ACUTE ENDOCRINE AND FORCE RESPONSES AND LONG-TERM ADAPTATIONS TO SAME-SESSION COMBINED STRENGTH AND ENDURANCE TRAINING IN WOMEN

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ABSTRACT

Eklund, D, Schumann, M, Kraemer, WJ, Izquierdo, M, Taipale, RS, and Häkkinen, K. Acute endocrine and force responses and long-term adaptations to same-session combined strength and endurance training in women. J Strength Cond Res 30(1): 164–175, 2016—This study examined acute hormone and force responses and strength and endurance performance and muscle hypertrophy before and after 24 weeks of same-session combined strength and endurance training in previously untrained women. Subjects were assigned 1 of 2 training orders: endurance preceding strength (E + S, n = 15) or vice versa (S + E, n = 14). Acute force and hormone responses to a combined loading (continuous cycling and a leg press protocol in the assigned order) were measured. Additionally, leg press 1 repetition maximum (1RM), maximal workload during cycling (Wmax), and muscle cross-sectional area (CSA) were assessed. Loading-induced decreases in force were significant (p < 0.01–0.001) before (E + S = 20 ± 11%, S + E = 18 ± 5%) and after (E + S = 24 ± 6%, S + E = 22 ± 8%) training. Recovery was completed within 24 hours in both groups. The acute growth hormone (GH) response was significantly (p < 0.001) higher after S + E than E + S at both weeks 0 and 24. Testosterone was significantly (p < 0.001) elevated only after the S + E loading at week 24 but was not significantly different from E + S. Both groups significantly (p < 0.001) improved 1RM (E + S = 13 ± 12%, S + E = 16 ± 10%), Wmax (E + S = 21 ± 10%, S + E = 16 ± 12%), and CSA (E + S = 15 ± 10%, S + E = 11 ± 8%). This study showed that the acute GH response to combined endurance and strength loadings was significantly larger in S + E compared with E + S both before and after 24 weeks of same-session combined training.

Key Words: concurrent training, testosterone, growth hormone, performance adaptations, order effect

INTRODUCTION

It has been well established in male populations that metabolically demanding resistance exercise elicits large acute elevations of serum testosterone (T), growth hormone (GH), and cortisol (C) (18,26,35). These acute anabolic responses in men have in some studies been linked to long-term physiological adaptations, such as gains in muscle strength and hypertrophy (22,31,41), whereas in other studies, this phenomenon has not been found (47). Even though the magnitude of exercise-induced elevations in hormonal concentrations may not be correlated to long-term adaptations per se, the hormonal responses are known to create the metabolic environment involved in tissue remodeling (19,45).

The hormonal responses to resistance exercise in women are similar to those of men, albeit smaller in magnitude. Typically, only minor or no acute elevations in T concentrations are reported in women after strenuous resistance exercise protocols (9,19,29). These limited magnitudes of T responses are likely related to the intensity of exercise and amount of activated muscle mass (10,25,29,32), but may possibly be counterbalanced by acute GH release to meet the anabolic needs of resistance exercise sessions (26).

When combining strength (S) and endurance (E) into the same training session, the question arises regarding which exercise order (i.e., E + S or S + E) should be preferred. The acute effect of the exercise order on circulating hormones is of relevance considering the possible implications for long-term adaptations. As data from female
populations are scarce, current knowledge of the hormonal responses to combined loadings relies mainly on findings from men. Based on earlier reports, a bout of endurance exercise seems to blunt the GH response to subsequent resistance exercise, thus resulting in lower postexercise concentrations than in the opposite order (16,39). The findings regarding C and T (4,37,39) are less conclusive and may be related to the intensity or volume of the used exercise protocols or the training status of the subjects (4,16,39). Because most of these studies have incorporated a cross-sectional design, possible changes in the exercise-induced hormonal responses are not well understood. A previous study by our group noted that the S + E order could initially result in faster recovery of T in men compared with the opposite order, possibly indicating different recovery needs (39). However, this difference was found to diminish with prolonged training and did not influence the long-term strength gains. Furthermore, even though endurance exercise acutely impairs subsequent force production (12,28) and has been suggested to attenuate strength development after prolonged E + S training (3), recent reports from both men and women show similar strength gains after long-term training (11,14).

Despite a growing interest toward research regarding concurrent training in female populations (11,40), there is currently paucity in the knowledge regarding hormonal responses to combined exercise sessions in women. Although strength and endurance performance and lean mass are likely to increase to a similar extent after training in either order (11), the effects of prolonged training on exercise-induced hormonal responses and the relevance for training adaptations have not been elucidated. Thus, the main purpose of the present study was to investigate the influence of the exercise order of combined strength and endurance loadings on acute hormone and force responses both before and after 24 weeks of combined training. The secondary purpose was to investigate whether the acute exercise–induced changes in hormone concentrations are associated with long-term training adaptations in strength and endurance performance or muscle cross-sectional area (CSA).

**Methods**

**Experimental Approach to the Problem**

To examine the effect of prolonged training on acute exercise–induced force and hormone responses to combined E + S or S + E loadings and the chronic adaptations in strength and endurance performance and muscle CSA, a 24-week training intervention was conducted. As the focus of this study was to compare training-induced adaptations in acute loading responses, a crossover design was not used and the subjects performed the experimental loadings in their assigned loading order only. The acute loading responses and long-term adaptations in strength and endurance performance were determined before (week 0) and after (week 24) the intervention (Figure 1).

**Subjects**

Twenty-nine women (age range 18–40 years) participated in the present study. Recruitment was conducted by several public postings. Subjects were (a) recreationally physically active but without systematic strength or endurance training for at least 1 year before participation, (b) below a body mass index (BMI) of 30 kg·m⁻², (c) nonsmokers, (d) free from chronic illnesses and injuries, and (e) not pregnant or lactating. A resting electrocardiogram screening was approved by a cardiologist. The subjects were informed about the study design, measurements, and procedures. The subjects were matched by physical fitness at baseline into 2 training groups: endurance preceding strength (E + S, n = 15, 29.1 ± 5.6 years, 168 ± 7 cm, 67 ± 10 kg, and BMI = 23.7 ± 3.3 kg·m⁻²) and strength preceding endurance (S + E, n = 14, 28.9 ± 4.4 years, 164 ± 5 cm, 62.4 ± 8 kg, and BMI = 23.2 ± 3.4 kg·m⁻²). Because of organizational constraints, acute loading responses were assessed from 23 subjects (E + S, n = 12; S + E, n = 11), whereas changes in strength and endurance performance and muscle CSA were assessed for all subjects. The study received ethical approval from the Ethics Committee of the University of Jyväskylä, Finland, and was conducted in accordance with the Declaration of Helsinki. After written and verbal information about the study and its procedures had been provided, written informed consent was obtained from all the subjects.

**Procedures**

Before the start of the measurements and training, subjects reported to the laboratory for a familiarization session during which the strength measurements were practiced and the equipment was adjusted to the specifics of the individual. Subjects wore the same shoes for all measurements and loading sessions. Blood sampling and all physical tests were conducted at the same time of the day ±1 hour throughout the study. The measurements of maximal strength and endurance performance were separated from each other and the loading measurements by at least 2 days. The last training session of the 24-week training intervention was separated from the following basal measurements by 2–4 days of rest. Nutritional information according to the national guidelines was provided before the start of the study, and the subjects were asked to keep their energy intake constant throughout the intervention. Ingestion of caffeine and alcohol was not allowed 12 and 24 hours before the measurements, and subjects were required to keep the nutritional intake before the measurements similar in weeks 0 and 24.

As recent reports have shown minimal influence of the menstrual cycle phase on anabolic hormone responses to strength (43) and endurance exercise (33), the measurements were conducted across several phases of the menstrual cycle. Four subjects from the E + S and 3 subjects from the S + E groups reported oral contraceptive use.

**Basal Measurements**

**Strength.** Maximal bilateral dynamic leg press 1 repetition maximum (1RM) was measured using a David 210 weight

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**Declaration of Helsinki.** After written and verbal information about the study and its procedures had been provided, written informed consent was obtained from all the subjects.
stack horizontal leg press device (David Health Solutions Ltd., Helsinki, Finland). Three warm-up sets (5 × 70–75%, 3 × 80–85%, and 2 × 90–95% of estimated 1RM) with 1 minute of rest between sets were performed before the 1RM trials. Upon verbal instruction, subjects performed a full leg extension (knee angle 180°) from a starting knee angle of below 60° (58° ± 2°). After each successful completion, the load was increased. Subjects were allowed a maximum of 5 trials. The trial with the highest completed load was accepted as the 1RM.

**Endurance.** The maximal endurance test was conducted on a cycle ergometer (Ergometrics 800; Ergoline, Bitz, Germany) using a graded exercise protocol. The test was initiated at 50 W for all subjects with 25-W increments applied every 2 minutes until volitional exhaustion. Maximal workload (Wmax) was calculated as $W_{\text{max}} = W_{\text{com}} + (t/120) \times 25$ (39), where $W_{\text{com}}$ represents the load of the last completed and $t$ the time of the last incomplete stage. Aerobic and anaerobic thresholds were determined for each subject based on the points of deflection in the curves of ventilation, oxygen consumption, production of carbon dioxide, and blood lactate (2).

**Muscle Cross-Sectional Area.** Cross-sectional area of the vastus lateralis muscle of the right limb was measured using a B-mode axial plane ultrasound device (SSD-a10; Aloka, Tokyo, Japan) and a panoramic imaging technique (1). Images were taken at both 50 and 70% of the muscle length. Images were analyzed using ImageJ software version 1.44 (National Institutes of Health, Bethesda, MD, USA) by manually marking the outlines of the muscles onto the image. The means of the 2 closest values of 50 and 70%, respectively, were averaged and used in the statistical analyses to assess total CSA. The reproducibility of the measurement has been reported earlier by our research group (38).

**Experimental Loading Protocol**

The experimental loading was intended to reflect the content of the 24-week training program, which was designed to reflect the exercise recommendations for physically active individuals (42). The loading consisted of both endurance cycling and a leg press protocol. Loadings were conducted for each subject at the same time of the day (±1 hour) at weeks 0 and 24 and were performed in the order specific to the training. Measurements of force and hormone responses during the loading were conducted before the initiation of the loading (Pre), after the first part (Mid: E or S, respective to order), and after the complete loading (Post). Recovery was monitored 24 ± 1 and 48 ± 1 hours after the cessation of exercise (24 and 48 hours, respectively).

Subjects were verbally encouraged throughout the loadings. Proper hydration on the day preceding the loading was encouraged. Consumption of 0.2 L of water was allowed between the 2 loading modes, after the Mid blood sample was taken.

**Strength Loading.** A David 210 weight stack horizontal leg press (David Health Solutions Ltd.) was used to conduct the strength loading. A detachable handle was available for assistance if necessary. The loading consisted of 3 protocols typically used in training for explosive strength ($3 \times 10$ repetitions at 40% 1RM with 3 minutes rest between sets), maximal strength ($4 \times 3$ repetitions at 75–90% 1RM with
3 minutes rest between sets), and muscle hypertrophy (4 × 10 repetitions at 75–80% 1RM with 2 minutes rest between sets). Loads were calculated from the 1RM obtained during the basal measurements. Additional resistance was added to at least one maximal and one hypertrophic set to complete a true RM and standardize the loading conditions. In the explosive sets, subjects were instructed to perform the concentric phase as fast as possible and the eccentric phase in a controlled manner, without pausing between repetitions. For the hypertrophy and maximal sets, subjects were instructed to fully extend their legs without locking their knees and to keep an even pace throughout the movement.

**Endurance Loading.** The endurance loading consisted of 30 minutes of continuous cycling at an intensity of 65% Wmax (39) on a Monark cycling ergometer (Ergomedic 839E; Monark Exercise AB, Vansbro, Sweden) equipped with electric resistance. The intensity was calculated based on Wmax from the basal measurement. Subjects were instructed to keep the pedaling pace at 70 revolutions per minute (rpm). The rpm was visible to the subjects throughout the loading and was additionally monitored by a member of staff. In case of the rpm dropping below 65 with the subject unable to increase it, the workload was lowered by 15 W. If the subject was unable to keep up the pace for a full minute after the reduction, the workload was further reduced by 15 W. If necessary, the procedure was repeated until the subject was able to keep up the required pace and complete the loading.

**Measurements During the Experimental Loading**

**Isometric Force Production.** Maximal isometric force (MVC) was measured on a leg press device (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland) with a knee joint angle of 107° (180° angle representing full extension) (17). The greater trochanter of the femur and lateral malleolus of the ankle of the right limb were used as anatomical reference points.

Subjects were instructed to perform an isometric bilateral leg press action as rapidly as possible with the aim of reaching the maximum force at the beginning of the trial and maintaining it for a duration of approximately 3 seconds. At Pre, 24- and 48-hour subjects were allowed to perform 3 trials with 1 minute rest between trials. At Mid and Post, subjects immediately proceeded to the measurement and performed 2 trials with only 10 seconds rest between trials for the purpose of recording exercise-induced fatigue. The trial with the highest force was selected for analysis. Force signals were recorded with Signal 2.16 software (CED, Cambridge, United Kingdom), sampled at 2,000 Hz and processed with a low-pass filter of 20 Hz. Trials were analyzed for MVC and average force produced between 0 and 500 milliseconds (MVC500).

**Blood Samples.** To determine the blood lactate concentrations, capillary blood samples were taken from the fingertip at Pre, Mid, and Post into a reaction tube containing an anticoagulant and a hemolyzing agent. The samples were analyzed using a Biosen lactate analyzer (S-line Lab + EKF, Magdeburg, Germany). In addition, venous blood samples were drawn at Pre, Mid, Post, 24, and 48 hours for determination of total T, C, and GH (22 kDa) concentrations. Resting concentrations of the same hormones and sex hormone-binding globulin (SHBG) and T/C and T/SHBG ratios were determined on the morning of the loading (7:00–9:00 AM) in a fasted state. Samples were drawn by a laboratory technician from the antecubital vein into a serum tube (Venosafe; Terumo Medical Co., Leuven, Belgium). Samples were centrifuged for 10 minutes at 3,500 rpm, after which serum was removed and frozen until analyzed. The hormones were analyzed with a chemical luminescence technique (Immulite 1000; Siemens, New York, NY, USA) using hormone-specific immune assay kits (Siemens). Creatine kinase (CK) was analyzed using chemical analysis (KoneLab 20 XT; Thermo Fisher Scientific Oy, Vantaa, Finland). Sensitivities for T, C, GH, SHBG, and CK were 0.5 nmol·L⁻¹, 5.5 nmol·L⁻¹, 0.03 mlU·L⁻¹, 0.02 nmol·L⁻¹, and 0.7 mlU·L⁻¹, respectively. Intra-assay coefficients of variation for T, C, GH, SHBG and CK were 9.8 ± 3.9%, 7.1 ± 1.1%, 6.0 ± 0.5%, 3.1 ± 1.3%, and 1.5 ± 0.7%, respectively. Inter-assay coefficients of variation for T, C, GH, SHBG and CK were 12.0 ± 6.3%, 7.9 ± 1.2%, 5.8 ± 0.3%, 5.0 ± 1.0%, and 3.6 ± 0.8%, respectively. Serum hormone concentrations were not corrected for changes in plasma volume. To monitor hemococoncentration (13), hemoglobin (HGB) and hematocrit (HCR) were analyzed with Systex X21 N (Systex America Inc., Mundelein, IL, USA) automated hematology analyzer with a cyanide-free and cumulative pulse height detection method, respectively.

**Training**

The training program has been described in detail previously (14). Briefly, the training was aimed to reflect recommendations for physically active individuals (42) and was targeted at improving both maximal strength and endurance performance. During the first 12 weeks, the subjects completed 2 weekly sessions of (1E + 1S) or (1S + 1E) (respectively to the assigned training order) and 5 sessions per 2 weeks (5 × [1E + 1S] or [1S + 1E]) during weeks 13–24. Time between training modes was 5–10 minutes and recovery time between training sessions 48–72 hours. Training sessions were supervised by research staff. Maintenance of normal daily activity was encouraged.

Strength training mainly targeted knee extensors and flexors and hip extensors. Exercises consisted of horizontal leg press, seated hamstring curls, and seated knee extensions. The program was initiated with the exercises performed in a circuit (2–4 sets of 15–20 repetitions with up to 60% of 1RM) and continued through hypertrophy-inducing training (2–5 × 8–12 at 80–85% of 1RM, 1–2 minutes rest) toward maximal strength training (2–5 × 3–5 at 85–95% of 1RM.

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**Page 167**

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3–4 minutes rest). A similar pattern of periodization was used for the upper body. Dumbbells and cable pulley machines were used for the upper body exercises and both machines and body weight for exercises of the trunk. The periodization was repeated during weeks 13–24 with increased training intensity and volume. The duration of each strength session was 50–60 minutes.

Endurance training sessions were performed on a cycle ergometer. Training intensities were controlled by heart rate zones corresponding to the threshold values of aerobic and anaerobic thresholds. Training consisted of 30–50 minutes continuous cycling near the AT (weeks 1–7 and 13–16), including interval training at and above the anaerobic threshold from weeks 8 and 17. The interval sessions were initiated and ended with 10- to 15-minute bouts below the aerobic threshold with 5-minute altering bouts on the anaerobic threshold and below the aerobic threshold in between.

Statistical Analyses
Data are presented as mean ± SD. Statistical analysis for changes during the experimental loadings at weeks 0 and 24 was performed using a 5-level analysis of covariance (ANCOVA) (i.e., Pre, Mid, Post, 24, and 48 hours) with absolute values for within-group changes and values relative to Pre for between-group differences, with Pre values used as covariates. As GH during the experimental loading and basal SHBG were nonnormally distributed even after a log transformation, nonparametric statistics were used both for the within-group changes (Wilcoxon signed rank test) and between-group comparisons (Mann-Whitney U-test). For the nonparametric tests, a Bonferroni adjustment was applied by multiplying the pairwise p values with the number of comparisons. To compare the experimental loading–induced within-group changes (i.e., Mid, Post, 24 hours, and 48 hours) across 24 weeks, paired samples t-tests were applied for each measurement point.

Training-induced changes in basal hormones and basal measurements of IRM, Wmax, and CSA were analyzed with a 2-way ANCOVA with baseline values used as covariates and between-group differences with an independent samples t-test. The individual ratios of changes in IRM and Wmax were calculated as the percent change in IRM divided by the percent change in Wmax.

Reported effect sizes (ES) are Cohen's d except for non-normally distributed data, where ES was defined as Z score/√n. Associations between exercise-induced changes in serum hormone concentrations and training-induced adaptations were examined using bivariate Pearson correlation coefficient for normally and Spearman's rank correlation coefficient for nonnormally distributed data. A trend was accepted for p values <0.06.

RESULTS
Training adherence was 99% in both E + S and S + E groups. All subjects completed at least 90% of the training sessions.

Acute Loading Responses at Week 0
No significant changes in body weight (−0.3 ± 0.3%), HGB (+3.6 ± 3.0%), or HCR (+2.6 ± 2.9%) were observed during the loading.

Maximal isometric force decreased significantly during the loading in both groups by Mid (E + S = −18 ± 13% from 1,740 ± 235 N, p < 0.01, ES = −1.305; S + E = −17 ± 7% from 1,810 ± 633 N, p < 0.01, ES = −0.515) and by Post (E + S = −20 ± 11%, p < 0.01, ES = −1.587; S + E = −18 ± 5%, p < 0.001, ES = −0.532) (Figure 2). Maximal isometric force between 0 and 500 milliseconds decreased significantly in E + S by Mid (E + S = −18 ± 12%, p < 0.01, ES = −1.24) and Post (−20 ± 14%, p < 0.01, ES = −1.15) and for S + E by Post (−16 ± 7%, p = 0.05, ES = −0.611). No significant differences of MVC or MVC 500 to Pre were observed for either group at 24 or 48 hours.
A significant increase in T was observed in E + S at Mid (from 0.5 ± 0.4 to 8.9 ± 1.1 nmol·L$^{-1}$, $p < 0.05$, ES = 0.513) (Figure 3). Cortisol remained statistically unaltered throughout the loading for both groups (Table 1). A trend ($p = 0.051$, ES = 0.256) was observed in C for E + S at Mid. At 24 and 48 hours, C was significantly lowered at Pre for S + E (24 hours: $-29 \pm 23\%$, $p < 0.01$, ES = $-1.53$, and 48 hours: $-29 \pm 14\%$, $p < 0.01$, ES = $-1.70$). A 5.6-fold increase from Pre in GH was observed at Mid for E + S ($p < 0.05$, ES = 0.888) and a 5.2-fold increase at Post for S + E ($p < 0.05$, ES = 0.830) (Figure 4). A trend was found in S + E from Mid to Post ($p = 0.055$, ES = 0.402). The change from Mid to Post was significantly different between groups ($p < 0.001$, ES = 0.886).

**Table 1.** Exercise-induced changes in serum cortisol (C), creatine kinase (CK), and blood lactate (La) during loading and recovery for E + S and S + E at weeks 0 and 24.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 24</th>
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<tbody>
<tr>
<td></td>
<td>E + S</td>
<td>S + E</td>
</tr>
<tr>
<td></td>
<td>($n = 10$)</td>
<td>($n = 10$)</td>
</tr>
<tr>
<td>C (nmol·L$^{-1}$)</td>
<td>397 ± 128</td>
<td>479 ± 105</td>
</tr>
<tr>
<td>Pre</td>
<td>347 ± 176</td>
<td>367 ± 139</td>
</tr>
<tr>
<td>Mid</td>
<td>426 ± 207</td>
<td>435 ± 211</td>
</tr>
<tr>
<td>Post</td>
<td>312 ± 111 (*)</td>
<td>332 ± 87†</td>
</tr>
<tr>
<td>24 h</td>
<td>347 ± 141</td>
<td>333 ± 60†</td>
</tr>
<tr>
<td>48 h</td>
<td>143 ± 36*</td>
<td>181 ± 89§</td>
</tr>
<tr>
<td></td>
<td>128 ± 38</td>
<td>132 ± 55**</td>
</tr>
<tr>
<td>CK (mlU·L$^{-1}$)</td>
<td>($n = 9$)</td>
<td>($n = 9$)</td>
</tr>
<tr>
<td>Pre</td>
<td>93 ± 21</td>
<td>95 ± 31</td>
</tr>
<tr>
<td>Mid</td>
<td>103 ± 19†</td>
<td>106 ± 32</td>
</tr>
<tr>
<td>Post</td>
<td>105 ± 17†</td>
<td>118 ± 35†</td>
</tr>
<tr>
<td>24 h</td>
<td>143 ± 36*</td>
<td>181 ± 89§</td>
</tr>
<tr>
<td>48 h</td>
<td>128 ± 38</td>
<td>132 ± 55**</td>
</tr>
<tr>
<td>La (mmol·L$^{-1}$)</td>
<td>($n = 12$)</td>
<td>($n = 11$)</td>
</tr>
<tr>
<td>Pre</td>
<td>1.1 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Mid</td>
<td>4.7 ± 1.0§</td>
<td>5.0 ± 1.9§</td>
</tr>
<tr>
<td>Post</td>
<td>5.6 ± 2.2§</td>
<td>5.2 ± 2.3§</td>
</tr>
</tbody>
</table>

*Within-group differences significant from Pre, $p = 0.051$.
†$p < 0.01$.
§$p < 0.001$.
††Within-group differences significant from Mid, $p \leq 0.05$ and $p < 0.01$.
‡Within-group differences significant from Post, $p \leq 0.05$.
**Within-group differences significant from 24 hours, $p < 0.05$.
††Within-group differences significant from corresponding value at week 0, $p \leq 0.05$. 
Blood lactate increased significantly in both groups by Mid (E + S by 4.2-fold, \( p < 0.001 \), ES = 4.80; S + E by 4.0-fold, \( p < 0.001 \), ES = 2.8) and Post (E + S by 5.0-fold, \( p < 0.001 \), ES = 2.84; S + E by 4.0-fold, \( p < 0.001 \), ES = 2.05) (Table 1). Creatine kinase was significantly elevated in comparison with Pre in E + S at Mid (13 ± 9% from 93 ± 621 mlU·L\(^{-1}\), \( p < 0.01 \), ES = 0.544), Post (16 ± 9%, \( p < 0.01 \), ES = 0.681), and 24 hours (57 ± 32%, \( p < 0.05 \), ES = 1.73) and for S + E at Post (26 ± 17% from 95 ± 31 mlU·L\(^{-1}\), \( p < 0.01 \), ES = 0.679) but not at 24 hours (96 ± 89%, ES = 1.29) (Table 1). Creatine kinase further increased in both groups between Post and 24 hours (E + S = \( p < 0.05 \), ES = 1.337; S + E = \( p < 0.05 \), ES = 0.935). No between-group differences were observed during loading or recovery.

**Acute Loading Responses at Week 24**

No significant changes in body weight (−0.5 ± 0.2%), HGB (+4.2 ± 3.8%), or HCR (+4.4 ± 2.9%) were observed during loading.

Maximal isometric force decreased significantly for both groups by Mid (E + S = −16 ± 9% from 1,833 ± 322 N, \( p < 0.001 \), ES = −0.890; S + E = −21 ± 8% from 1,966 ± 690 N, \( p < 0.001 \), ES = −0.935). No between-group differences were observed during loading or recovery.

**Order Effect in Women: Loading and Training**

Figure 4. Growth hormone responses for E + S and S + E during loading at weeks 0 and 24. Within-group differences: *significant from Pre, †significant from Mid, §significant from Post, ‡significant from week 0; #between-group difference at given time point. *\( p \leq 0.05 \), ††\( p < 0.01 \), †††\( p < 0.001 \), *\( p < 0.05 \), ††††\( p < 0.01 \), †††††\( p < 0.001 \).

Figure 5. Changes in 1 repetition maximum (1RM) (left), maximal workload (middle) during the cycling endurance test, and the individual ratios of the magnitude of gains in 1RM and workload (right). *Significant from week 0. ***\( p < 0.001 \).
The main findings of the present study were that after experimental loading at week 0, significantly elevated serum GH was observed only in S + E, whereas serum T remained unchanged in both groups. At week 24, both T and GH were significantly elevated in S + E but not in E + S at Post. The exercise order did not affect the magnitude of loading-induced fatigue measured as maximal voluntary isometric force and rapid force production either at week 0 or at week 24. Additionally, muscle force production was recovered by
24 hours after both exercise orders both at week 0 and 24. The present 24-week combined strength and endurance training period resulted in significant increases in 1RM strength, Wmax, and muscle cross section of similar magnitudes in both groups. These chronic adaptations were not associated with the acute exercise-induced changes of serum hormones in either order.

In accordance with our previous study with men performing the same experimental loading with the same relative intensity (39), we observed no acutely elevated concentrations of T in the present study at Post before the prolonged training period after either order. This outcome was expected, considering the combination of explosive, maximal, and hypertrophic sets in the present strength loading. Thus, the protocol was likely not strenuous enough to elicit acute anabolic responses (39), as large elevations in T would be expected in women mainly after hypertrophic type protocols with a large stress on the metabolic system (25,29). However, this design was purposefully chosen to reflect the content of the 24-week training program, which was created based on common exercise recommendations (42).

Interestingly, as elevated concentrations of serum T were observed during loading for E + S at Mid (weeks 0 and 24) and for S + E at Post (week 24), our results suggest that the observed elevations may primarily have been a result of the present endurance exercise. Considering the likely absence of hemocoagulation in the present study, this supports previous findings of endurance exercise inducing elevations in T in female populations (15,24). The lack of significantly increased T at Post for the S + E group at week 0 is in line with earlier investigations in men (4,37,39), with unchanged concentrations of T after a combined loading in the S + E order. However, the significantly elevated concentration of serum T in S + E at week 24 could be related to training-induced increased sensitivity to adrenocorticotropic hormone (30), which stimulates the adrenal cortex and releases androgens as a by-product of C secretion (23,34). This together with the relatively higher rise in lactate after training could be related to why elevated T was observed in the S + E group at Post at week 24, but not at week 0. However, no such observation was made in the E + S group. Furthermore, as we only measured total T and did not detect any significant elevations in C during the loadings in either order, this hypothesis remains speculative.

It also needs to be acknowledged that the underlying causes of exercise-induced elevations in T in women are not fully comprehended, and not all plausible mechanisms were monitored in the present study. Possible mechanisms include the time course of androgen receptor regulation (44) and reduced hepatic clearance as observed in men (5). Hemocoagulation as an indirect cause for elevated T can likely be ruled out in the present study because of unchanged hemoglobin and hematocrit during loading. It is also possible that oral contraceptive use affects the secretion of T and the metabolites of dehydroepiandrosterone (15) and, consequently, the biosynthesis pathway of T during exercise. However, in the present study, a similar number of subjects in both groups reported oral contraceptive use and, on a group level, the pattern of T response to endurance exercise was comparable in both orders.

Similar to the exercise-related variables affecting the acute responses of T, the intensity of exercise is a major contributor to the magnitude of responses of GH in women (29,46). As expected based on previous findings (16), the GH concentrations in the E + S order both before and after training were significantly elevated after endurance exercise but diminished after the strength loading. This pattern in the kinetics of exercise-induced GH release was significantly different between groups both before and after training as S + E demonstrated elevated GH throughout loading in contrast to E + S. These differing GH responses may have been caused by endurance exercise-induced lipolysis (36). The release of free fatty acids (FFA) is likely a major influence for suppressed GH release, possibly by affecting anterior pituitary function (6). It needs to be noted that even though oral contraceptive use could amplify lipolysis during continuous cycling exercise, the FFA concentration is likely to remain unaffected (7).

Because of a critical relative threshold for GH secretion, the intensity of training would need to be continuously progressive in order for significant GH responses to occur within a loading session after prolonged training (8). In the present study, the loading was conducted with values relative to 1RM and Wmax to keep the relative intensity the same at both weeks 0 and 24 and to be matched for the current training status and improved performance level of the subjects. This was reflected in the GH responses in both groups at week 24 as higher absolute concentrations at both Mid and Post in comparison with corresponding time points at week 0. In the S + E order, despite the fact that the magnitudes of the loading-induced changes (Pre-Mid and Pre-Post) were not statistically larger than the corresponding magnitudes at week 0, the GH responses during loading at week 24 were statistically significant. This serves as an indication of adaptation to training, as the same relative exercise intensity was potent in significantly elevating serum GH concentrations. Similar indications for training adaptations were found in the E + S group, as the magnitudes of the Pre-Mid and Pre-Post changes were significantly larger at week 24 than at week 0. Interestingly, although the adaptations in GH release seem to indicate that the loadings were still strenuous at week 24, changes in the behavior of CK may suggest better tolerance of the experimental loading. Creatine kinase was slightly elevated both during loading and recovery in both groups at week 0 but during week 24 only elevated in S + E during loading and similar to resting levels during recovery. As CK can be considered to be an indirect indicator of muscle damage, the lack of its presence during recovery after training may indicate an increased tolerance for combined strength and
endurance loadings, similar to what was recently observed in men (39).

Interestingly, although the GH responses clearly differed between the present exercise orders during loading, no between-group differences were observed in training-induced increases in muscle CSA. The implications of the present findings thus require further clarification through examining additional forms of GH than solely the present 22 kDa variant. Although an acute bout of exercise may stimulate variants of GH that are incapable of generating increases in biological activity, chronic resistance exercise may increase the circulating concentrations of biologically active GH (27). This may in part explain why the GH responses in the present study were not related to the changes in muscle CSA in either group and warrant further investigation of the mechanisms of several GH variants both during combined strength and endurance loadings and prolonged combined training.

In addition to a lack of relationship between changes in muscle CSA and the magnitudes of acute GH release, we also observed no associations between acute responses of T and long-term performance adaptations. Although it has been suggested that tissue exposure to acute elevations in anabolic hormones would not be associated with hypertrophy or strength performance (47), such correlations have been demonstrated in male populations after pure strength training (22,31). Furthermore, previously reported correlations of basal levels of T and T/SHBG ratios and strength development and changes in CSA after strength training in women (2021) were not found in the present study despite significantly increased basal levels of T. Thus, it seems reasonable to suggest that the detection of possible linkages of resistance exercise-induced anabolic responses and gains in strength and hypertrophy may be interfered when strength training is simultaneously accompanied by endurance training both in men (39) and women. However, further studies with different training protocols are needed for more definite conclusions.

It is noteworthy that both exercise orders resulted in similar training-induced long-term gains in 1RM, thus challenging earlier suggestions of the order of S + E being superior to E + S in terms of adaptations in strength performance (3,28). The experimental loading showed that the acute fatigue in terms of exercise-induced decreases in MVC and MVC500 was of similar magnitude after both loading conditions both before and after training. Furthermore, as neither MVC nor MVC500 was no longer significantly depressed from Pre loading values by 24 hours, the experimental loadings indicate that the recovery of maximal and rapid strength performance was completed within 24 hours of cessation of exercise. Consequently, it can be assumed that the recovery between individual training sessions was sufficient, as the sessions were consistently separated by at least 48–72 hours. Even though we only monitored recovery before and after 24 weeks of training, the loads used in the experimental loadings were similar to those used during training. This may, in part, explain similar gains in 1RM in both groups.

However, it also needs to be noted that the findings regarding the effect of mode of endurance exercise (i.e., running or cycling) on changes in strength performance are to date equivocal (40,48). Thus, comparisons of the present training program, consisting of cycling endurance training, with other protocols should be done with caution. Interestingly, although no between-group differences were observed in the long-term training-induced changes, the magnitude of gains in strength in relation to endurance performance was highly individual in both exercise orders (Figure 5). This warrants further investigation regarding the mechanisms of the underlying adaptations to same-session combined strength and endurance training in women.

To conclude, this study demonstrated that the acute hormone and force responses to a combined strength and endurance loading were by large similar between exercise orders in previously untrained women both before and after training, with the exception of differences in the kinetics of serum concentrations of GH during exercise. Furthermore, our results showed that strength and endurance performance and muscle CSA after 24 weeks of same-session combined strength and endurance training were similar in both exercise orders. Therefore, our data indicate that despite some differences in the acute anabolic responses to exercise, the present 24-week combined strength and endurance training program resulted in similar long-term performance and morphological adaptations in both groups.

**Practical Applications**

As the present study did not show order-specific responses of recovery of force, the findings indicate that the exercise order does not seem to be of great importance for previously untrained women when combining strength and endurance into the same training session. Even though the GH responses to exercise were significantly larger in S + E compared with E + S both before and after training, this was not reflected in or associated with the long-term adaptations. Consequently, the gains in strength and endurance performance and muscle size were of similar magnitudes in the 2 training groups after 24 weeks of combined strength and endurance training. Thus, previously untrained women can achieve performance improvements and increases in muscle size by combining strength and endurance into the same training session with either exercise order, when sufficient recovery is allowed.

**REFERENCES**


