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Title: Hormonal responses during two different concurrent-training trials in youth elite soccer players: does changing the organisation of training impact the hormonal response to concurrent exercise?

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Abstract

BACKGROUND: There are no data describing the acute hormonal responses to concurrent-training programmes in youth elite soccer players. Therefore, the aim of this study was to describe the total testosterone (T), cortisol (C), and growth hormone (hGH) responses during two same-day concurrent-training (CT) trials in elite soccer players. **METHODS:** n=13 youth elite players (age: 17.0 ± 0.2 yrs; height, 1.80 ± 0.07 m; body mass, 73.1 ± 5.7 kg; $\dot{V}O_2^{\max}$, 64.4 ± 4.8 ml \cdot kg $^{-1}$ \cdot min $^{-1}$) from an English premier league soccer club completed two CT trials. ‘Trial 1’ (CT1); E (10.30h) followed by S (14.00h) and Trial 2 (CT2); strength-training (S) 09.00h followed by a soccer-specific endurance-training session (E) at 10.30h. Venous blood samples were collected at 5 time-points around training and food intake (T1; 08.00h, T2; 09.45h, T3; 12.30h, T4; 13.45h and T5; 15.15h) and analysed for T (nmol/L) and C (nmol/L) and hGH (ug/L). **RESULTS:** There was no main effects found between exercise conditions for any hormones (T; P=0.22, C; P=0.07, hGH; P=0.21). Effect size analysis revealed a moderate effect for T at T3 (ES=0.63, CT1; 18.4 ± 3.8 , CT2; 15.7 ± 4.7 nmol/L-1). A moderate effect for T area under the curve (AUC) was observed between conditions (CT1; 300 ± 76 versus CT2; 244 ± 81 [AU]; ES=0.71). A moderate effect was apparent for C concentrations T4 in (ES=-0.95, CT1; 230 ± 69 , CT2; 314 ± 105 nmol/L-1). Moderate effect sizes were observed at T3 and T4 (ES=0.82, CT1; 1.28 ± 1.17 , CT2; 0.47 ± 0.75 , ES=0.72, CT1; 0.11 ± 0.05 , CT2; 0.07 ± 0.06 ug/L-1 respectively). A moderate effect for hGH AUC was observed between trials (CT1; 14 ± 11 versus CT2; 5 ± 9 ; [AU], ES=-1.08). **CONCLUSIONS:** The organisation of the concurrent-training protocols used in this study has a negligible impact upon the acute T, C and hGH in youth elite soccer players.

Keywords: Soccer, Endocrinology, Resistance-training, Endurance-training, Concurrent-training, Youth.

Introduction

The elite soccer training environment has been described as a dynamic and challenging setting for practitioners and coaches [1]. In this regard, the design and implementation of all aspects of training can be influenced/dictated by a variety of complex factors [2]. An obvious limiting factor is the intensive competition schedule, which restricts training time, subsequently limiting the coaches' ability to deliver all aspects of training across each micro-cycle with adequate recovery time. As a result, it is common for players to perform multiple diverse training sessions on the same day (e.g. strength-training and endurance-training) [3] a training arrangement traditionally referred to as 'concurrent-training' [4].

Testosterone (T), cortisol (C) and human, Growth hormone (hGH) are said to play an important role in the daily physiological function of humans [5]. For example; T has been shown to be indirectly involved in a number of short-term process which support healthy muscle development (e.g. regulation of lipid/protein, neural activity and energy metabolism) [6]. Cortisol has been shown to influence a number acute metabolic events during/following a training session (e.g. increasing blood glucose levels via gluconeogenesis) and to be associated with tissue remodeling [7]. Whereas, hGH has been shown to effect neural function, IGF-1 release, activation of calcium retention, and stimulation of hepatic gluconeogenesis (amongst others) (for review see; [8]). Subsequently, in a bit to describe the endocrine responses to different training methods researchers have measured various hormones during/following many training paradigms [9–16]

Although, at present there are few data describing the acute hormonal responses to concurrent-training in elite soccer players [17,18]. Moreover, no studies have investigated how elite players respond to the unique concurrent-training programmes used in the English professional soccer leagues [2,19]. Particularly, few data exist describing the impact of performing concurrent training in different exercise orders and with different recovery

periods between exercise bouts. Of the available studies, results suggest that when two diverse training bouts are performed on the same day, the sequence of aerobic and resistance-training and the recovery duration between training bouts can influence the response of T, C and hGH [11,12,15,16,20]. Although, as the endocrine response can be modulated by a variety of factors (e.g. gender, training status, exercise intensity and exercise mode) [21] and the participants in the above studies were both untrained and not familiar with concurrent-training per se, it is plausible to suggest that elite soccer players might exhibit different acute hormonal responses than those previously described. To the authors' knowledge, no studies have investigated the acute responses of T, C and hGH to the concurrent strength and endurance-training programmes used by youth elite soccer players in England. Therefore, the present study aimed to describe the hormonal response during two concurrent-training scenarios previously observed at a professional soccer club in England [2,19].

Materials and Methods

Participants.

Thirteen elite post-adolescent soccer players who competed in the under-18 English Premier League were recruited for this study (age: 17.0 ± 0.2 years; height, $1.80 \text{ m} \pm 0.07$ m; body mass, 73.1 ± 5.7 kg; $\dot{V}O_2^{\text{max}}$, $64.4 \pm 4.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). All participants had a minimum of 2 years strength-training history (full-time professional athletes) and were familiar with the present strength-training protocols. After receiving oral and written information concerning any possible risks associated with the training and testing protocols, all participants gave their written informed consent to participate in the study. As the participants were under 18 years of age additional consent of parents or those with legal responsibility for the individual was also gained. The study conformed to the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Institutional Review Board (IRB) at a University within the United Kingdom.

Overview of experimental procedures.

The participants attended the laboratory on four occasions (Monday/Thursday on two consecutive weeks). On day one and two, anthropometric, strength and aerobic capacity assessments were carried out. On days three and four participants completed one of two 'concurrent-training trials'; concurrent-training trial 1 (CT1) on day 3 and concurrent-training trial 2 (CT2) on day 4. During each trial, venous blood samples were collected from each participant before, between and following each exercise bout and subsequently analysed for growth hormone (hGH), testosterone (T) and cortisol (C). The participants in this study were well-trained athletes with a minimum of two years full-time professional training under the instruction of the same football/strength and conditioning coach. To mitigate any hormonal

responses being a result of unfamiliarity the participants completed alternating patterns of CT1 and CT2 in the training cycle leading up to this investigation.

Baseline testing (days 1 & 2).

One week prior to the first trial each participant reported to the laboratory and completed a 6-repetition maximum (6-RM) strength testing session. The testing session involved the same strength-training exercises used during the experimental trials; half-back squat, stiff leg dead lift, front lunge & the dead-lift exercise. Prior to testing the participants warmed up by performed each exercise above. The warm-up intensity was calculated using previous training data and consisted of 6-10 repetitions at and intensities of 50-90% of 6-RM [22]. This was followed by a series of maximal repetitions, separated by 3-5 minute rest periods. Each time the participant successfully completed the weight was increased by 2.5 or 5kg until the participant could not complete the exercise with correct technique. The maximum weight in kilograms lifted was recorded and used as the training load during experimental trials one and two. Strength assessments were carried out at 12.00h. On the second visit to the laboratory (3 days later) a direct assessment of the participants $\dot{V}O_2^{\max}$ and maximum heart rate was carried out using a modified 'Bruce test' for athletes [23]. Here, an incremental exercise protocol was performed on a motorised treadmill to volatile exhaustion. Throughout the test heart rate and gas exchange was sampled using a heart rate monitor (Polar, Kempele, Finland) and a MetaLyzer 3B-R3 (Cortex , Leipzig Germany) respectively. Following the test each participants $\dot{V}O_2^{\max}$ and maximum heart rate (HR^{\max}) was recorded. The maximal treadmill test was carried out between 10.00h and 14.00h.

Experimental trials 1 & 2 (days 3 and 4).

Four days later participants completed concurrent-training trial 1 (CT1); a soccer-specific endurance-training session at 10.30h and a strength-training session at 14.00h (see below for specific detail). Three days later the players completed CT2; a strength-training session at 9.00h followed by a soccer-specific endurance-training session at 10.30h. Throughout each day venous blood samples were collected on 5 occasions before and after exercise and nutrition. Controlled nutrition was provided before, between and after training in both trials. The times of training described above were decided following an observation of the concurrent-training pattern within this soccer club and have previously been studied across a 5-week training intervention [19].

Procedures

Training programmes.

We employed a within subject design whereby each athlete completed two concurrent-training sessions thus acting as their own control. On each trial the participants completed the same strength-training (S) and soccer-specific endurance-training (E) training sessions at the same exercise intensity on both observational days. Soccer-specific endurance-training stimuli 'E' consisted of a dynamic warm up (~20 minutes), small-sided games (~25 minutes) and technical / tactical work (~50 minutes). To allow the players to rehydrate regular intervals were also provided throughout each 'E' training session. Small-sided games involved a '4 v 4', possession format. Each game lasted 4 minutes and was performed at an intensity of ~85-95% HR^{max}. Between each game 3 min of active recovery was allocated. Games were performed on a 37m x 27m pitch (for more detail on this game format and reliability statistics please refer to (Little & Williams 2007)). During each 'E' training session the participants wore a global positioning system (GPS) device (StatSports, Ireland) and a heart rate monitor

(Polar, Kempele, Finland). Acute training data recorded during each experimental trial is presented in table 1. The mean duration of the entire session 'E' was 90 min and players' average perceived exertion score using 'Borg's 1-10 rating of perceived exertion scale' was 6 ± 1 for the 'E' component of training there were no statistical differences in objective or subjective 'training load' indices between trials. The 'S' training session consisted of 4 sets of 6 maximal repetitions (85% of 1RM) of the following exercises: parallel back squat, dead-lift, stiff-leg dead-lift and front-lunge. Participants also completed 3 sets of 8 repetitions of the Nordic hamstring exercise. Training compliance and individual workout data (weight lifted, number of sets and repetitions completed), was recorded for each participant for all sessions. The mean duration of S was 40 min and players' average perceived exertion score was 8 ± 1 . The 'S' component of training was identical between trials and therefore there were no statistical differences in objective or subjective 'training load' indices between conditions (table 1).

INSERT TABLE 1 HERE

Blood sampling.

Venous blood samples were collected on five occasions by a qualified phlebotomist during each experimental trial (8.00-8.15h, 9.45-10.00h, 12.30-12.45h, 13.45-14.00h and 15.15-15.30h) (Figure 1). After each sample was collected, the blood was maintained in ambient temperature for 45 min and then centrifuged for 10 min at 4.000 rpm at 4 degrees. Following separation, serum was removed and frozen at -70°C for later analysis. Each blood sample was subsequently analysed for concentrations of serum growth hormone (hGH) ($\mu\text{g/L}$), total testosterone (T) (nmol/L) and cortisol (C) (nmol/L). Testosterone and Cortisol were measured using Cobas 6000 analyser series radioimmunoassay kits (Hoffmann-La Roche Ltd, Switzerland). The 22 kD monomer of human growth hormone (hGH) was measured using the

Access 2 immunoassay System, (Beckman Coulter, USA). To eliminate inter-assay variance, all samples were analysed within the same assay batch, and all intra-assay variances were \leq 5.0%. Antibody sensitivities were; 0.7 nmol/L for T, 1.4 nmol/L for C and 0.07 ug/L for hGH.

INSERT FIGURE 1 HERE

Dietary Controls.

A standardised breakfast was consumed at 0745 and 0900 h for CT2 and CT1 respectively (~540 Calories: 100 g carbohydrate, 15 g protein and 7 g fat). All players also consumed a standardised lunch at 1230 h (~1000 calories 140 g carbohydrate, 60 g protein and 25 g fat). Finally, all players consumed a standardised recovery shake on completion of strength-training (~220 calories: 25 g whey protein, 13 g CHO, 0.5 g fat, Multipower, UK).

Statistical Analysis.

The software package SPSS (Version 17.0 SPSS inc. Chicago, IL) was used for statistical analysis. After normality (i.e Shapiro Wilk) and variance assurance (i.e. Levene) comparisons between different exercise orders were assessed using a two-way analysis of variance (ANOVA) with repeated measures (time v exercise condition). For each hormone the area under the curve (AUC) was calculated using a trapezoidal method and subsequently analysed using a paired t-test. Effect sizes were calculated for each time point between and within condition using methods previously described by Hopkins [24] with values of <0.2, 0.2 to 0.6, 0.6 to 1.2, 1.2 to 2.0 and >2.0 considered to represent very small, small, medium, large and very large effects, respectively. All data in text, figures are presented as means \pm SD. Statistical significance (P) was set at \leq 0.05).

Results

Total Testosterone.

Between trial comparisons: When compared between trials there was no main effects observed between trials ($P=0.22$). When compared between trials a moderate effect was observed at time-point 3 '12.30h', ($ES=0.63$). Small and very small effect sizes were observed at other time points (T1; 08.00h, $ES=-0.26$, T2; 09.45h, $ES=-0.05$, T4, 13.45h, $ES=0.09$, T5; 15.15, $ES=0.10$). A moderate effect for testosterone AUC was observed between conditions (CT1; 300 ± 76 versus CT2; 244 ± 81 ; $ES=0.71$). In CT1, testosterone did not increase from before training to post training in either exercise mode (S; $ES=0.04$, E; $ES=-0.11$). There was a reduction in testosterone (large effect) from time-point 3 (12.30h) to time-point 4 (13.45h) in CT1 ($P = 0.01$; $ES=1.34$). In CT2, a reduction in testosterone was observed from time-point 2 (09.45h) to time-point 3 (12.30h) and from time-point 3 (12.30h) to time-point 4 (13.45h). Each reduction was classified as a small ($ES=0.55$) and moderate effect size ($ES=0.62$) respectively. No change in testosterone was observed from time-point 4 (13.45h) to time-point 5 (15.15h) (i.e. pre to post S) ($ES=0.16$).

INSERT FIGURE 2 NEAR HERE

Cortisol.

Between trial comparisons: No main effect was apparent between trials ($P=0.07$). A moderate effect size between conditions was apparent at time point 4 ($ES=-0.95$). Small and Trivial effects were observed at all other time-point between conditions (T1; 08.00h, $ES=-0.50$, T2; 09.45h, $ES=0.11$, T3, 12.30h, $ES=0.08$, T5; 15.15, $ES=-0.07$). When the AUC was compared between trials there was no differences apparent (small effect). (CT1; 4913 ± 1228 versus CT2; 5382 ± 1449 ; $ES=-0.35$). In CT1, a very large effect (reduction) in cortisol was observed from time-point 1 (08.00h) to time-point 2 (09.45h) ($ES=2.17$) from time-point 3

(12.30h) to time-point 4 (13.45h) (large effect) (ES=1.24) and from time-point 4 (13.45h) to time-point 5 (15.15h) (large effect) (ES=1.14). Cortisol did not change following E (i.e. time-point 3 to time-point 4) (ES=-0.35). In the CT2 trial, cortisol reduced from time-point 1 (08.00h) to time-point 2 (09.45h) (large effect) (ES=1.9). Cortisol also decreased (very large effect) from time-point 4 (13.45) to time-point 5 (15.15h) (ES=2.10).

Human Growth Hormone (hGH).

Between trial comparisons: When compared between conditions there were no statistical differences (main effects) in growth hormone (P=0.21). Moderate effect sizes were observed at both time-point 3 and 4 (ES=0.82, ES=0.72 respectively). A moderate effect for growth hormone AUC was observed between trials (CT1; 14 ± 11 versus CT2; 5 ± 9 ; ES=-1.08). In CT1, a reduction in growth hormone was observed from time-point 3 (12.30h) to time-point 4 (13.45h) (large effect) (ES=1.38). An increase in growth hormone was observed from time-point 4 (13.45h) to time-point 5 (15.15h) approaching significance (moderate effect size) (ES=-0.86). No other differences were observed in growth hormone in CT1. In CT2 growth hormone increased (large effect size) from time-point 4 (13.45h) to time-point 5 (15.15h) (ES=-1.08). An increase in growth hormone was observed from time-point 1 (08.00h) to time-point 2 (09.45h) approaching significance (moderate effect size) (ES=-0.77).

Discussion

We aimed to describe and compare the hormonal responses to two same-day concurrent-training scenarios previously observed at a professional soccer club. To the best of our knowledge, this is the first study that has described the hormonal responses to concurrent-training programmes typically seen in professional soccer clubs. There were no main effects observed between conditions, and in general, the hormonal responses observed in this study similar to a typical diurnal response (particularly T and C) in the absence of exercise. Therefore, our data suggest that the organisation of the concurrent-training protocols used in this study has a negligible impact upon the acute T, C and hGH in youth elite soccer players. It is thought that the small between group differences observed (effect sizes), were transient and therefore, unlikely to play a key role in regulating indirect physiological processes associated with acute and chronic training responses. Therefore, the organisation of concurrent-training may not influence metabolic processes which support muscle adaptation beyond the typical diurnal fluctuations previously seen in healthy males.

An acute increase in T concentration immediately following an isolated bout of exercise is a typical observation [25], however, in the present study T did not increase immediately following endurance or strength-training in either training trial. In fact, a moderate reduction in T following endurance-training in CT2 (i.e. time-point 2 to time-point 3; 09.45h - 12.30h), was observed (ES=0.55). A reduction in T from time-point 3 to time-point 4 (12.30h - 13.45h) was also observed in both concurrent training scenarios (CT1; ES=1.34, large decrease, CT2; ES=0.62, moderate decrease) but no statistical between group differences were found. Our findings therefore, suggest that the exercise protocols used in the present study do not influence the hormonal response out with the normal diurnal fluctuations typically seen in healthy males [26]. Therefore, the organisation of concurrent-training bouts may not be an

important 'training variable' that modulates testosterone secretion in well trained youth elite soccer players.

The steroid, cortisol, has previously been shown to acutely increase in response to both psychological and physiological stress [27,28], although was not apparent in the present study. Again, we observed a pattern similar to that of the normal typical diurnal pattern for cortisol [29], where cortisol was initially high at the first sample point (08.00h ~450-480 nmol/L⁻¹) and then decreasing throughout the day. As such, large, and very large effect sizes (reductions) in cortisol occurred regardless of the organisation of concurrent-training (i.e. CT1 or CT2). Our observation is similar to that of Horne, [30] and Rosa et al., [11,12] who have observed a normal diurnal pattern for cortisol when participating in strength and endurance exercise in healthy participants. In the present study, when cortisol was compared between trials there was only a moderate effect was observed at time-point 4 (ES=-0.95). Here, cortisol appeared to remain elevated from time-point 3 to time-point 4 in CT2 (i.e. when resistance-training was performed before endurance-training). This response could be expected following the completion of a large physical stressor such as is associated with the consecutive exercise bouts. Although, it is acknowledged that less is clear about cortisol's direct role in substrate utilisation in the present study with respect to acute muscle performance. Therefore, future studies could investigate the effects of multiple training bouts on the same day and their impact upon cortisol, muscle performance.

It is well documented that a single bout of high-intensity resistance exercise will result in an increase in serum hGH concentration [31]. In the present study we also observed increased hGH following training. Changes were classified as 'moderate effect sizes' (CT1; ES=-0.86 moderate increase, CT2; ES=-0.77, moderate increase). When compared between conditions hGH increased from time-point 2 to 3 in CT1 whereas in CT2 hGH decreased

across the same time-points. The attenuation of hGH in the CT2 condition from time-point 2 to time-point 3 could potentially be in part explained by the organisation of training and nutrition (i.e. the sequence of exercise, the recovery period). Other studies have reported an attenuation in growth hormone when multiple exercise bouts are performed within close proximity of each other [32–36]. Therefore, the relatively short recovery period between training bouts in the ‘CT2’ trial (~60 min vs. ~105 mins) could have influenced the response of hGH. It is thought that the increased demand for energy substrates and enhanced rate of lipolysis during prolonged exercise periods are thought to inhibit hGH secretion [35]. This is somewhat supported by the fact that hGH was not attenuated in the CT1 condition at the same time-point (i.e. when training was separated and a meal was consumed between training bouts). Collectively, suggesting, that the ingestion of carbohydrate between training bouts may be an important variable to regulate hGH responses between concurrent-training sessions. However, more research is required to fully understand the impact of different concurrent-training and nutritional situations in elite athletes.

It must be noted that large intra and inter-individual variations were observed within the present data-set (e.g. T; ~20-60%, C; ~40-80%, hGH; ~100-1000%). Large variations in hormonal responses to training programmes have already been shown to exist in elite athletes [37], as such authors have proposed that suggesting that individuals may be able to engage in individualised training protocols to promote specific metabolic responses that promote specific acute and /or chronic responses [38]. Therefore, future studies could aim to further investigate this phenomenon by investigating the individual hormonal responses to concurrent exercise in elite soccer players.

Limitations.

It is acknowledged that this study is not without limitation. Ideally, isolated observations of the strength and soccer-specific endurance-training would have allowed us to comprehensively compare each condition and make better conclusions. However, such studies using the present population are difficult to conduct in elite training setting where the environment is less controllable and involves a small sample. The time-course of blood collection in the present study may also be a limitation of the present study. Indeed, given that the players were due to attend training sessions the following day exploring the responses across a longer period may offer new insights into how these athletes respond to their habitual concurrent-training programmes across extended periods (e.g. 12h, 24h, 48h). It is therefore recommended that future studies investigating the hormonal responses to concurrent exercise should study this response across a longer period of time following training.

Conclusion.

The aim of this study was to characterise the acute hormonal responses to two concurrent-training protocols previously observed in an elite soccer club. Our results suggest the organisation of concurrent-training has a negligible impact upon the acute T, C and hGH in youth elite soccer players. Although, due to the lack of control in the present study, more work is required to understand the acute and chronic impact of altering the recovery duration between concurrent-training sessions. This work may allow us to better understand how individuals respond to various exercise stimuli and could one day allow us to formulate personalised training interventions.

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Conflict of interest

The authors have no conflict of interest to report.

TITLES OF TABLES

Table 1: Objective and subjective measurements of endurance (E), strength (S) ‘training load’ completed during concurrent trial 1 (CT1) and concurrent trial 2 (CT2).

	CT1	CT2	P
Endurance-training (E)			
Distance Covered (m)	5767 ± 2012	6986 ± 1012	0.06
Distance > 5.5m/s (m)	198 ± 88	210 ± 80	0.77
Minutes > 85% HR MAX	00:03:23 ± 00:05:08	00:02:11 ± 00:04:05	0.44
Duration (min)	90	90	0
RPE (1-10)	5.9 ± 0.7	6.0 ± 0.7	0.72
Strength-training (S)			
Volume (AU) (reps • sets • load)	8000 ± 494	8000 ± 494	0
Half Back Squat 6RM	102 ± 15	102 ± 15	0
SLDL 6RM	82 ± 8	82 ± 8	0
Lunge 6RM	70 ± 5	70 ± 5	0
Deadlift 6RM	80 ± 15	80 ± 15	0
Duration (min)	40	40	0
RPE (1-10)	7.0 ± 0.7	7.2 ± 0.7	0.61

TITLES OF FIGURES

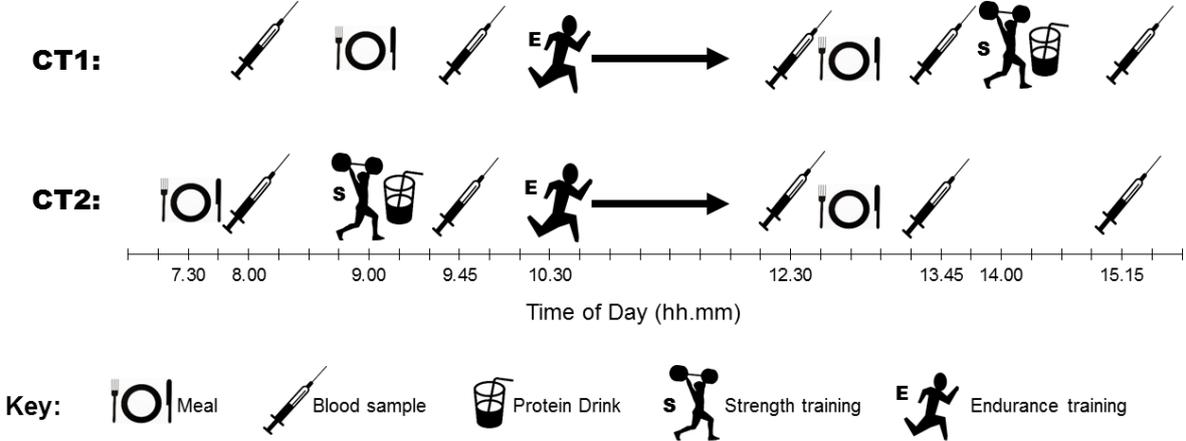


Figure 1: Schematic describing the organization of training and time-course of blood collection during concurrent trial 1 (CT1) and concurrent trial 2 (CT2).

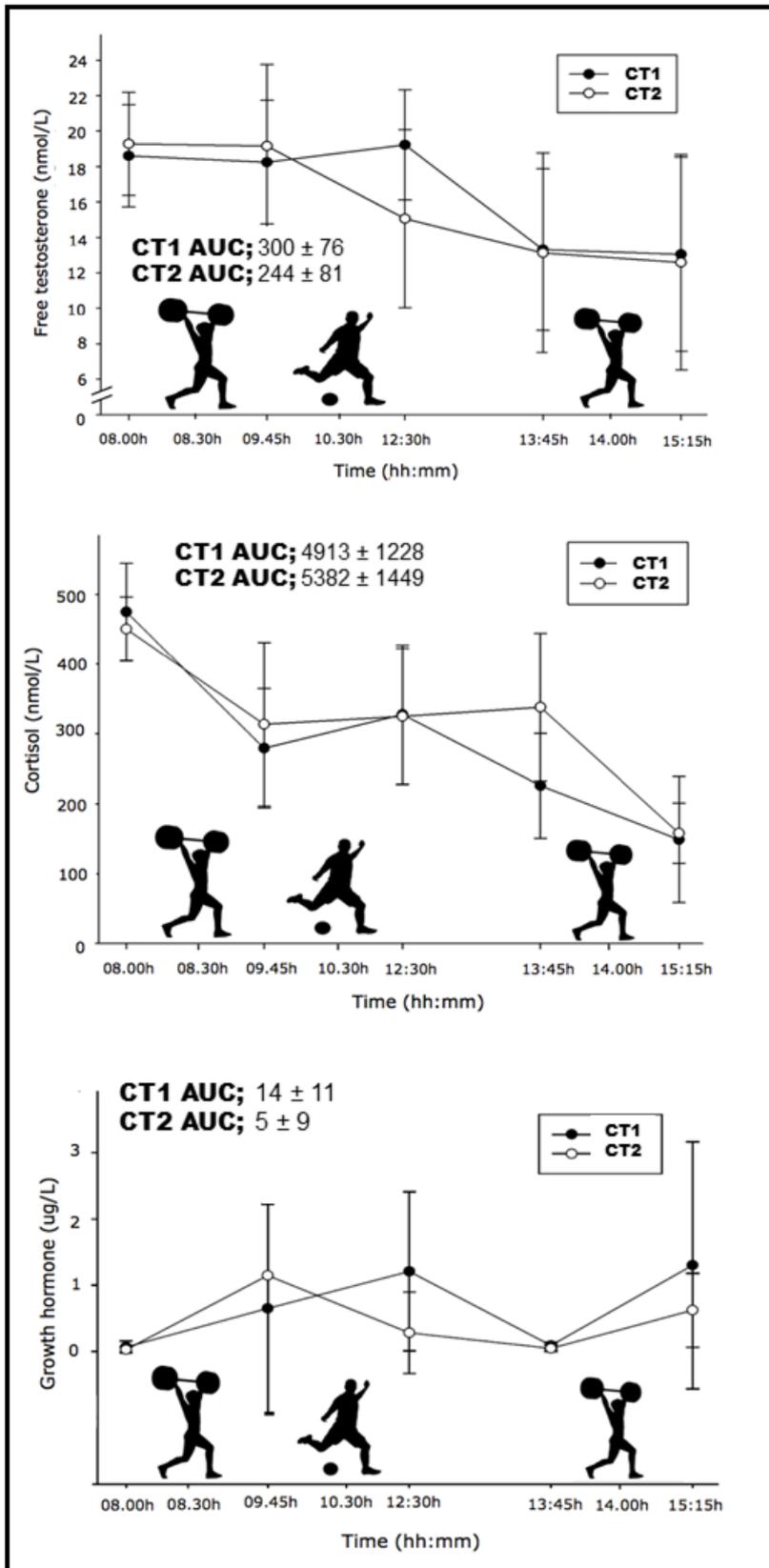


Figure 2: Total Testosterone (T), Cortisol (C) and Growth Hormone (hGH) responses to concurrent trial 1 (CT1) and concurrent trial 2 (CT2).

