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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Running title

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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

biochemical pathways. Cortisol plays a role in stimulation of gluconeogenesis and mobilisation of free fatty acids to initiate glucose maintenance (Salway, 2006), and this is particularly important in response to exercise. Furthermore, acute stress has also been shown to increase circulating levels of testosterone (Sutton, Coleman, Casey, & Lazarus, 1973). Proposed mechanisms for release include lactate stimulated secretion (Farrell, Garthwaite, & Gustafson, 1983; Lin, Wang, Wang, & Wang, 2001; Lu et al., 1997; Port, 1991) and an increase in circulating catecholamines (Chrousos, 1998; Jezova & Vigas, 1981). However, the glucocorticoid and catecholamine responses to stress appear to interact in complex and opposing ways (Komesaroff & Funder, 1994).

Many studies have investigated the effect of acute continuous exercise on cortisol and testosterone levels; with most reporting increases post-exercise (Allgrove, Gomes, Hough, & Gleeson, 2008; Budde et al., 2010; Jacks, Sowash, Anning, McGloughlin, & Andres, 2002; Kokalas, Tsalis, Tsigilis, & Mougios, 2004; O'Connor & Corrigan, 1987; Rudolph & McAuley, 1998). An increase in cortisol has also been observed after intermittent exercise (Dimitriou, Sharp, & Doherty, 2002; Hough, Papacosta, Wraith, & Gleeson, 2011; Vuorimaa, Ahotupa, Hakkinen, & Vasankari, 2008). However, some studies have shown no change in cortisol levels (Eliakim et al., 2009; Moreira, Arsati, de Oliveira Lima Arsati, da Silva, & de Araujo, 2009), suggesting athletes may become accustomed a certain type and intensity of exercise and require extra stress to elicit a hormone response (Vuorimaa et al., 2008). A threshold of exercise at 60% VO_{2max} for >20 minutes has been proposed to elicit an increase in cortisol levels; however, this is contentious, with observation that exercise above 60% VO_{2max} for 30 minutes failed to elicit an increase in cortisol levels (VanBruggen, Hackney, McMurray, & Ondrak, 2011) and an increase seen after shorter duration exercise, such as a 30 second Wingate test (Crewther, Lowe, Ingram, & Weatherby, 2010). However, is it very difficult to compare studies, given the range of sample timing and examination of different exercise intensities and modes of exercise, consequently negating any large general meta-analyses.

Much research has focused on the hormonal response to resistance training, particularly protocols to increase strength and hypertrophy. The protocol design, including intensity and volume of training, appears to underpin the hormonal response (Crewther, Keogh, Cronin, & Cook, 2006). Schemes designed to induce hypertrophy have been shown to result in a larger increase in cortisol and testosterone than those designed to elicit neural or strength adaptations (Hakkinen & Pakarinen, 1993; Kraemer et al., 1991; Kraemer et al., 1990; Linnamo, Pakarinen, Komi, Kraemer, & Hakkinen, 2005; McCaulley et al., 2009; Raastad, Bjoro, & Hallen, 2000; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003). The intensity of the exercise must be sufficient to elicit a significant hormonal response (Beaven, Gill, & Cook, 2008b; Cadore et al., 2009; Fry & Lohnes, 2010; Smilios et al., 2003) and training status may also be important (Ahtiainen, Pakarinen, Kraemer, & Hakkinen, 2004; Kraemer et al., 1999). However, despite the number of studies concerned with resistance weight training protocols; to date, no studies have examined the response to bodyweight-only circuit training; a common training method devised in the 1960's that is employed by endurance athletes (Morgan & Adamson, 1965).

Previous research has established that many hormones can be measured from both blood and saliva, with strong correlations between salivary and total blood measures of cortisol (r=0.81 to 0.86) and weak to moderate correlations for testosterone (r=0.57 to 0.87) (Aardal & Holm, 1995; Crewther et al., 2010; O'Connor & Corrigan, 1987; Vittek, L'Homedeiu, Gordon, Rappaport, & Southren, 1985). Acute levels of free cortisol in saliva have been shown to be 70% of the free cortisol in the serum, due to a relative abundance of the cortisol-metabolising enzyme 11-β hydroxysteroid dehydrogenase in the salivary gland (Kirschbaum & Hellhammer, 2000; Obmiński, 1998). Serum and salivary cortisol have also shown a stronger correlation at lower serum levels (Aardal & Holm, 1995; Obmiński & Stupnicki, 1997; VanBruggen et al., 2011). It is suggested that an exponential relationship may be appropriate, as the free cortisol levels increase more rapidly once the binding capacity of

cortisol binding globulin (CBG) is exceeded (Gozansky, Lynn, Laudenslage, & Kohurt, 2005; Port, 1991).

Furthermore, studies examining the hormonal response to exercise have reported delays of five to 30 minutes for peak cortisol and testosterone levels in saliva after exercise (Crewther, Cronin, Keogh, & Cook, 2008; Crewther et al., 2010; Hough et al., 2011; O'Connor & Corrigan, 1987; VanBruggen et al., 2011). It is important to realise that measurement of hormones in saliva is complex, individual and situation dependent; therefore, clarification of optimum post-exercise sampling time can be difficult. However, there is evidence that salivary measures are a more sensitive marker of the hormonal response to acute exercise than blood (Crewther et al., 2010; Gozansky et al., 2005; Obmiński & Stupnicki, 1997) as salivary measures are often indicative of the free or 'biologically active' biomarkers as they diffuse from the blood into the oral cavity and are not bound to albumin (Humphrey & Williamson, 2001).

The aims of the present study were; firstly, to examine the salivary cortisol and testosterone response to three different training sessions in runners, notably the effect of bodyweight circuit exercise, chosen as they are common sessions undertaken by endurance athletes. Secondly, to investigate the correlation between blood and salivary hormone measures and timing of post-exercise peak hormone levels in both media.

Methods

Participants

Ten healthy male runners participated in the study. All competed regularly in running, triathlon and ironman competitions and trained 4-8 times per week. The main participant characteristics are presented in table 1. The study was approved by the University of

Greenwich ethics committee and participants received written and verbal instructions and gave their written informed consent.

Procedures

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

Preliminary measures

On the first visit participants provided a fingertip fingertip capillary blood sample for measurement of blood lactate before they undertook an incremental maximal oxygen uptake (VO_{2max}) test on a pre-programmed treadmill (Woodway ELG55, Weil am Rhein, Germany). In order to establish each participant's lactate threshold, during the VO_{2max} test an interrupted incremental speed protocol was employed similar to that described by others (Vuorimaa et al., 2008). Following a five minute warm up (5-10 $km.hr^{-1}$), participants commenced running at 2 $km.hr^{-1}$ below predicted 10 mile pace. Each stage was two minutes in duration and the treadmill speed increased 1 $km.hr^{-1}$ per stage and incline remained 1% throughout. After each stage participants stopped for 45 seconds for a fingertip capillary blood sample to be collected. Expired gas was analysed with a calibrated automatic gas analyser to determine oxygen consumption (VO_2). Heart rate (HR) was measured with a Polar HR monitor (Polar Electro Oy, Kempele, Finland) and rating of perceived exertion (RPE) on a 6-20 Borg scale (Borg, 1982), both recorded in the final 15 seconds of each stage, participants continued running until volitional exhaustion.

Blood lactate measures were analysed with a BioSen C line machine (EFK Diagnostic, Barleben, Germany). Lactate threshold was deemed to be 1 mmol.L^{-1} above the resting value (Yoshida, Chida, Ichioka, & Suda, 1987). Speed and percentage VO_{2max} at lactate threshold were then calculated.

Main trials

On visits 2-5, participants undertook three main training protocols and a rest trial, in a randomised order, separated by at least three days. During all trials heart rate was recorded every 1.5 minutes and RPE every three minutes during the exercise trials. The trials were as follows:

A. Circuit training consisting of three sets of ten exercises with 30 minutes total session duration. The exercises were: sit ups, press ups, squat jumps, back raises, burpees, plank, bicycle exercise, stationary running, tricep dips and step ups. Exercises did not involve any external weights and were performed on a mat where necessary or while standing on the floor. Tricep dips were performed on a box (30 cm) and the step ups on a bench (35 cm). Each exercise was performed for 30 seconds with 30 seconds recovery between exercises and began with sit-ups. Participants were told to perform as many repetitions as possible in each 30 second period.

B A tempo run performed for 30 minutes at a constant speed which coincided with lactate threshold established during the VO_{2max} test.

C An interval session (31 minutes), consisting of six intervals of 3.5 minutes duration at the treadmill speed equivalent to 90% VO_{2max}, interspersed by recovery periods of two minutes duration at the speed equivalent to 30% VO_{2max}. Mean heart rate was adjusted for the proportion of time spent during recovery (32.3%) and repetitions (67.7%).

D Participants sat and rested for the 30 minutes duration of this trial.

Saliva collection and analysis

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

Blood collection and analysis

Prior to the interval session, tempo run and rest trial participants were fitted with a cannula in the forearm (21G Venflon, Becton, Dickinson and Co., Oxford, United Kingdom). During and after the circuit session all blood samples were taken by venepuncture (21G BD Vacutainer Safety-Lok blood collection set; Becton, Dickinson and Co.) from an antecubital vein as there was a risk the cannula could be dislodged during the activities. Blood samples were collected into 6 mL tripotassium ethylenediaminetetraacetic acid (K_3EDTA) Vacutainers (Becton, Dickinson and Co.), pre-exercise and 0, 15, 30 and 60 minutes post-

exercise. Blood samples were refrigerated at 4°C until the end of each trial (for no longer than two hours). Samples were identified as being stable for up to four hours at 4°C prior to centrifugation and freezing (Tuck et al., 2008). After each trial blood samples were centrifuged at 1,500g for 10 minutes, and the plasma was divided into aliquots and stored at -80 °C until analysis. Plasma cortisol and testosterone concentrations were determined using commercially available ELISA kits (DRG Instruments, Germany). The sensitivity of the kits was 2.5 $ng.mL^{-1}$ (plasma cortisol) and 0.083 $ng.mL^{-1}$ (plasma testosterone). The mean intra assay coefficients of variation were 9.3% amd 6.1% for cortisol testosterone, respectively. The mean inter assay coefficients of variation were 6.2% and 7.5% for cortisol and testosterone, respectively.

Statistical analysis

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

Results

Trial characteristics

The characteristics in terms of running speed, percentage VO_{2max} 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% ± 220% after INT, 106 ± 156% after TEMP and 82 ± 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% ± 39% after INT, 63 ± 40% after TEMP and 30 ± 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 ± 68.0 $ng.mL^{-1}$ vs. 2.41 ± 1.89 $ng.mL^{-1}$ respectively). This trend was mirrored in salivary (145.7 ± 48.1 $pg.mL^{-1}$) and plasma (5518.9 ±1873.0 $pg.mL^{-1}$) 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 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

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

Discussion

The aims of the present study were firstly, to investigate the salivary cortisol and testosterone response to three different training protocols in runners, particularly a bodyweight only circuit session and secondly, to assess the correlation of plasma and salivary hormone measures and timing of post-exercise peak hormone concentrations.

A significant increase in salivary cortisol concentration was observed after the INT session compared to rest. Participants reported the INT session to be more strenuous overall with a significantly higher mean RPE and the intermittent nature of the INT trial, with periods at 90% VO_{2max}, may have contributed to the higher perceived exertion. However, the INT and TEMP trials showed no difference in mean maximum HR (%), but a significant weak to moderate correlation was revealed between mean maximum HR (%) and mean RPE ($r=0.52$), suggesting HR (%) was linked with perceived exertion. The prolonged increase in salivary cortisol levels seen after INT, are supported by other researchers (Elloumi, Maso, Michaux, Robert, & Lac, 2003; Hough et al., 2011) and may reflect the high metabolic demand of the trial, which in turn led to activation of the HPA axis and an increase in cortisol secretion. There is evidence that cortisol levels are correlated with blood lactate (Farrell et al., 1983; Port, 1991) and suggestion that lactate may activate chemoreceptors in the working muscle and stimulate the HPA axis (Farrell et al., 1983). Although highly speculative, given the lack of blood lactate measurements in the study, the periods of exercise above 90% VO_{2max} during INT may have contributed to a higher level of blood lactate accumulation than TEMP or CIR. In all exercise trials there was a significant increase in peak post-exercise salivary cortisol, compared to pre-exercise values, with the largest increase seen after INT; this suggests all trials were of sufficient intensity to elicit a hormonal response. These findings reflect those reported by another group who examined

athletes completing a 40 minute tempo run at 80% velocity of VO_{2max} (vVO_{2max}) and a 40 minute repetition session, which consisted of two minutes run ($100\%vVO_{2max}$) and two minutes recovery (slow walk) (Vuorimaa et al., 2008). Both trials showed a significant increase in serum cortisol concentration post-exercise. However, the authors postulated that if the total work output of tempo and intervals runs were equated, the serum cortisol response to the interval session may have been greater than tempo; therefore, the closely matched work outputs of TEMP and INT in the present study reflect this suggestion.

There was an increase in salivary testosterone post-exercise in both running based exercise trials, with post-exercise peak testosterone over 50% higher than pre-exercise. These findings are supported by studies in runners (Vuorimaa et al., 2008), cyclists (Hough et al., 2011) and rowers (Kokalas et al., 2004). Possible mechanisms for this change include increased production of testosterone by sympathetic stimulation of the testes (Fahrner & Hackney, 1998). Furthermore, activation of the sympathetic nervous system and increased lactate accumulation may have contributed to the increase in testosterone concentration, although supporting evidence is limited to rats (Lu et al., 1997). There is speculation that protein binding affinity can be affected by changes in pH and temperature elicited by exercise, this in turn may lead to a higher free proportion of cortisol and testosterone in the blood and increased levels in saliva (Obminski & Stupnicki, 1996; Rosner, 1990). However, a more recent study showed no binding affinity changes after endurance exercise (Fahrner & Hackney, 1998) and further research is required to support this mechanism. Given evidence of reduced blood flow to the liver during exercise (Rowell, Blackmon, & Bruce, 1964); reduced hepatic clearance of testosterone is another possible reason for the increase, rather than a higher secretion rate (Cadoux-Hudson, Few, & Imms, 1985; Sutton et al., 1973). Dissimilarly to cortisol, salivary testosterone returned to baseline 60 minutes post-exercise in all trials, which suggests different mechanisms of release or clearance of these hormones occurring in response to exercise stress (Jessop, 1999).

In the present study, novelty exists with investigation of the acute cortisol and testosterone response to bodyweight-only circuit training. There was a significant increase in post-exercise peak salivary cortisol (82%) and testosterone (31%) in response to CIR. The presence of a concomitant increase in cortisol and testosterone mimics the hormonal response to hypertrophy based resistance training protocols, with those designed to increase strength showing little or no change (Crewther et al., 2008; Hakkinen & Pakarinen, 1993; Linnamo et al., 2005; McCaulley et al., 2009; Smilios et al., 2003). It has also been suggested that the absolute workload is important to elicit an increase in cortisol and testosterone, but the design of the session including recovery time should also be considered (Cadore et al., 2009; Hickson, Hidaka, Foster, Falduto, & Chatterton, 1994; Kraemer et al., 1993; Kraemer et al., 1991; Smilios et al., 2003). The increase in cortisol and testosterone is thought to represent a catabolic and anabolic state, which is essential to initiate an increase in muscle growth (Crewther et al., 2006).

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

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

However, despite the similar response to hypertrophy resistance schemes, the bodyweight-only protocol undertaken in this study is unlikely to have elicited large strength or hypertrophy gains and with regular training the most likely outcome would be small increases in muscular strength and vVO2max (Taipale et al., 2010).

When considering the role of testosterone changes, most studies have found that increased levels do not directly cause muscle hypertrophy (West et al., 2010a; Wilkinson, Tarnopolsky, Grant, Correia, & Phillips, 2006a), in contrast to the response seen after exogenous administration (Bhasin et al., 1996). It has also been suggested that an increase in testosterone could be a result of decreased muscle utilisation (Kraemer et al., 1990) or, reduced hepatic clearance. Furthermore, research has postulated that testosterone may play a permissive role in physiological adaptations to resistance training (Crewther, Cook, Cardinale, Weatherby, & Lowe, 2011; Viru & Viru, 2005). Currently, the current biological roles of exercise induced hormone changes remain somewhat uncertain (West & Phillips, 2012). Recent studies have suggested that testosterone may contribute to behavioural adaptations driving greater voluntary effort; exemplified in a study linking pre-exercise salivary testosterone levels with self selected resistance training workloads and performance in female netball players (Cook & Beaven, 2013). Given the element of self-selected effort during circuit training, it would be interesting to examine this concept further in future research, especially given evidence that non-physical psychological priming, such as watching a video can positively influence pre-exercise testosterone levels and subsequent voluntary exercise performance (Cook & Crewther, 2012). The current consensus is that the adaptive response to strength training is likely to be multi-faceted, with several acute training factors (one of them hormonal), rather than a single factor. However, given the lack of data, further investigation is required to examine the hormonal response to bodyweight-only circuit training and concurrent physiological adaptations and mechanisms.

The second aim of the study was to compare salivary and plasma hormone levels and for pooled samples, plasma and salivary cortisol showed a moderate positive correlation ($r>0.80$). Similar correlations have been demonstrated in other studies at rest (Aardal & Holm, 1995), after 30 minutes of cycling at 75% VO_{2max} (O'Connor & Corrigan, 1987) and a Wingate test (Crewther et al., 2010). Furthermore, comparison within individuals revealed a correlation was very similar to the pooled data ($r>0.80$). Previous studies have shown a stronger correlation with saliva at serum levels below the CBG limit (approximately 400 nmol/L) than above (Aardal & Holm, 1995; Obmiński & Stupnicki, 1997; VanBruggen et al., 2011). However, the present study disagrees with these findings; as a stronger correlation was revealed at plasma cortisol levels above 400 nmol/L (145 ng/mL). Furthermore, an exponential relationship showed the same r value as linear regression; therefore, linear correlation appears suitable for the comparison of salivary and plasma cortisol levels.

Plasma and salivary testosterone levels showed a weak to moderate correlation between individuals ($r>0.55$) with a large inter individual variation, similar to other published studies (Crewther et al., 2010; Vittek et al., 1985). However, others have shown no correlation between salivary and serum testosterone pre and post resistance exercise (Cadore et al., 2008). As previously discussed, it is important to realise that measurement of hormones in saliva is individual and situation dependent and therefore a variation in response is likely. Results from the present study confirm the validity of using salivary measures to monitor the cortisol response to exercise; however, the weaker correlations observed between saliva and total plasma testosterone should be approached with caution with further validation warranted.

Additionally, plasma and salivary cortisol and testosterone levels peaked simultaneously in all trials, with the exception of a slightly later plasma cortisol peak after TEMP. A post-exercise delay of up to 20 minutes between peak cortisol and testosterone levels in saliva compared to blood has previously been reported at rest (Kirschbaum & Hellhammer, 1989)

and after exercise (Hough et al., 2011; O'Connor & Corrigan, 1987). The present results reflect reports observing an immediate diffusion from blood into saliva after intravenous injection (Kirschbaum & Hellhammer, 2000; Wang, Plymate, Nieschlag, & Paulsen, 1981). The lack of lag time in the current trial may reflect the complex relationship between salivary and blood hormone levels. Furthermore, unlike previous studies, the present study used stimulated saliva sampling, therefore hormones levels in saliva are unlikely to have been affected by changes in salivary volume in response to exercise, caused by vasoconstriction of the arterioles in response to sympathetic stimulation (Chicharro, Lucia, Perez, Vaquero, & Urena, 1998).

Overall, peak salivary cortisol and testosterone levels occurred immediately post-exercise for TEMP and CIR; however, after INT salivary cortisol peaked at ~15 minutes post-exercise. However, there was variation between individuals in peak time for INT and CIR. For these trials there was a large inter-individual difference for post-exercise hormone peaks (ranging from immediately after to ~30 minutes post-exercise). The individual differences may have been caused by the intermittent exercise and complex nature of hormone measurement. Studies examining the time for hormones to peak post-exercise, have shown peak testosterone occurs earlier than cortisol, within 10 minutes of cessation of exercise (Hough *et al., 2*011; Daly *et al., 2*005); however, the present study did not show this trend. Given the variation between individuals after intermittent exercise, post-exercise peak values may be a better indicator of the hormonal response than the mean peak response.

Conclusion

In conclusion, results suggest that in typical training sessions undertaken by endurance trained athletes, cortisol concentration may be an indicator of acute exercise stress. A bodyweight-only aerobic circuit session elicited an increase in cortisol and testosterone levels and further investigation is required to establish the mechanisms for this response. Salivary and plasma cortisol levels were correlated in response to acute exercise; and this

supports the use of saliva as an alternative sampling technique to blood for measurement of cortisol; however, weaker correlations for testosterone require further investigation. Finally, the results revealed evidence of no delay in hormone tracking between plasma and saliva and importance should be given to the highly complex, individual and situational nature of the hormone response to acute exercise.

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Tables

Table 1 Descriptive characteristics of participants

	Mean (± SD)
Age (y)	39.3 ± 6.6
Body mass (kg)	76.6 ± 8.7
Height (m)	1.78 ± 0.06
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	59.2 ± 5.9
Maximum heart rate (bpm)	180 ± 11

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

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

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

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

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

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

VO_2 – volume of oxygen uptake

VO_{2max}- maximal oxygen uptake

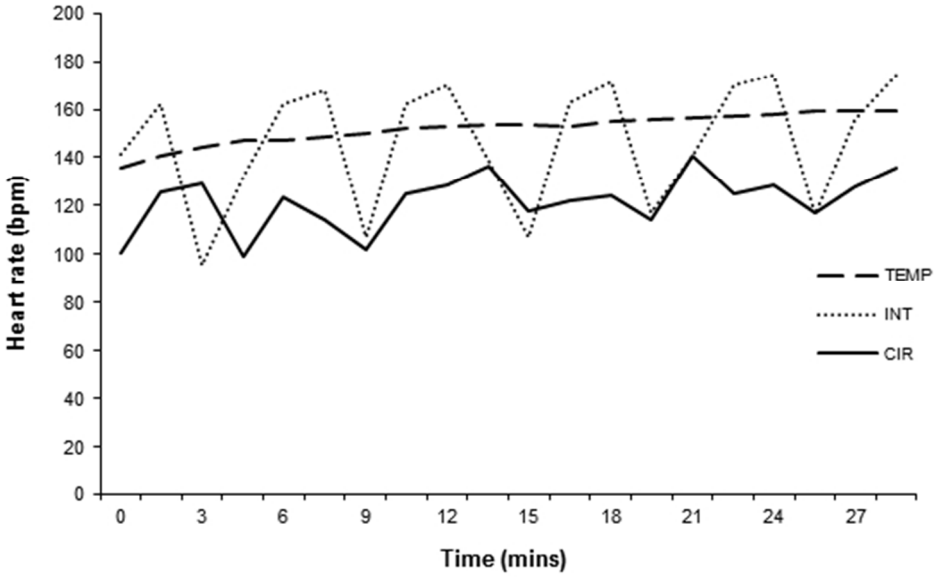

Fig. 1 Mean HR during INT (dotted line), TEMP (dashed line) and CIR (solid line) trials (n=8)
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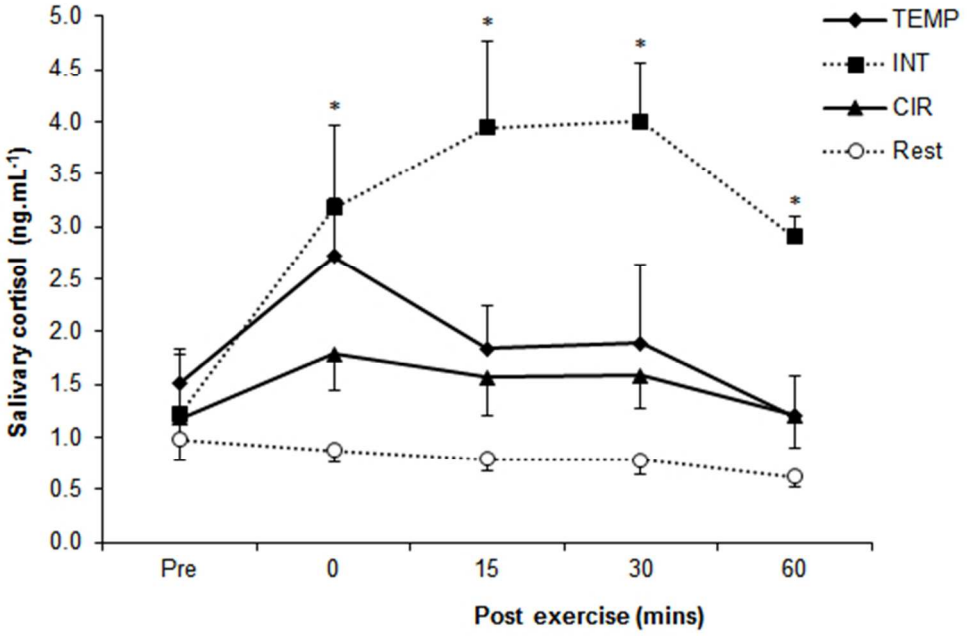

Fig. 2 Salivary cortisol response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean +/- S.E.M) (n=10) *denotes significant difference from pre exercise value ($p<0.05$)
147x99mm (96 x 96 DPI)

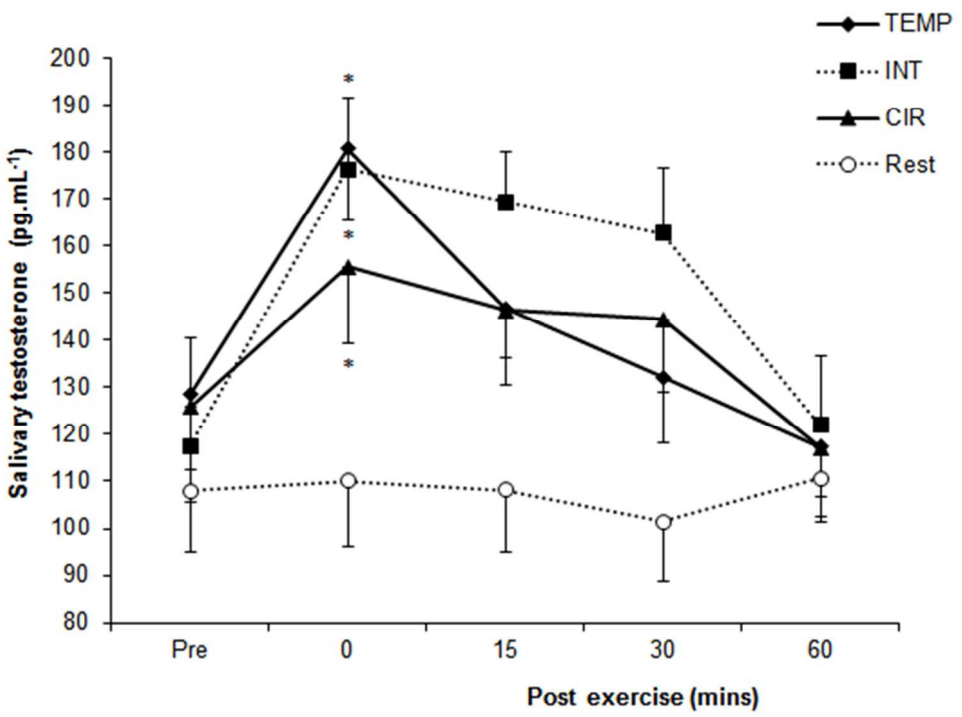

Fig 3. Salivary testosterone response to rest (open circles), TEMP (closed diamonds), INT (closed squares) and CIR (closed triangles) trials (mean +/- S.E.M) (n=10) *denotes significant difference from pre exercise value ($p<0.05$)
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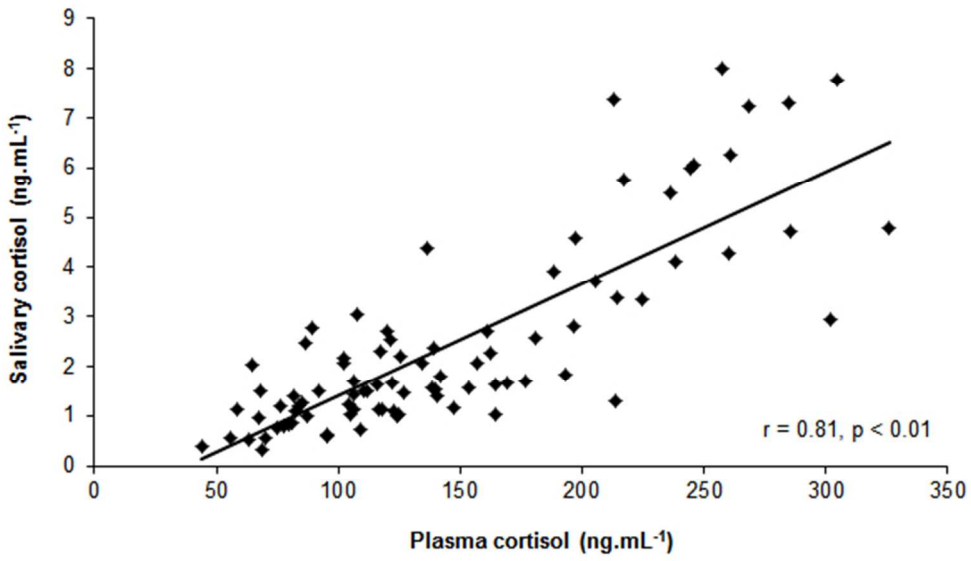

Fig. 4 Relationship between plasma and salivary cortisol concentrations from INT and TEMP sessions (n=87)
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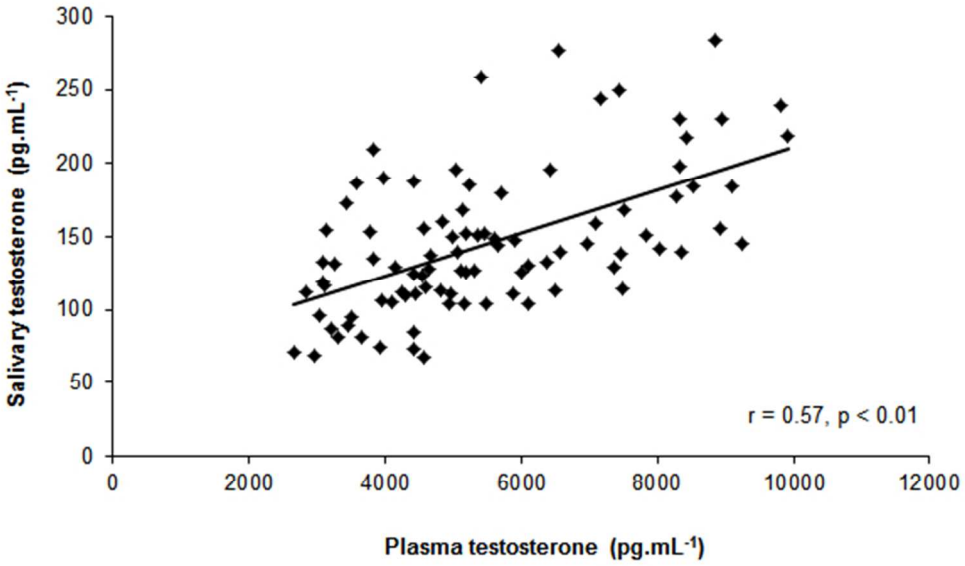

Fig. 5 Relationship between plasma and salivary testosterone concentrations from INT and TEMP sessions (n=91)
157x95mm (96 x 96 DPI)