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Title: Acute endocrine and force responses and long-term adaptations to same-session combined strength and endurance training in women

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Running head: Order effect in women: loading and training

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ABSTRACT

This study examined acute hormone and force responses as well as 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 one of two 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 one-repetition maximum (1RM), maximal workload during cycling (W_{max}) 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 24h in both groups. The acute growth hormone response was significantly ($p<0.001$) higher after S+E than E+S at both Week 0 and Week 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%), W_{max} (E+S 21±10%, S+E 16±12%) and CSA (E+S 15±10%, S+E 11±8%). This study showed that the acute growth hormone response to combined endurance and strength loadings was significantly larger in S+E compared to E+S both before and after 24 weeks of same-session combined training. Strength and endurance performance and CSA increased to similar extents in both groups during 24 weeks despite differences in the kinetics of growth hormone. Previously untrained women can improve performance and increase muscle CSA utilizing either exercise order.

Keywords: 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, growth hormone and cortisol (18, 25, 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), while 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 remodelling (e.g. 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 testosterone concentrations are reported in women following strenuous resistance exercise protocols (e.g. (9, 19, 29). These limited magnitudes of testosterone responses are likely related to the intensity of exercise and amount of activated muscle mass (10, 26, 29, 32), but may possibly be counterbalanced by acute growth hormone release to meet the anabolic needs of resistance exercise sessions (25).

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 is 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 growth hormone response to subsequent resistance exercise, thus resulting in lower post-exercise concentrations than in the opposite order (16, 39). The findings regarding cortisol and testosterone (4, 37, 39) are less conclusive and may be related to the intensity or volume of

the utilized exercise protocols or the training status of the subjects (4, 16, 39). Since 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 testosterone in men in comparison to 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 following prolonged E+S training (3), recent reports from both men and women show similar strength gains following long-term training (11, 14).

Despite a growing interest towards research regarding concurrent training in female populations (e.g. 11, 40), there is currently paucity in the knowledge regarding hormonal responses to combined exercise sessions in women. Although strength and endurance performance as well as lean mass are likely to increase to a similar extent following training in either order (11), the effects of prolonged training on exercise induced hormonal responses and the relevance for training adaptations has 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. A 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.

METHODS

Experimental approach to the problem

In order 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 cross-sectional area, a 24-week training intervention was conducted. As the focus of this study was to compare training-induced adaptations in acute loading responses, a cross-over design was not used and the subjects performed the experimental loading 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 participated in the present study. Recruitment was conducted by several public postings. Subjects were 1) recreationally physically active but without systematic strength or endurance training for at least 1 year prior to participation, 2) below a body mass index of 30 m^2/kg, 3) non-smokers 4) free from chronic illnesses and injuries and 5) not pregnant or lactating. A resting ECG 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 two 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). Due to organizational constraints, acute loading responses were assessed from 23 subjects (E+S n=12, S+E n=11), while changes in strength and endurance performance as well as muscle cross-sectional area 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 subjects.

Procedures

Prior to 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 day ± 1 h throughout the study. The measurements of maximal strength and endurance performance were separated from each other and the loading measurements by at least two 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 h and 24 h (respectively) prior to the measurements and subjects were required to keep the nutritional intake prior to the measurements similar at 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 three subjects from the S+E groups reported oral contraceptive use.

*** Figure 1 near here ***

Basal measurements

Strength. Maximal bilateral dynamic leg press one-repetition maximum (1RM) was measured using a David 210 weight stack horizontal leg press device (David Health Solutions Ltd., Helsinki, Finland). Three warm-up sets (5 x 70-75%, 3 x 80-85% and 2 x 90-95% of estimated 1RM) with 1 min of rest between sets were performed before the 1 RM trials. Upon

verbal instruction, subjects performed a full leg extension (knee angle 180°) from a starting knee angle of below 60º (58º±2º). After each successful completion the load was increased. Subjects were allowed a maximum of five trials. The trial with the highest completed load was accepted as the 1 RM.

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 watts (W) for all subjects with 25W increments applied every 2 min until volitional exhaustion. Maximal workload (W_{max}) was calculated as $W_{max} = W_{com} + (t/120)*25$ (39), where W_{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 (CSA) 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 50% and 70% of the femur length, i.e. the distance between the greater trochanter and the joint space on the lateral side of the knee. The 10 MHz linear-array probe was moved across the thigh from the medial to the lateral side with the subject lying in a supine position. Leg position was fixed using a Styrofoam support. Lines perpendicular to the measurement table were drawn across the thigh to ensure that the probe was moved in a straight line. Three images were taken at both 50% and 70% of the muscle length. Images were analyzed using ImageJ -software version 1.44 (National Institute of Health, USA) by manually marking the outlines of the muscles onto the image. The mean of the two 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 day (±1h) at Week 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±1h and 48±1h after the cessation of exercise ("24h" and "48h", 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 two loading modes, after the "Mid" blood sample was taken.

Strength loading. A David 210 weight-stack horizontal leg press (David Health Solutions Ltd., Helsinki, Finland) was used to conduct the strength loading. A detachable handle was available for assisting if necessary. The loading consisted of three protocols typically used in training for explosive strength (3x10 repetitions at 40% 1RM with 3 min rest between sets), maximal strength (4x3 repetitions at 75-90% 1RM with 3 min rest between sets) and muscle hypertrophy (4x10 repetitions at 75-80% 1RM with 2 min rest between sets). Loads were calculated from the 1 RM obtained during the basal measurements. Additional resistance was added to at least one maximal and one hypertrophic set in order to complete a true repetition maximum 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% W_{max} (39) on a Monark cycling ergometer (Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) equipped with electric resistance. The intensity was calculated based on W_{max} from the basal measurement. Subjects were instructed to keep the pedalling 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 15W. If the subject was unable to keep up the pace for a full minute after the reduction, the workload was further reduced by 15W. 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 107º (180º representing full extension) (20). 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 3s. At Pre, 24h and 48h subjects were allowed to perform three trials with 1 min rest between. At Mid and Post subjects immediately proceeded to the measurement and performed two trials with only 10s rest between 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, UK), sampled at 2000 Hz and processed with a low-pass filter of 20 Hz. Trials were analyzed for MVC and average force produced between 0 and 500 ms (MVC_{500}).

Blood samples. To determine blood lactate concentrations, capillary blood samples were taken from the fingertip at Pre, Mid and Post into a reaction tube containing an anti-coagulant and 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, 24h and 48h for determination of total testosterone (T), cortisol (C) and growth hormone (GH, 22 kDa) concentrations. Resting concentrations of the same hormones as well as 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, Terum Medical Co, Leuven, Belgium). Samples were centrifuged for 10 min at 3500 rpm, after which serum was removed and frozen until analysed. The hormones were analysed with a chemical luminescence technique (Immulite 1000, Siemens, New York, USA) using hormone-specific immune-assay kits (Siemens, New York, USA). Creatine kinase (CK) was analysed using chemical analysis (KoneLab 20 XTi, 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} and 0.02 nmol l^{-1} and 0.7 mlU l^{-1}, respectively. Intra-assay coefficients of variation for T, C, GH and SHBG 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 haemoconcentration (13), haemoglobin (HGB) and haematocrit (HCR) were analysed with Sysmex KX 21 N (Sysmex America Inc., Mundelein, IL, USA) automated haematology analyser 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 (e.g. 42) and was targeted at improving both maximal strength and endurance performance. During the first 12 weeks,

the subjects completed two weekly sessions of [1E+1S] or [1S+1E] (respective to the assigned training order) and five sessions per two weeks (5x [1E+1S] or [1S+1E]) during weeks 13-24. Time between training modes was 5-10 min and recovery time between training sessions 48-72 h. Training sessions were supervised by research staff. Maintenance of normal daily activity was encouraged.

Strength training mainly targeted knee extensors and flexors as well as 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 x 8-12 at 80-85% of 1RM, 1-2 min rest) towards maximal strength training (2-5 x 3-5 at 85-95% of 1RM, 3-4 min 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 min.

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 min 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 onwards. The interval sessions were initiated and ended with 10-15 minute bouts below the aerobic threshold with 5-minute altering bouts on the anaerobic threshold and below the aerobic threshold in between.

Statistical analysis. Data are presented as means±SD. Statistical analysis for changes during the experimental loadings at Week 0 and 24 was performed using a five-level ANCOVA (i.e. Pre, Mid, Post, 24h and 48h) 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 non-normally distributed even after a log transformation, non-parametric statistics were used both for the within-group changes (Wilcoxon signed-rank test) and between-group comparisons (Mann-Whitney U-test). For the non-parametric 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, 24h and 48h) across 24 weeks, paired-samples t-tests were applied for each measurement point.

Training induced changes in basal hormones and basal measurements of 1 RM, W_{max} and CSA were analyzed with a two-way ANCOVA with baseline-values used as covariates, and between-group differences with an independent-samples t-test. The individual ratios of changes in 1 RM and W_{max} were calculated as the percentage change in 1 RM divided by the percentage change in W_{max}.

Reported effect sizes (ES) are Cohen's d except for non-normally distributed data, where ES was defined as Z-score/$\sqrt{n}$. Associations between the 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 non-normally distributed data. A trend was accepted for p-values <0.06.

RESULTS

Training adherence was 99% in both E+S and S+E. 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.

MVC decreased significantly during the loading in both groups by Mid (E+S -18±13% from 1740±235 N, P<0.01, ES=-1.305; S+E -17±7% from 1810±633 N, P<0.01, ES=-0.515) and by Post (E+S -20±11%, P<0.001, ES=-1.587; S+E -18±5% P<0.001, ES=-0.532) (Figure 2). MVC_{500} 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 24h or 48h.

*** Figure 2 near here ***

A significant increase in T was observed in E+S at Mid (from 0.5±0.4 to 8.9±1.1 $nmol \cdot l^{-1}$, P<0.05, ES=0.513) (Figure 3). C 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 24h and 48h, C was significantly lowered from Pre for S+E (24h: -29±23%, P<0.01, ES=-1.53 and 48h: -29±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).

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). CK was significantly elevated in comparison to Pre in E+S at Mid (13±9% from 93±21 $mlU \cdot l^{-1}$, P<0.01, ES=0.544), Post (16±9%, P<0.01, ES=0.681) and 24h (57±32%, P<0.05, ES=1.73) and for S+E at Post (26±17% from 95±31 $mlU \cdot l^{-1}$, P<0.01, ES=0.679) but not at 24h (96±89%, ES=1.29) (Table 1). CK further increased in both groups between Post and 24h (E+S P<0.05, ES=1.337 and S+E P<0.05, ES=0.935). No between-group differences were observed during loading or recovery.

*** Figure 3 and 4 near here ***

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 the loading.

MVC decreased significantly for both groups by Mid (E+S -16±9% from 1833±322 N, $P<0.001$, ES=-0.890; S+E -21±8% from 1966±690 N, $P<0.001$, ES=-0.623) and by Post (E+S -24±6%, $P<0.001$, ES=-1.66; S+E -22±8%, $P<0.01$, ES=-0.731) (Figure 2). MVC_{500} decreased significantly for both groups by Mid (E+S -18±17% $P<0.05$, ES=-0.683 and S+E -20±12%, $P<0.05$, ES=-0.678) and post (E+S -24±6%, $P<0.001$, ES=-1.43 and S+E -25±23% $P<0.05$, ES=-0.637). Both groups recovered significantly ($P<0.05$-0.001) from Post to 24h and 48h. No between-group differences were observed during loading or recovery.

A significant increase in T was found in E+S at Mid (from 0.8±0.3 to 1.2±0.4 $nmol \cdot l^{-1}$, $P<0.05$, ES=1.247) and S+E at Post (from 0.9±0.9 to 1.5±1.2 $nmol \cdot l^{-1}$, $P<0.001$, ES=0.536) (Figure 3). For S+E, T significantly increased from Mid to Post ($P<0.01$, ES=0.371). C was decreased for S+E from Pre at 48h (-28%, $P<0.001$, ES=-0.974) and near-significantly decreased at 24h (-26%, $P=0.051$, ES=-0.8254) in comparison to Pre (Table 1). A significant increase in GH was noted for both groups at Mid (E+S 7.0-fold from, $P<0.01$, ES=0.885 and S+E 2.5-fold, $P<0.05$, ES=0.790) and at Post for S+E (3.6-fold, $P<0.05$, ES=0.886) (Figure 4). For E+S a decrease took place from Mid to Post (from 68.3±31.7 to 14.7±11.3 $mlU \cdot l^{-1}$, $P<0.01$, ES=-0.885). A between-group difference was observed at Mid ($P<0.01$), and the change from Mid to Post was significantly different between groups ($P<0.001$).

Blood lactate increased 5-fold for E+S by Mid ($P<0.001$, ES=3.03) and 6-fold for S+E ($P<0.001$, ES=3.24) (Table 1). Lactate at Post was increased 5-fold for E+S ($P<0.001$,

ES=4.81) and 5.5-fold for S+E (P<0.001, ES=3.23) (Table 1). CK was significantly (P<0.05) elevated in comparison to Pre for S+E at Mid (14±9%, from 108±72 $mlU \cdot l^{-1}$, P<0.001, ES=0.169) and Post (25±17%, P<0.01, ES=0.299), but not at 24h or 48h (Table 1). No between-group differences were observed during loading or recovery.

*** Table 1 near here ***

Differences in the acute loading responses before and after training

In E+S, the GH response was significantly different at Pre-Mid (P<0.01), Pre-Post and Mid-Post (P<0.05) in comparison to the corresponding changes at Week 0 (Figure 4). The relative change in blood lactate was significantly larger after 24 weeks of training than the corresponding change at Week 0 for S+E during Pre-Mid (P<0.01), Pre-Post (P<0.05) and Mid-Post (P<0.05) (Table 1).

Basal measurements

Both groups increased 1RM (E+S by 13±12% from 102±21 kg, P<0.001, ES=0.569 and S+E by 17±10% from 99±18 kg, P<0.001, ES=0.884) (Figure 5), W_{max} (E+S by 21±10% from 170±26 W, P<0.001, ES=1.36 and S+E by 16±12% from 182±27 W, P<0.001 ES=1.05) and CSA (E+S by 15±10% from 17±2cm^2, P<0.001, ES=1.32 and S+E by 11±8% from 19±3cm^2, P<0.001, ES=0.680). Basal hormone concentrations are presented in Table 2.

Correlations

No significant correlations were observed between the acute changes in testosterone, cortisol or growth hormone and long-term 1RM, W_{max} or CSA development in either E+S or S+E. Basal levels of T, T/SHBG and T/C were not correlated with changes in 1 RM or W_{max} or CSA.

*** Figure 5 and Table 2 near here ***

DISCUSSION

The main findings of the present study were that following the experimental loading at Week 0, significantly elevated serum GH was observed only in S+E, while 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 24. Additionally, muscle force production was recovered by 24 h following 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, W_{max} and muscle cross-sectional 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 following 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 following hypertrophic type protocols with a large stress on the metabolic system (26,

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 (Week 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 haemoconcentration 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 following 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 byproduct of cortisol 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 testosterone and did also not detect any significant elevations in cortisol 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 e.g. the time course of androgen receptor regulation (44) and reduced hepatic clearance as observed in men (5). Haemoconcentration as an indirect cause for elevated T can likely be ruled out in the present study due to unchanged hemoglobin and hematocrit during loading. It is also possible that oral contraceptive use affects the secretion of T as well as 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.

Similarly 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 following the strength loading. This pattern in the kinetics of exercise-induced growth hormone release was significantly different between groups both before and after training as S+E demonstrated elevated GH throughout the 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 through 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).

Due to 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 W_{max} in order 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 to corresponding time points at Week 0. In the S+E order, despite the fact that the magnitude 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, while the adaptations in GH release seem to indicate that the loadings were still strenuous at Week 24, changes in the behaviour of CK may suggests better tolerance of the experimental loading. CK 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, similarly 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 e.g. through examining additional forms of GH than solely the present 22 kDa variant. While 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 growth hormone (27). This may in part explain why the GH responses in the present study were not related to the changes in muscle cross-sectional area in either group and warrants further investigation of the mechanisms of several GH variants both during combined strength and endurance loadings as well as prolonged combined training.

In addition to a lack of a relationship between changes in muscle cross-sectional area and the magnitudes of acute GH release, we also observed no associations between acute responses of T and long-term performance adaptations. While 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 following pure strength training (e.g. 22, 31). Furthermore, previously reported correlations of basal levels of T and T/SHBG-ratios and strength development and changes in CSA following strength training in women (e.g. 17, 21) were not found in 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 MVC_{500} were of similar magnitudes following both loading conditions both before and after training. Furthermore, as neither MVC nor MVC_{500} were no longer significantly depressed from Pre-loading values by 24h, 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 48-72h. Even though we only monitored recovery before and after 24 weeks of training, the loads utilized in the experimental loading 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 the 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, to other protocols should be done with caution. Interestingly, while no between-group differences were observed in 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 as well as muscle cross-sectional area following 24 weeks of same-session combined strength and endurance training were similar in both exercise orders. Therefore, our data indicates 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 growth hormone responses to exercise were significantly larger in S+E compared to E+S both before and after the training, this was not reflected in or associated with the long-term adaptations. Consequently, the gains in strength and endurance performance as well as muscle size were of similar magnitudes in the two training groups following 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.

Captions and legends for figures

Figure 1. Overview of the experimental design. E+S = Endurance preceding strength, S+E = Strength preceding endurance.

Figure 2. MVC for E+S and S+E during loading and recovery at Week 0 and at Week 24. Within-group differences: *=Significant from Pre, §=Significant from Post. **P<0.01, ***P<0.001, § P<0.05, §§ P<0.01 and §§§ P<0.001.

Figure 3. Responses in total testosterone for E+S and S+E during loading and recovery at Week 0 and at Week 24. Within-group differences: *=Significant from Pre, +=Significant from Mid, §=Significant from Post. *P<0.05, ***P<0.001, +P<0.05 and §§§ P<0.001.

Figure 4. Growth hormone responses for E+S and S+E during loading at Week 0 and at Week 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<0.05. **P<0.01, +P0.05, +P<0.01, •P<0.05, ••P<0.01, #P<0.05, ##P<0.01 and ###P<0.001.

Figure 5. Changes in 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.

Captions and legends for tables

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 Week 0 and 24. Within-group differences: *=significant from Pre, +=significant from Mid, §=significant from Post, ¤ = significant from 24 h, •=significant from corresponding value at Week 0. *P<0.05. **P<0.01, ***P<0.001, +P0.05, +P<0.01, •P<0.05, ••P<0.01, ¤ P=0.05, § P<0.05. §§ P<0.01, §§§ P<0.001.

Table 2. Basal serum concentrations of total testosterone (T), cortisol (C), growth hormone (GH), sex-hormone binding globulin (SHBG), and ratios of testosterone and cortisol (T/C) and testosterone and sex-hormone binding globulin (T/SHBG) before and after the training intervention.

* within-group difference to Week 0; *P<0.05 and ***P<0.001

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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 Week 0 and 24. Within-group differences: *=significant from Pre, +=significant from Mid, §=significant from Post, ¤ = significant from 24 h, •=significant from corresponding value at Week 0. *P<0.05. **P<0.01, ***P<0.001, +P0.05, +P<0.01, •P<0.05, ••P<0.01, ¤ P=0.05, § P<0.05. §§ P<0.01, §§§ P<0.001.

	W E E K 0		**W E E K 2 4**	
	E+S	**S+E**	**E+S**	**S+E**
C (nmol·l^{-1})	*(n=10)*	*(n=10)*	*(n=8)*	*(n=10)*
Pre	397±128	479±105	413±154	616±178
Mid	437±176	367±139	471±96	567±199
Post	426±207	435±211	423±108	778±252
24 h	312±111 [*] p=0.051	332±87 **	284±134	459±200 [*] p=0.051§§
48 h	347±141	333±60 **	322±116	445±170*** §§§
CK (mlU·l^{-1})	*(n=9)*	*(n=9)*	*(n=9)*	*(n=9)*
Pre	93±21	95±31	103±44	109±72
Mid	103±19 **	106±32	121±49	121±71 ***
Post	105±17 **	118±35 ** +	123±55	131±75 **
24 h	143±36 *	181±89 §	145±80	148±81
48 h	128±38	132±55 ¤	141±116	123±70
La (mmol·l^{-1})	*(n=12)*	*(n=11)*	*(n=12)*	*(n=11)*
Pre	1.1±0.3	1.3±0.3	1.2±0.4	1.1±0.4
Mid	4.7±1.0 ***	5.0±1.9 ***	6.3±2.3 ***	6.8±2.4 *** ••
Post	5.6±2.2 ***	5.2±2.3 ***	6.5±1.5 ***	6.0±2.1 *** •

Table 2. Basal serum concentrations of total testosterone (T), cortisol (C), growth hormone (GH), sex-hormone binding globulin (SHBG), and ratios of testosterone and cortisol (T/C) and testosterone and sex-hormone binding globulin (T/SHBG) before and after the training intervention.

* within-group difference to Week 0; *P<0.05 and ***P<0.001

	WEEK 0		**WEEK 24**	
	E+S	S+E	E+S	S+E
T (nmol·l^{-1})	0.6±0.3	0.6±0.4	1.1±0.3 ***	1.3±0.9 ***
C (nmol·l^{-1})	546±186	564±185	614±225	673±136 *
GH (mlU·l^{-1})	7.9±12.1	11.0±10.0	6.1±11.1	14.0±20.6
SHBG (nmol·l^{-1})	52.3±17.5	65.3±42.0	68.6±12.7 *	81.6±43.4 *
T/C *10^{3}	1.4±1.1	1.3±1.0	1.9±1.1 *	2.1±1.8 *
T/SHBG *10^{3}	15.3±8.8	13.1±10.7	17.3±6.7	21.5±11.4