Order Effects of Combined Strength and Endurance Training on Testosterone, Cortisol, Growth Hormone, and IGF-1 Binding Protein 3 in Concurrently Trained Men

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[1]*Sport Sciences Department, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal;* [2]*Research Center in Sports Sciences, Health Sciences and Human Development, Vila Real, Portugal; and* [3]*Exercise Research Laboratory, Physical Education School, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*

Abstract

Rosa, C, Vilaça-Alves, J, Fernandes, HM, Saavedra, FJ, Pinto, RS, and dos Reis, VM. Order effects of combined strength and endurance training on testosterone, cortisol, growth hormone, and IGF-1 binding protein 3 in concurrently trained men. *J Strength Cond Res* 29(1): 74–79, 2015—Concurrent training (CT) has been widely used in fitness centers to simultaneously optimize cardiovascular and neuromuscular fitness, and induce a high-energy expenditure. Therefore, the aim of this study was to compare the acute effects of 2 different orders of CT on hormonal responses in concurrently trained men. Fourteen men (mean ± *SD*: 24.7 ± 5.1 years) were randomly divided into 2 groups: endurance training followed by strength (ES, $n = 7$) and strength training followed by endurance (SE, $n = 7$). Serum concentrations of testosterone, cortisol, growth hormone, and IGF-1 binding protein 3 (IGFBP-3) were measured before and after both training orders. A significant interaction between exercise order and time was only found in the IGFBP-3 levels ($p = 0.022$). The testosterone and IGFBP-3 concentrations significantly increased in the ES group after the exercise trainings (57.7 ± 35.1%, $p = 0.013$ and 17.0 ± 15.5%, $p = 0.032$, respectively) but did not change significantly in the SE group (15.5 ± 36.6%, $p = 0.527$ and −4.2 ± 13.9%, $p = 0.421$, respectively). Conversely, cortisol and growth hormone concentrations significantly increased in both ES (169.2 ± 191.0%, $p = 0.021$ and 13,296.8 ± 13,009.5%, $p = 0.013$, respectively) and SE (92.2 ± 81.5%, $p = 0.017$ and 12,346.2 ± 9714.1%, $p = 0.001$, respectively) groups compared with baseline values. No significant correlations were found between the changes in the hormonal concentrations. In conclusion, these results suggest that immediately postexercise testosterone and IGFPB-3 responses are significantly increased only after the ES order. Therefore, an ES training order should be prescribed if the main focus of the training intervention is to induce an acute postexercise anabolic environment.

Key Words concurrent training, acute hormonal response, strength training

Introduction

The combination of endurance training (ET) and strength training (ST) in the same training session, so-called concurrent training (CT), is an effective strategy to improve both cardiorespiratory and neuromuscular functions (21), as well to induce a high-energy expenditure (2,17). However, because of the specificity of the stimulus provided either by the ET or by the ST, the intrasession concurrent exercise order may cause "interferences" on the responses and adaptations to both modes of training (13). These interferences may be associated with several factors, such as the low content of muscle glycogen leading to a chronic catabolic state, especially in type I fibers (3), fatigue in the neural system resulting from ST (6,8), and peripheral fatigue as a consequence of ET, which can subsequently impair the performance during ST (20).

With respect to the specific exercise effects of different body sites (lower body vs. upper body), Cadore et al. (7) observed, in elderly men, that 12 weeks of CT, with different exercise order, interfered in the magnitude of muscle dynamic strength gains 1 repetition maximum [1RM], in the lower limbs, when the aerobic training was performed on a cycle ergometer, but had no influence on the upper limbs. Furthermore, in a study with recreationally strength-trained men (5), an acute improvement in the testosterone/cortisol ratio was observed at the end of the first exercise mode (ET or ST), independently of the exercise order.

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29(1)/74–79

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Regarding the acute effects of CT, Goto et al. (11) found a decrease of growth hormone in response to ST as influenced by ET. However, the protocol used did not provide any significant increase in the levels of cortisol and testosterone. In another study, Cadore et al. (5) found that testosterone level remained significantly higher than resting levels after the second exercise modality (ET or ST) only in the endurance-strength (ES) order as compared with the strength-endurance (SE) order.

Analyzing the longitudinal (chronic) effects of CT, Häkkinen at al. (13) observed significant increases in strength, motor skills, and aerobic endurance on the first weeks of training, but with prolongation of the training period, these increases were limited or null.

Kraemer et al. (17) have suggested that a great training volume can acutely cause a high elevation in the circulating concentrations of cortisol, inducing an unfavorable environment for the repair and development of the muscle mass, and a consequent imbalance between the anabolic hormones (testosterone and growth hormone), which are stimulating factors of protein synthesis. Conversely, Bell et al. (4) have suggested that CT can attenuate the muscle hypertrophy process, although the physiological reasons for this negative influence are still unknown.

To date, the available evidence on the hormonal responses to intrasession exercise order (ES or SE) has included trained (5) and untrained men (29), as well as both genders (31). Participants in these studies were regularly involved in endurance (31) or ST (5), but none were specifically familiar to CT. Moreover, and to the best of our knowledge, only 1 recent study (31) has examined a wider set of hormonal indicators, consisting of testosterone, growth hormone, IGF-1 binding protein 3 (IGFBP-3), and cortisol. As suggested in the previous study, the 2 first hormones (testosterone and growth hormone) and the IGF-1 are some of the most important anabolic hormonal indicators associated with muscle hypertrophy, whose concentrations usually increase as an acute response to endurance and strength exercise. More precisely, the distribution and transport of most of the IGF-1 in the circulation is made by the IGFBP-3, making it relevant to assess its underlying concentration changes due to the effects of different exercise order. In addition, cortisol is one of the most influential catabolic hormones, which interferes in the anabolic process associated with muscle hypertrophy. However, the acute programming of the ET and ST in the previous studies was not designed to promote a high-energy expenditure, which is a common goal in fitness settings. In this respect, Alves et al. (2) observed that 3 combinations of ST and ET, with a methodological structure based on promoting a high-energy expenditure, did not promote different effects on overall oxygen consumption during the exercise sessions. Given that lean body mass is an important factor in promoting a higher daily energy expenditure and taking into account the specific nature of adaptation to different exercise stimulus, further investigation on the acute effects of different intrasession order of CT on the hormonal concentrations in concurrently trained individuals is warranted. Moreover, such research is expected to provide further insight into the most beneficial order of execution of the exercise modes, and consequently, a rationale for future exercise prescriptions oriented to promote higher energy expenditure, as well as a favorable acute anabolic environment that maintains or promotes muscle hypertrophy.

Therefore, the aim of this study was to investigate and compare the hormonal responses (testosterone, cortisol, growth hormone, and IGFBP-3) to different intrasession endurance and ST order in concurrently trained men.

Methods

Experimental Approach to the Problem

To investigate the effect of exercise order manipulation on the acute hormonal responses to a CT session, subjects attended the laboratory for 5 different sessions. The measurement of the anthropometric measures (body mass, height, and skin folds) was performed in the first visit to the laboratory. In this same session, the 1RM test for all strength exercises was determined. The 1RM retests were performed 72 hours later and showed high reliability (intraclass correlation coefficients [ICCs]): squat (SQ) (ICC >0.96), leg press (LP) (ICC >0.98), bench press (BP) (ICC >0.97), and lat pull down (ICC >0.96). In the following week, a submaximal running test was performed on a treadmill and was repeated after 72 hours, showing a high reliability: velocity at 2 $m \cdot mol^{-1}$ blood lactate (ICC >0.90) and at 4 $m \cdot mol^{-1}$ (ICC >0.93). Four days later, the subjects returned to the laboratory and performed 1 single session of concurrent exercises in the order of the assigned group, that is, ES or SE. Blood samples were collected before and immediately after the training sessions. All subjects performed the strength and aerobic tests and training sessions at the same time of the day (between 8 and 10.30 AM).

Subjects

Fourteen healthy men, aged between 20 and 35 years, volunteered for this study. They were randomly assigned to one of the 2 groups, ES ($n = 7$; 24.1 ± 4.1 years; 82.5 ± 7.9 kg; 175.9 ± 5.7 cm and 12.0 ± 2.3% of body fat) or SE ($n = 7$; 25.6 ± 5.2 years; 80.7 ± 7.7 kg; 177.9 ± 5.5 cm and 11.8 ± 3.2% of body fat). All participants were physically active and had engaged in CT at least 3 times a week, during the previous 12 months. All participants fulfilled the inclusion criteria: not to report positive responses on the PAR-Q test, not to intake food supplements and anabolic steroids for at least 3 months before and throughout the study, and absence of any kind of musculoskeletal or metabolic conditions that could limit exercise (33). All subjects read and signed a consent form to participate in the study, and the procedures were approved by the institutional Research Ethics Committee.

TABLE 1. One repetition maximum and endurance performance (mean ± *SD*) in ES and SE group.*

Variables	ES ($n = 7$) (mean ± *SD*)	SE ($n = 7$) (mean ± *SD*)
SQ 1RM (kg)	160.00 ± 16.33	168.57 ± 12.15
BP 1RM (kg)	114.57 ± 8.92	109.14 ± 7.82
LP 1RM (kg)	354.29 ± 31.55	353.57 ± 40.90
FLPD 1RM (kg)	90.71 ± 9.76	83.57 ± 6.90
Running velocity at 2 $mmol \cdot L^{-1}$ ($km \cdot h^{-1}$)	7.73 ± 0.39	7.73 ± 0.39
Running velocity at 4 $mmol \cdot L^{-1}$ ($km \cdot h^{-1}$)	11.04 ± 0.78	11.73 ± 1.44

*ES = endurance-strength; SE = strength-endurance; SQ = squat; BP = bench press; LP = leg press; FLPD = front lat pull down.

Measurements

Estimation of Body Fat. Body fat was estimated from measurements of skinfold thickness, using a skinfold caliper (Cescorf, Porto Alegre, Brazil), with an accuracy of 1 mm, and performed by an experienced examiner. The following skinfolds were assessed: subscapularis, tricipital, pectoralis, midaxillary, suprailiac, abdominal, and femoris (13).

Assessment of One Repetition Maximum. The 1RM values were obtained over 3–5 attempts for each exercise, at the moment when the evaluated participant presents a concentric phase failure in the movement, using the protocol described by Kraemer and Fry (16). To minimize the possible errors, the following strategies were adopted: (a) basic information was provided about the test's procedures; (b) subjects were reminded about the correct technical execution of the exercises; (c) verbal stimulation was used to promote maximal effort; (d) the same barbells and weight plates were used in all tests sessions; and (e) performance time for each repetition (concentric and eccentric contraction) was 3 seconds, controlled by an electronic metronome (Quartz, São Paulo, Brazil).

Endurance Test. Every subject performed a warm-up for 3 minutes at a velocity of 5 $km \cdot h^{-1}$ followed by a progressive submaximal discontinuous test on a treadmill (Embreex 568, Brusque, Brazil). Initial velocity ranged from 7.5 to 8.3 $km \cdot h^{-1}$ and was raised 1.6 $km \cdot h^{-1}$ at every stage. At the end of each stage, which lasted 3 minutes, a blood sample was taken from the right earlobe, to assess blood lactate concentrations (Accutrend Plus, Roche Diagnostic, Penzberg, Germany). The corresponding running velocities at selected blood lactate concentrations of 2 and 4 $mmol \cdot L^{-1}$ were recorded and used to individually prescribe the ET (28).

Collection and Analysis of Venous Blood. The blood samples were taken before and immediately after the CT sessions. Each individual was placed comfortably seated with a tourniquet tied around his arm and a collection of 10 ml of blood from the median cubital vein was obtained, using sterile needles into serum tubes (Venosafe, Terumo Medical Co., Leuven, Belgium), by a qualified laboratory technician. To control for the circadian variation of hormones, all collections were performed between 08:00 and 10:30 hours. After collection, the blood sample was kept at an ambient temperature of 20–25° C for 45 minutes and centrifuged for 10 minutes at 1500 rpm (Megafuge 1.0R, Heraeus, Langenselbold, Germany). The serum was then removed and kept at −55° C for later analysis. The serum concentrations of testosterone, cortisol, growth hormone, and IGFBP-3 were determined using radioimmunoassay kits (Cortisol Coat-Count-RIA; DPC Medlab, Los Angeles, CA, USA). All assays were performed according to the instructions of the manufacturers by licensed and certified laboratory technicians.

Concurrent Training Protocols

The protocol of the ST sessions was designed to consider an appropriate and efficient manipulation of several training variables, which have been associated in the literature with higher energy expenditure during and after the exercise(s), namely multiple sets (12), exercises recruiting large-muscle mass (23), movement speed (23,30), number of repetitions (27), the intensity and volume of the workload (15,32), resting time between sets (32), and the execution order of the resistance exercises (9). Additionally, the effect of the aerobic and strength exercise order on the oxygen uptake was also taken into consideration as suggested by Alves et al. (2).

The ET exercise was performed on a treadmill (Embreex, model 568, Brusque, Brazil). The training included 32 minutes of intermittent exercise, with 2 minutes at a speed corresponding to a blood lactate concentration of 2 $mmol \cdot L^{-1}$, alternating with 1 minute at a speed corresponding to a blood lactate concentration of 4 $mmol \cdot L^{-1}$.

The ST included 3 sets of 10 repetitions with 70% of 1RM for the following exercises: SQ, BP, LP, and front lat pull down (FLPD). Moreover, subjects were also required to perform 3 sets of 30 repetitions of the abdominal crunch (AC) and lumbar extension (LE) exercise with their own body weight. All sets of an exercise were completed before changing exercise. The ST exercises always followed this order: SQ, BP, LP, AC, FLPD, and LE. A rest interval of 1 minute was maintained between sets. A metronome (Quartz, São Paulo, Brazil) was set and used at a cadence of 40 $b \cdot min^{-1}$ to establish a rate of 20 exercise repetitions per minute.

Table 2. Hormone concentrations (mean ± *SD*) obtained before and after different exercise order.*

Variables	ES ($n = 7$) Pre	ES ($n = 7$) Post	SE ($n = 7$) Pre	SE ($n = 7$) Post
Testosterone (nmol·L^{-1})	10.08 ± 3.45	15.80 ± 6.12†	16.03 ± 7.21	17.36 ± 7.71
Cortisol (nmol·L^{-1})	286.11 ± 204.17‡	484.20 ± 180.71†	511.79 ± 178.51	937.51 ± 426.54†
Growth hormone (μg·L^{-1})	0.09 ± 0.08	8.33 ± 6.19†	0.31 ± 0.65	8.65 ± 3.95†
IGFBP-3 (mg·L^{-1})	4.93 ± 0.82	5.78 ± 1.35†	5.01 ± 0.80	5.27 ± 1.21

*ES = endurance-strength; SE = strength-endurance; IGFBP-3 = IGF-1 binding protein 3.
†Significant difference from baseline (pre) values ($p \leq 0.05$).
‡Significant difference between the ES and SE groups ($p \leq 0.05$).

Statistical Analyses

Data are presented as mean ± *SD*. Percent changes (Δ%) and respective *SD* from the beginning to the end of the training session were calculated in the hormonal concentrations. Normality assumption was checked with the Shapiro-Wilk test. Test-retest reliability was examined by using the ICC. To investigate changes in the outcome measures over exercise order and time, 2 (groups) × 2 (time) repeated-measure analyses of variance were used. Paired *t*-tests were used to examine specific exercise order changes in the hormonal indicators. Pearson correlation coefficients were calculated to measure the associations between changes (pretraining-posttraining) in the outcome measures. The significance level was set at 5%. The statistical powers of all main and simple significant effects were above 0.95, whereas the statistical power of the significant interaction effect was 0.68. All data were analyzed using the IBM SPSS 19.0 (SPSS, Inc., Chicago, IL, USA).

Results

Results presented in Table 1 show that the baseline strength and endurance values did not differ across different exercise order groups ($p > 0.05$).

No significant differences in concentrations of the serum hormones were observed at the beginning of the training sessions across groups, with the exception of the cortisol levels ($p = 0.048$). Thus, further comparative analysis of the cortisol concentrations included the baseline values as covariate.

Regarding the changes in the concentrations of serum hormones (Table 2), a significant effect was found for the main effect of time in the testosterone ($p = 0.017$) and growth hormone concentrations ($p < 0.001$), as well as a significant interaction between exercise order groups and time in the IGFBP-3 levels ($p = 0.022$). There was no significant interaction or main effects in the cortisol concentration ($p > 0.05$). In addition, no significant main effect was observed for exercise order groups ($p > 0.05$).

The testosterone and IGFBP-3 concentrations significantly increased in the ES group after the exercise trainings (57.7 ± 35.1%, $p = 0.013$ and 17.0 ± 15.5%, $p = 0.032$, respectively) but did not change significantly in the SE group (15.5 ± 36.6%, $p = 0.527$ and −4.2 ± 13.9%, $p = 0.421$, respectively). Conversely, cortisol and growth hormone concentrations significantly increased in both ES (169.2 ± 191.0%, $p = 0.021$ and 13,296.8 ± 13,009.5%, $p = 0.013$, respectively) and SE (92.2 ± 81.5%, $p = 0.017$ and 12,346.2 ± 9714.1%, $p = 0.001$, respectively) groups compared with baseline values.

No significant correlations were found between the changes in the hormonal concentrations.

Discussion

This study investigated the hormonal responses to different exercise order (ES vs. SE) in concurrently trained men. To the best of our knowledge, this is the first study that examines the influence of manipulating ET and ST exercises order on the acute hormonal responses, with a methodological structure based on promoting a high-energy expenditure and with a sample comprised by subjects trained in endurance and ST. The main results of this study demonstrate that there was a significant increase in testosterone and IGFPB-3 concentrations levels in the ES order when compared with the SE order, which suggests the occurrence of a higher anabolic environment when ET is performed before ST.

In relation to the serum testosterone levels, the results of this study corroborate the findings reported by Cadore et al. (5), when ET is performed before the ST exercises. However, in both studies, when exercises were performed in a reverse order (SE), no significant differences in the testosterone concentrations were observed, when compared with the baseline. In contrast, other studies (29,31), found no significant differences in this outcome variable, regardless of the intra-session exercise order. These mixed findings may be attributed in part to different methodologies used in ST exercises (22) and to the training level of the participants (33). In relation to the ST methodologies, while Cadore et al. (5)

used a hypertrophy type training, Schumann et al. (29) used a combination of exercises and methodologies based on explosive, maximal strength and hypertrophy training, and Taipale and Häkkinen (31) used a strength and explosive methodology. The use of ST methodologies with moderate intensity, multiple sets, and low rest intervals between sets, like the typical session to promote muscular hypertrophy as well as to induce a high-energy expenditure, tend to promote higher acute levels of serum testosterone after exercise (14,18,24,26,33), contrary to the power and submaximal training methods (22). This may explain the differences found in this study when compared with other investigations (5,29,31). Another possible cause to explain the differences between those studies and this study may be due to the training levels of the subjects. In our study, subjects were trained in both ET and ST at least 12 months, and in the other studies, subjects were physically active but untrained (29), or trained in ET only (31), or trained in ST only (5). Previous research demonstrated that a greater acute elevation of serum testosterone post-ST is expected in ST subjects, but not in ET subjects (33). For that reason, greater acute responses of post-exercise testosterone levels might be expected, such as with the study of Cadore et al. (5) and presently.

Regarding GH levels, a significant increase was observed in both exercise order (ES and SE) in our study, without differences between them, that is in accordance with Schumann et al. (29) In contrast, Taipale and Häkkinen (31) found a significantly higher increase when the ET was performed after ST, although both exercise order groups increased their baseline GH levels. GH levels depend on several factors such as the volume and intensity of exercise (1,10,26). Additionally, the fitness level and the type of training usually performed also tend to influence the GH postexercise responses (26). As mentioned earlier, there were differences in the level and type of exercise practiced between this study and the studies of Schumann et al. (29) and Taipale and Häkkinen (31). The recreational endurance running background of the subjects from Taipale and Häkkinen's (31) study, may have influenced the better response of GH when ET was performed after ST. Moreover, the different responses in the cortisol levels, in comparison with this study, may probably be related to this sample effect. Kraemer and Ratamess (14,18) indicated that endurance athletes tend to show less-pronounced changes in hormone concentrations in response to ST training when compared with strength-trained subjects. This fact was supported by the responses to ST, independently of the exercise order used (31).

The serum IGFBP-3 levels tend to reflect the spontaneous endogenous GH secretion, and this binding protein is considered the most important of the carrier proteins for IGF-1, an important anabolic biomarker (25), modulating the interaction with the receptors and accounting for 95% of the circulating IGF-1 (32). With respect to IGFBP-3 levels in this study, a significant interaction between exercise order and time was observed. The ES order promoted a significant increase, whereas a significant decrease was observed in the SE order. These results are in accordance with the ones reported by Taipale and Häkkinen (31) for ES order, but not for the SE order. These last differences may be due to the distinct methods used in the ET exercise across the studies. In this study, an interval training methodology was used, whereas Taipale and Häkkinen (31) used continuous methods. Meckel et al. (25) observed a significant increase of the IGFBP-3 levels after 4 sprints of 250 m at 80% of maximum velocity, with an interval of 3 minutes between the sprints. These results seem to indicate that the interval and the high-intensity training methods promote increases in IGFBP-3 levels.

However, some limitations should be considered when interpreting the results of this study. First, blood samples were collected from 2 separate groups due to logistic difficulties in maintaining the same research conditions for the global sample in a controlled and crossover design. Second, logistical and financial limitations made it impossible to collect and analyze additional blood samples between different modes of training and after recovery.

In conclusion, the results of this study suggest that the immediately postexercise testosterone and IGFPB-3 response is increased only after the ES order, whereas both ES and SE order induce similar cortisol and growth hormone responses. However, different ET and ST training methods, as well, as the modality and physical level of the subjects may influence the acute postexercise hormonal responses. As such, additional studies on hormonal responses to distinct training methods, throughout different postexercise time course, as well as considering each subject's physical activity levels are warranted.

Practical Applications

The present findings demonstrate an order effect in hormone responses to different intrasession order of CT in concurrently trained subjects. More precisely, significant increases in testosterone and IGFPB-3 concentrations were only observed after the ES training order. Therefore, these results thus suggest that performing ET before ST in a single session of CT induces an acute hormonal response that results in a more favorable anabolic environment immediately after the workout. Nevertheless, further research is needed on the investigation and relevance of the present findings regarding prolonged training adaptations in physically active and sedentary populations.

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