilkinson, Tarnopolsky, Grant, Correia, & Phillips, 2006b).

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3 However, despite the similar response to hypertrophy resistance schemes, the bodyweight-
4 only protocol undertaken in this study is unlikely to have elicited large strength or
5 hypertrophy gains and with regular training the most likely outcome would be small
6 increases in muscular strength and vVO₂max (Taipale et al., 2010).
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13 When considering the role of testosterone changes, most studies have found that increased
14 levels do not directly cause muscle hypertrophy (West et al., 2010a; Wilkinson,
15 Tarnopolsky, Grant, Correia, & Phillips, 2006a), in contrast to the response seen after
16 exogenous administration (Bhasin et al., 1996). It has also been suggested that an increase in
17 testosterone could be a result of decreased muscle utilisation (Kraemer et al., 1990) or,
18 reduced hepatic clearance. Furthermore, research has postulated that testosterone may play a
19 permissive role in physiological adaptations to resistance training (Crewther, Cook,
20 Cardinale, Weatherby, & Lowe, 2011; Viru & Viru, 2005). Currently, the current biological
21 roles of exercise induced hormone changes remain somewhat uncertain (West & Phillips,
22 2012). Recent studies have suggested that testosterone may contribute to behavioural
23 adaptations driving greater voluntary effort; exemplified in a study linking pre-exercise
24 salivary testosterone levels with self selected resistance training workloads and performance
25 in female netball players (Cook & Beaven, 2013). Given the element of self-selected effort
26 during circuit training, it would be interesting to examine this concept further in future
27 research, especially given evidence that non-physical psychological priming, such as
28 watching a video can positively influence pre-exercise testosterone levels and subsequent
29 voluntary exercise performance (Cook & Crewther, 2012). The current consensus is that the
30 adaptive response to strength training is likely to be multi-faceted, with several acute training
31 factors (one of them hormonal), rather than a single factor. However, given the lack of data,
32 further investigation is required to examine the hormonal response to bodyweight-only
33 circuit training and concurrent physiological adaptations and mechanisms.
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3 The second aim of the study was to compare salivary and plasma hormone levels and for
4 pooled samples, plasma and salivary cortisol showed a moderate positive correlation
5 ($r>0.80$). Similar correlations have been demonstrated in other studies at rest (Aardal &
6
7 Holm, 1995), after 30 minutes of cycling at 75% VO_{2max} (O'Connor & Corrigan, 1987) and a
8
9 Wingate test (Crewther et al., 2010). Furthermore, comparison within individuals revealed a
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11 correlation was very similar to the pooled data ($r>0.80$). Previous studies have shown a
12
13 stronger correlation with saliva at serum levels below the CBG limit (approximately 400
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15 nmol/L) than above (Aardal & Holm, 1995; Obmiński & Stupnicki, 1997; VanBruggen et
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17 al., 2011). However, the present study disagrees with these findings; as a stronger correlation
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19 was revealed at plasma cortisol levels above 400 nmol/L (145 ng/mL). Furthermore, an
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21 exponential relationship showed the same r value as linear regression; therefore, linear
22
23 correlation appears suitable for the comparison of salivary and plasma cortisol levels.
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29 Plasma and salivary testosterone levels showed a weak to moderate correlation between
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31 individuals ($r>0.55$) with a large inter individual variation, similar to other published studies
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33 (Crewther et al., 2010; Vittek et al., 1985). However, others have shown no correlation
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35 between salivary and serum testosterone pre and post resistance exercise (Cadore et al.,
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37 2008). As previously discussed, it is important to realise that measurement of hormones in
38
39 saliva is individual and situation dependent and therefore a variation in response is likely.
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41 Results from the present study confirm the validity of using salivary measures to monitor the
42
43 cortisol response to exercise; however, the weaker correlations observed between saliva and
44
45 total plasma testosterone should be approached with caution with further validation
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47 warranted.
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51 Additionally, plasma and salivary cortisol and testosterone levels peaked simultaneously in
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53 all trials, with the exception of a slightly later plasma cortisol peak after TEMP. A post-
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55 exercise delay of up to 20 minutes between peak cortisol and testosterone levels in saliva
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57 compared to blood has previously been reported at rest (Kirschbaum & Hellhammer, 1989)
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3 and after exercise (Hough et al., 2011; O'Connor & Corrigan, 1987). The present results
4 reflect reports observing an immediate diffusion from blood into saliva after intravenous
5 injection (Kirschbaum & Hellhammer, 2000; Wang, Plymate, Nieschlag, & Paulsen, 1981).
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7 The lack of lag time in the current trial may reflect the complex relationship between
8
9 salivary and blood hormone levels. Furthermore, unlike previous studies, the present study
10 used stimulated saliva sampling, therefore hormones levels in saliva are unlikely to have
11 been affected by changes in salivary volume in response to exercise, caused by
12 vasoconstriction of the arterioles in response to sympathetic stimulation (Chicharro, Lucia,
13 Perez, Vaquero, & Urena, 1998).
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23 Overall, peak salivary cortisol and testosterone levels occurred immediately post-exercise for
24 TEMP and CIR; however, after INT salivary cortisol peaked at ~15 minutes post-exercise.
25 However, there was variation between individuals in peak time for INT and CIR. For these
26 trials there was a large inter-individual difference for post-exercise hormone peaks (ranging
27 from immediately after to ~30 minutes post-exercise). The individual differences may have
28 been caused by the intermittent exercise and complex nature of hormone measurement.
29
30 Studies examining the time for hormones to peak post-exercise, have shown peak
31 testosterone occurs earlier than cortisol, within 10 minutes of cessation of exercise (Hough *et*
32 *al.*, 2011; Daly *et al.*, 2005); however, the present study did not show this trend. Given the
33 variation between individuals after intermittent exercise, post-exercise peak values may be a
34 better indicator of the hormonal response than the mean peak response.
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48 **Conclusion**

49 In conclusion, results suggest that in typical training sessions undertaken by endurance
50 trained athletes, cortisol concentration may be an indicator of acute exercise stress. A
51 bodyweight-only aerobic circuit session elicited an increase in cortisol and testosterone
52 levels and further investigation is required to establish the mechanisms for this response.
53 Salivary and plasma cortisol levels were correlated in response to acute exercise; and this
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3 supports the use of saliva as an alternative sampling technique to blood for measurement of
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5 cortisol; however, weaker correlations for testosterone require further investigation. Finally,
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7 the results revealed evidence of no delay in hormone tracking between plasma and saliva and
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9 importance should be given to the highly complex, individual and situational nature of the
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11 hormone response to acute exercise.
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13 14 15 **References**

- 16
17 Aardal, E., & Holm, A. C. (1995). Cortisol in saliva - reference ranges and relation to
18 cortisol in serum. *European Journal of Clinical Chemistry and Clinical
19 Biochemistry*, 33(12), 927-932.
- 20 Ahtiainen, J. P., Pakarinen, A., Kraemer, W. J., & Hakkinen, K. (2004). Acute hormonal
21 responses to heavy resistance exercise in strength athletes versus nonathletes.
22 *Canadian Journal of Applied Physiology*, 29(5), 527-543.
- 23 Allgrove, J. E., Gomes, E., Hough, J., & Gleeson, M. (2008). Effects of exercise intensity on
24 salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci*,
25 26(6), 653 - 661.
- 26 Beaven, C. M., Gill, N. D., & Cook, C. J. (2008a). Salivary Testosterone and Cortisol
27 Responses in Professional Rugby Players After Four Resistance Exercise Protocols.
28 *The Journal of Strength & Conditioning Research*, 22(2), 426-432
29 410.1519/JSC.1510b1013e3181635843.
- 30 Beaven, M. C., Gill, N. D., & Cook, C. J. (2008b). Salivary testosterone and cortisol
31 responses in professional rugby players after four resistance exercise protocols.
32 *Journal of Strength and Conditioning Research*, 22(2), 426-432.
- 33 Bhasin, S., Storer, T. W., Berman, N., Callegari, C., Clevenger, B., Phillips, J., . . . Casaburi,
34 R. (1996). The effects of supraphysiologic doses of testosterone on muscle size and
35 strength in normal men. *N Engl J Med*, 335(1), 1-7. doi:
36 10.1056/NEJM199607043350101
- 37 Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*,
38 14(5), 377-381.
- 39 Budde, H., Voelcker-Rehage, C., Pietrassyk-Kendziorra, S., Machado, S., Ribeiro, P., &
40 Arafat, A. M. (2010). Steroid hormones in the saliva of adolescents after different
41 exercise intensities and their influence on working memory in a school setting.
42 *Psychoneuroendocrinology*, 35(3), 382-391.
- 43 Cadore, E., Lhullier, F., Brentano, M., Silva, E., Ambrosini, M., Spinelli, R., . . . Krueel, L.
44 (2008). Correlations between serum and salivary hormonal concentrations in
45 response to resistance exercise. *J Sports Sci*, 26(10), 1067 - 1072.
- 46 Cadore, E. L., Lhullier, F. L., Alberton, C. L., Almeida, A. P., Sapata, K. B., Korzenowski,
47 A. L., & Krueel, L. F. (2009). Salivary hormonal responses to different water-based
48 exercise protocols in young and elderly men. *Journal of Strength and Conditioning
49 Research*, 23(9), 2695-2701.
- 50 Cadoux-Hudson, T. A., Few, J. D., & Imms, F. J. (1985). The effect of exercise on the
51 production and clearance of testosterone in well trained young men. *Eur J Appl
52 Physiol Occup Physiol*, 54(3), 321-325. doi: 10.1007/bf00426153
- 53 Chicharro, J. L., Lucia, A., Perez, M., Vaquero, A. F., & Urena, R. (1998). Saliva
54 composition and exercise. *Sports Med*, 26(1), 17-27.
- 55 Chrousos, G. P. (1998). Stressors, Stress, and Neuroendocrine Integration of the Adaptive
56 Response: The 1997 Hans Selye Memorial Lecture. *Ann N Y Acad Sci*, 851(1), 311-
57 335.
58
59
60

- 1
2
3 Cook, C. J., & Beaven, C. M. (2013). Salivary testosterone is related to self-selected training
4 load in elite female athletes. *Physiol Behav*, 116-117, 8-12. doi:
5 10.1016/j.physbeh.2013.03.013
- 6 Cook, C. J., & Crewther, B. T. (2012). Changes in salivary testosterone concentrations and
7 subsequent voluntary squat performance following the presentation of short video
8 clips. *Horm Behav*, 61(1), 17-22. doi: 10.1016/j.yhbeh.2011.09.006
- 9 Crewther, B., Cronin, J., Keogh, J., & Cook, C. (2008). The salivary testosterone and cortisol
10 response to three loading schemes. *J Strength Cond Res*, 22(1), 250-255.
- 11 Crewther, B., Keogh, J., Cronin, J., & Cook, C. (2006). Possible stimuli for strength and
12 power adaptation: acute hormonal responses. *Sports Med*, 36(3), 215-238.
- 13 Crewther, B., Lowe, T., Ingram, J., & Weatherby, R. (2010). Validating the salivary
14 testosterone and cortisol concentration measures in response to short high-intensity
15 exercise. *Journal of Sports Medicine and Physical Fitness*, 50(1), 85-92.
- 16 Crewther, B. T., Cook, C., Cardinale, M., Weatherby, R. P., & Lowe, T. (2011). Two
17 emerging concepts for elite athletes: the short-term effects of testosterone and
18 cortisol on the neuromuscular system and the dose-response training role of these
19 endogenous hormones. *Sports Med*, 41(2), 103-123. doi: 10.2165/11539170-
20 000000000-00000
- 21 Dimitriou, L., Sharp, N. C., & Doherty, M. (2002). Circadian effects on the acute responses
22 of salivary cortisol and IgA in well trained swimmers. *Br J Sports Med*, 36(4), 260-
23 264.
- 24 Eliakim, A., Portal, S., Zadik, Z., Rabinowitz, J., Adler-Portal, D., Cooper, D. M., . . .
25 Nemet, D. (2009). The effect of a volleyball practice on anabolic hormones and
26 inflammatory markers in elite male and female adolescent players. *Journal of*
27 *Strength and Conditioning Research*, 23(5), 1553-1559.
- 28 Elloumi, M., Maso, F., Michaux, O., Robert, A., & Lac, G. (2003). Behaviour of saliva
29 cortisol [C], testosterone [T] and the T/C ratio during a rugby match and during the
30 post-competition recovery days. *Eur J Appl Physiol*, 90(1-2), 23-28.
- 31 Fahrner, C. L., & Hackney, A. C. (1998). Effects of Endurance Exercise on Free
32 Testosterone Concentration and the Binding Affinity of Sex Hormone Binding
33 Globulin (SHBG). *Int J Sports Med*, 19(1), 12,15.
- 34 Farrell, P. A., Garthwaite, T. L., & Gustafson, A. B. (1983). Plasma adrenocorticotropin and
35 cortisol responses to submaximal and exhaustive exercise. *J Appl Physiol*, 55(5),
36 1441-1444.
- 37 Fry, A., & Lohnes, C. (2010). Acute testosterone and cortisol responses to high power
38 resistance exercise. *Human Physiology*, 36(4), 457-461. doi:
39 10.1134/s0362119710040110
- 40 Gozansky, W. S., Lynn, J. S., Laudenslage, M. L., & Kohurt, W. M. (2005). Salivary cortisol
41 determined by enzyme immunoassay is preferable to serum total cortisol for
42 assessment of dynamic hypothalamic-pituitary-adrenal axis activity. *Clinical*
43 *Endocrinology*, 63(3), 336-341.
- 44 Granger, D. A., Schwartz, E. B., Booth, A., & Arentz, M. (1999). Salivary Testosterone
45 Determination in Studies of Child Health and Development. *Horm Behav*, 35(1), 18-
46 27. doi: <http://dx.doi.org/10.1006/hbeh.1998.1492>
- 47 Hakkinen, K., & Pakarinen, A. (1993). Acute hormonal responses to two different fatiguing
48 heavy-resistance protocols in male athletes. *J Appl Physiol*, 74(2), 882-887.
- 49 Hickson, R. C., Hidaka, K., Foster, C., Falduto, M. T., & Chatterton, R. T. (1994).
50 Successive time courses of strength development and steroid hormone responses to
51 heavy-resistance training. *J Appl Physiol*, 76(2), 663-670.
- 52 Hough, J. P., Papacosta, E., Wraith, E., & Gleeson, M. (2011). Plasma and salivary steroid
53 hormone responses of men to high-intensity cycling and resistance exercise. *Journal*
54 *of Strength and Conditioning Research*, 25(1), 23-31.
- 55 Humphrey, S. P., & Williamson, R. T. (2001). A review of saliva: Normal composition,
56 flow, and function. *Journal of Prosthetic Dentistry*, 85(2), 162-169.
- 57
58
59
60

- 1
2
3 Jacks, D. E., Sowash, J., Anning, J., McGloughlin, T., & Andres, F. (2002). Effect of
4 exercise at three exercise intensities on salivary cortisol. *Journal of Strength and*
5 *Conditioning Research*, 16(2), 286-289.
- 6 Jessop, D. S. (1999). Stimulatory and inhibitory regulators of the hypothalamo-pituitary-
7 adrenocortical axis. *Best Practice & Research Clinical Endocrinology &*
8 *Metabolism*, 13(4), 491-501. doi: <http://dx.doi.org/10.1053/beem.1999.0039>
- 9 Jezova, D., & Vigas, M. (1981). Testosterone response to exercise during blockade and
10 stimulation of adrenergic receptors in man. *Hormone Research*, 15(3), 141-147.
- 11 Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological
12 research: an overview. *Neuropsychobiology*, 22(3), 150-169.
- 13 Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoendocrine
14 research: recent developments and applications. *Psychoneuroendocrinology*, 19,
15 313-333.
- 16 Kirschbaum, C., & Hellhammer, D. H. (2000). Salivary cortisol. In G. Fink (Ed.),
17 *Encyclopedia of Stress* (Vol. 3, pp. 379-383). San Diego: Academic Press.
- 18 Kokalas, N., Tsalis, G., Tsigilis, N., & Mougios, V. (2004). Hormonal responses to three
19 training protocols in rowing. *Eur J Appl Physiol*, 92(1-2), 128-132.
- 20 Komesaroff, P. A., & Funder, J. W. (1994). Differential glucocorticoid effects on
21 catecholamine responses to stress. *American Journal of Physiology - Endocrinology*
22 *And Metabolism*, 266(1), E118-E128.
- 23 Kraemer, W. J., Fleck, S. J., Dziados, J. E., Harman, E. A., Marchitelli, L. J., Gordon, S. E., .
24 . . Triplett, N. T. (1993). Changes in hormonal concentrations after different heavy-
25 resistance exercise protocols in women. *J Appl Physiol*, 75(2), 594-604.
- 26 Kraemer, W. J., Fleck, S. J., Maresh, C. M., Ratamess, N. A., Gordon, S. E., Goetz, K. L., . .
27 . Patton, J. F. (1999). Acute hormonal responses to a single bout of heavy resistance
28 exercise in trained power lifters and untrained men. *Canadian Journal of Applied*
29 *Physiology*, 24(6), 524-537.
- 30 Kraemer, W. J., Gordon, S. E., Fleck, S. J., Marchitelli, L. J., Mello, R., Dziados, J. E., . . .
31 Fry, A. C. (1991). Endogenous anabolic hormonal and growth factor responses to
32 heavy resistance exercise in males and females. *Int J Sports Med*, 12(2), 228-235.
- 33 Kraemer, W. J., Marchitelli, L., Gordon, S. E., Harman, E., Dziados, J. E., Mello, R., . . .
34 Fleck, S. J. (1990). Hormonal and growth factor responses to heavy resistance
35 exercise protocols. *J Appl Physiol*, 69(4), 1442-1450.
- 36 Lin, H., Wang, S. W., Wang, R. Y., & Wang, P. S. (2001). Stimulatory effect of lactate on
37 testosterone production by rat Leydig cells. *Journal of Cellular Biochemistry*, 83(1),
38 147-154.
- 39 Linnamo, V., Pakarinen, A., Komi, P. V., Kraemer, W. J., & Hakkinen, K. (2005). Acute
40 hormonal responses to submaximal and maximal heavy resistance and explosive
41 exercises in men and women. *Journal of Strength and Conditioning Research*, 19(3),
42 566-571.
- 43 Lu, S. S., Lau, C. P., Tung, Y. F., Huang, S. W., Chen, Y. H., Shih, H. C., . . . Wang, P. S.
44 (1997). Lactate and the effects of exercise on testosterone secretion: evidence for the
45 involvement of a cAMP-mediated mechanism. *Med Sci Sports Exerc*, 29(8), 1048-
46 1054.
- 47 McCaulley, G. O., McBride, J. M., Cormie, P., Hudson, M. B., Nuzzo, J. L., Quindry, J. C.,
48 & Travis Triplett, N. (2009). Acute hormonal and neuromuscular responses to
49 hypertrophy, strength and power type resistance exercise. *Eur J Appl Physiol*,
50 105(5), 695-704.
- 51 Moreira, A., Arsati, F., de Oliveira Lima Arsati, Y. B., da Silva, D. A., & de Araujo, V. C.
52 (2009). Salivary cortisol in top-level professional soccer players. *Eur J Appl Physiol*,
53 106(1), 25-30.
- 54 Morgan, R. E., & Adamson, G. T. (1965). *Circuit training*: G. Bell.
- 55 O'Connor, P. J., & Corrigan, D., L. (1987). Influence of short-term cycling on salivary
56 cortisol levels. *Medicine & Science in Sports & Exercise*, 19(3), 224-228.
- 57
58
59
60

- 1
2
3 Obmiński, Z. (1998). Changes in the free (unbound) fraction of testosterone in serum in vitro
4 as affected by pH and temperature. *Experimental and Clinical Endocrinology and*
5 *Diabetes*, 106(01), 85-88. doi: 10.1055/s-0029-1211956
- 6 Obminski, Z., & Stupnicki, R. (1996). Effect of temperature and pH on the magnitude of the
7 free fraction of cortisol in serum. *Experimental and Clinical Endocrinology and*
8 *Diabetes*, 104(4), 350-352.
- 9 Obmiński, Z., & Stupnicki, R. (1997). Comparison of the testosterone-to-cortisol ratio values
10 obtained from hormonal assays in saliva and serum. *J Sports Med Phys Fitness*,
11 37(1), 50-55.
- 12 Port, K. (1991). Serum and Saliva Cortisol Responses and Blood Lactate Accumulation
13 during Incremental Exercise Testing. *Int J Sports Med*, 12(05), 490-494. doi:
14 10.1055/s-2007-1024720
- 15 Raastad, T., Bjoro, T., & Hallen, J. (2000). Hormonal responses to high- and moderate-
16 intensity strength exercise. *Eur J Appl Physiol*, 82(1-2), 121-128.
- 17 Rose, R. M., Kreuz, L. E., Holaday, J. W., Sulak, K. J., & Johnson, C. E. (1972). Diurnal
18 Variation of Plasma Testosterone and Cortisol. *Journal of Endocrinology*, 54(1),
19 177-178. doi: 10.1677/joe.0.0540177
- 20 Rosner, W. (1990). The Functions of Corticosteroid-Binding Globulin and Sex Hormone-
21 Binding Globulin: Recent Advances. *Endocrine Reviews*, 11(1), 80-91. doi:
22 10.1210/edrv-11-1-80
- 23 Rowell, L. B., Blackmon, J. R., & Bruce, R. A. (1964). Indocyanine Green Clearance and
24 Estimated Hepatic Blood Flow during Mild to Maximal Exercise in Upright Man.
25 *Journal of Clinical Investigation*, 43, 1677-1690.
- 26 Rudolph, D. L., & McAuley, E. (1998). Cortisol and affective responses to exercise. *J Sports*
27 *Sci*, 16(2), 121-128.
- 28 Salway, J. G. (2006). *Medical Biochemistry at a Glance* (2nd ed.). Oxford: Blackwell.
- 29 Smilios, I., Pilianidis, T., Karamouzis, M., & Tokmakidis, S. P. (2003). Hormonal responses
30 after various resistance exercise protocols. *Medicine & Science in Sports & Exercise*,
31 35(4), 644-654.
- 32 Sutton, J. R., Coleman, M. J., Casey, J., & Lazarus, L. (1973). Androgen Responses during
33 Physical Exercise. *Br Med J (Clin Res Ed)*, 1(5852), 520-522. doi:
34 10.1136/bmj.1.5852.520
- 35 Taipale, R., Mikkola, J., Nummela, A., Vesterinen, V., Capostagno, B., Walker, S., . . .
36 Häkkinen, K. (2010). Strength training in endurance runners. *Int J Sports Med*,
37 31(7), 468-476.
- 38 Tremblay, M. S., Copeland, J. L., & Van Helder, W. (2004). Effect of training status and
39 exercise mode on endogenous steroid hormones in men. *J Appl Physiol*, 96(2), 531-
40 539. doi: 10.1152/jappphysiol.00656.2003
- 41 Tuck, M. K., Chan, D. W., Chia, D., Godwin, A. K., Grizzle, W. E., Krueger, K. E., . . .
42 Brenner, D. E. (2008). Standard Operating Procedures for Serum and Plasma
43 Collection: Early Detection Research Network Consensus Statement Standard
44 Operating Procedure Integration Working Group. *Journal of Proteome Research*,
45 8(1), 113-117.
- 46 VanBruggen, M. D., Hackney, A. C., McMurray, R. G., & Ondrak, K. S. (2011). The
47 relationship between serum and salivary cortisol levels in response to different
48 intensities of exercise. *Int J Sports Physiol Perform*, 6(3), 396-407.
- 49 Viru, A., & Viru, M. (2005). Preconditioning of the performance in power events by
50 endogenous testosterone: in memory of professor Carmelo Bosco. *J Strength Cond*
51 *Res*, 19(1), 6-8. doi: 10.1519/1533-4287(2005)19<6:POTPIP>2.0.CO;2
- 52 Vittek, J., L'Homedieu, D., Gordon, G., Rappaport, S., & Southren, A. (1985). Direct
53 radioimmunoassay (RIA) of salivary testosterone correlation with free and total
54 serum testosterone. *Life Sciences*, 37, 711-716.
- 55
56 Vuorimaa, T., Ahotupa, M., Häkkinen, K., & Vasankari, T. (2008). Different hormonal
57 response to continuous and intermittent exercise in middle-distance and marathon
58 runners. *Scandinavian Journal of Medicine and Science in Sports*, 18(5), 565-572.
- 59
60

- 1
2
3 Wang, C., Plymate, S., Nieschlag, E., & Paulsen, C. (1981). Salivary testosterone in men:
4 further evidence of a direct correlation with free serum testosterone. *Journal of*
5 *Clinical Endocrinology and Metabolism*, 53(5), 1021-1024.
- 6 West, D. W., Burd, N. A., Tang, J. E., Moore, D. R., Staples, A. W., Holwerda, A. M., . . .
7 Phillips, S. M. (2010a). Elevations in ostensibly anabolic hormones with resistance
8 exercise enhance neither training-induced muscle hypertrophy nor strength of the
9 elbow flexors. *J Appl Physiol*, 108(1), 60-67. doi: 10.1152/jappphysiol.01147.2009
- 10 West, D. W., Burd, N. A., Tang, J. E., Moore, D. R., Staples, A. W., Holwerda, A. M., . . .
11 Phillips, S. M. (2010b). Elevations in ostensibly anabolic hormones with resistance
12 exercise enhance neither training-induced muscle hypertrophy nor strength of the
13 elbow flexors. *J Appl Physiol*, 108(1), 60-67.
- 14 West, D. W., & Phillips, S. M. (2012). Associations of exercise-induced hormone profiles
15 and gains in strength and hypertrophy in a large cohort after weight training. *Eur J*
16 *Appl Physiol*, 112(7), 2693-2702. doi: 10.1007/s00421-011-2246-z
- 17 Wilkinson, S. B., Tarnopolsky, M. A., Grant, E. J., Correia, C. E., & Phillips, S. M. (2006a).
18 Hypertrophy with unilateral resistance exercise occurs without increases in
19 endogenous anabolic hormone concentration. *Eur J Appl Physiol*, 98(6), 546-555.
20 doi: 10.1007/s00421-006-0300-z
- 21 Wilkinson, S. B., Tarnopolsky, M. A., Grant, E. J., Correia, C. E., & Phillips, S. M. (2006b).
22 Hypertrophy with unilateral resistance exercise occurs without increases in
23 endogenous anabolic hormone concentration. *Eur J Appl Physiol*, 98(6), 546-555.
- 24 Yoshida, T., Chida, M., Ichioka, M., & Suda, Y. (1987). Blood lactate parameters related to
25 aerobic capacity and endurance performance. *Eur J Appl Physiol Occup Physiol*,
26 1(56), 7-11.
27
28
29
30
31
32
33
34
35
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Tables

Table 1 Descriptive characteristics of participants

	Mean (\pm SD)
Age (y)	39.3 \pm 6.6
Body mass (kg)	76.6 \pm 8.7
Height (m)	1.78 \pm 0.06
VO _{2max} (ml.kg ⁻¹ .min ⁻¹)	59.2 \pm 5.9
Maximum heart rate (bpm)	180 \pm 11

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Table 2 Mean (\pm SD) values for treadmill speed and VO_{2max} for TEMP and INT and CIR trials (n=10)

Trial (mean \pm SD)	Treadmill Speed (km.h ⁻¹)	VO_{2max} (%)	Maximum HR (%)
	¹⁾		
TEMP	13.0 \pm 1.4	74.7 \pm 1.6	87.1 \pm 6.1*
INT - repetition	15.3 \pm 1.6	88.3 \pm 3.2	
INT - recovery	3.6 \pm 0.7	30.6 \pm 3.3	
INT - mean	11.6 \pm 1.3	66.5 \pm 3.0	86.0 \pm 7.1*
CIR	n/a	n/a	67.4 \pm 7.5

*denotes values significant difference compared to CIR ($P < 0.05$)

Table 3 Mean and peak HR (\pm SD) (b.p.m) and mean RPE for all trials (n=10)

b.p.m (\pm SD)	Rest	TEMP	INT	CIR
Mean HR	59 \pm 6	155 \pm 10*#	145 \pm 12*#	116 \pm 10*
Peak HR	N/A	163 \pm 10	173 \pm 12§	148 \pm 10
RPE	6.0 \pm 0	14 \pm 2	15 \pm 2§	13 \pm 1

*denotes significant difference compared to rest ($P < 0.01$), # denotes significant difference compared to CIR ($P < 0.01$), § denotes significant

difference compared to CIR and TEMP ($P < 0.01$)

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Figure captions

Fig. 1 Mean HR during INT (dotted line), TEMP (dashed line) and CIR (solid line) trials (n=10)

Fig. 2 Salivary cortisol response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean +/- S.E.M) (n=10) *denotes significant difference from pre exercise value ($p < 0.05$)

Fig 3. Salivary testosterone response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean +/- S.E.M) (n=10) *denotes significant difference from pre exercise value ($p < 0.05$)

Fig. 4 Relationship between plasma and salivary cortisol concentrations from INT and TEMP sessions (n=87)

Fig. 5 Relationship between plasma and salivary testosterone concentrations from INT and TEMP sessions (n=91)

Conflict of interest

The authors have no conflicts of interest to declare

Abbreviations

ANOVA - analysis of variance

CIR – circuit session

ELISA – enzyme linked immunosorbent assay

INT – interval session

RPE – rating of perceived exertion

TEMP – tempo run

VO₂ – volume of oxygen uptake

VO_{2max}- maximal oxygen uptake

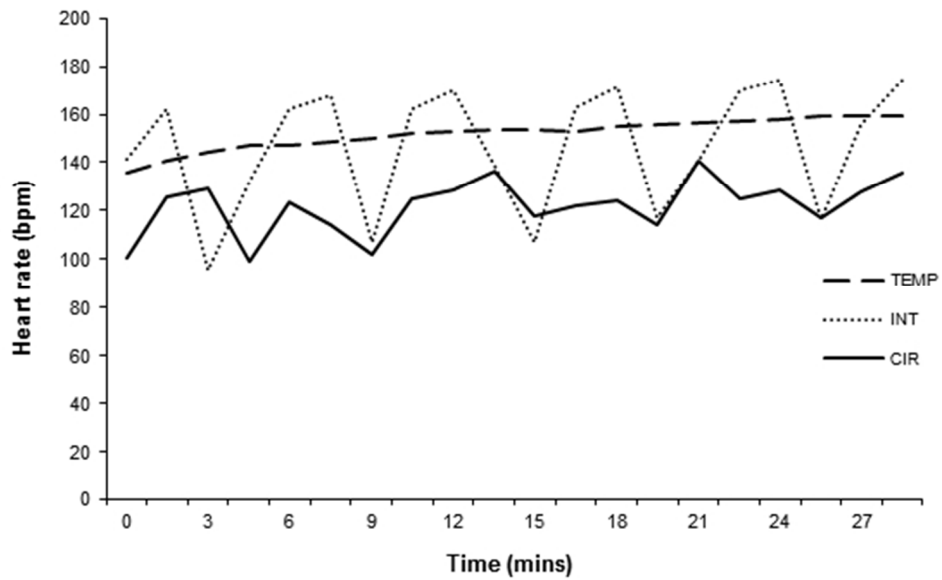


Fig. 1 Mean HR during INT (dotted line), TEMP (dashed line) and CIR (solid line) trials (n=8)
153x93mm (96 x 96 DPI)

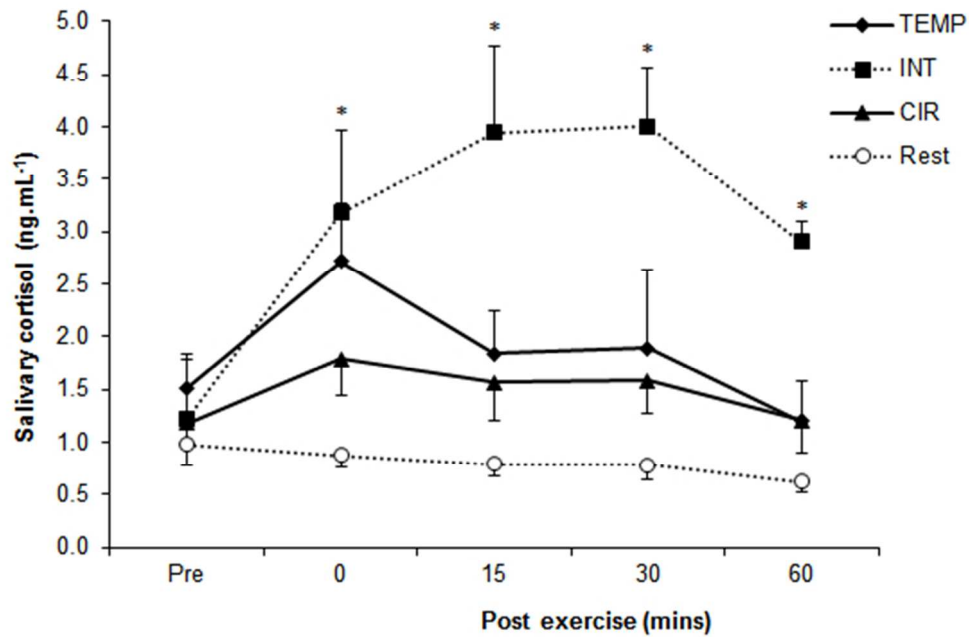


Fig. 2 Salivary cortisol response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean \pm S.E.M) (n=10) *denotes significant difference from pre exercise value ($p < 0.05$)
147x99mm (96 x 96 DPI)

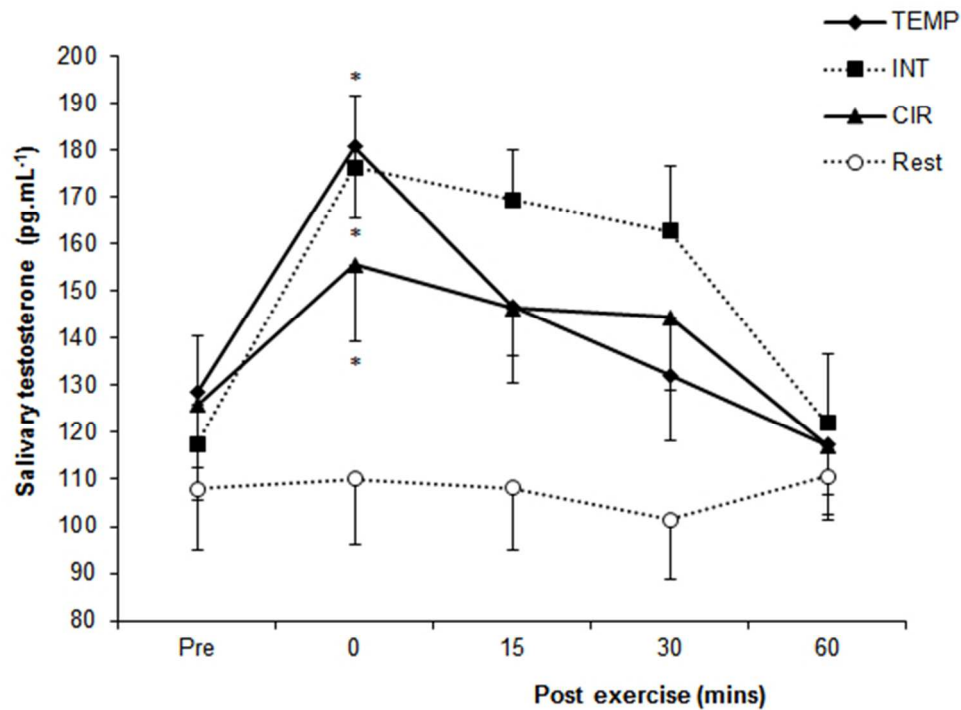


Fig 3. Salivary testosterone response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean \pm S.E.M) (n=10) *denotes significant difference from pre exercise value ($p < 0.05$)

146x108mm (96 x 96 DPI)

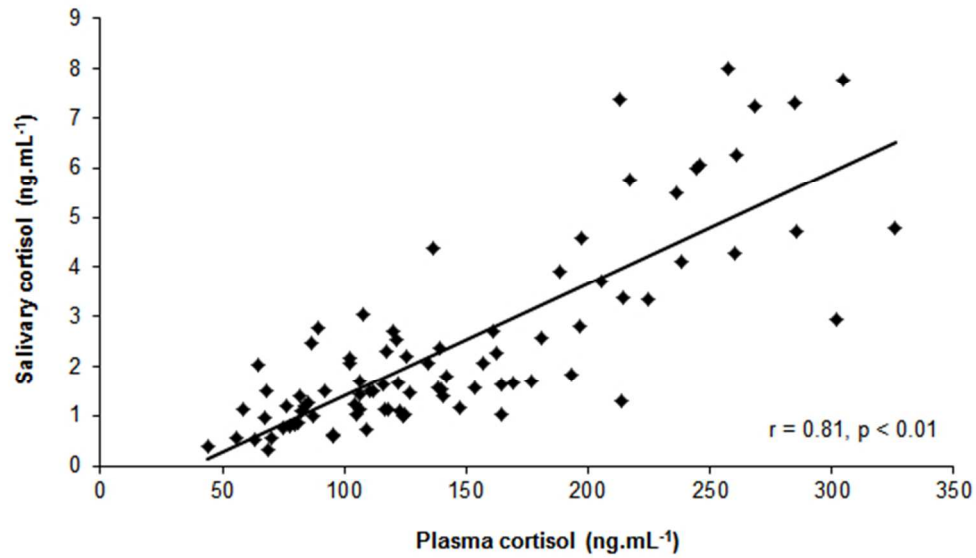


Fig. 4 Relationship between plasma and salivary cortisol concentrations from INT and TEMP sessions (n=87)
159x94mm (96 x 96 DPI)

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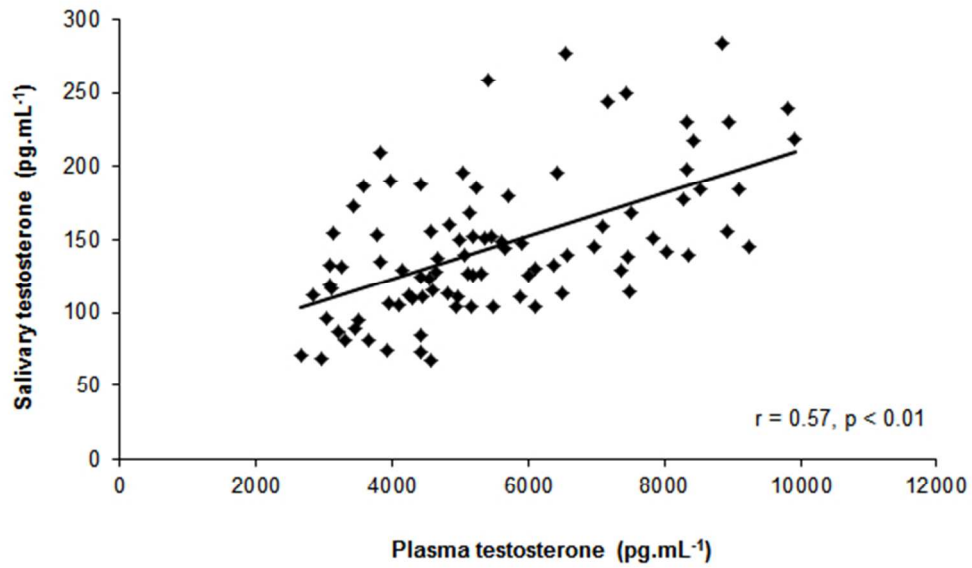


Fig. 5 Relationship between plasma and salivary testosterone concentrations from INT and TEMP sessions
(n=91)
157x95mm (96 x 96 DPI)