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The order effect of combined endurance and strength loadings on force and hormone responses: Effects of prolonged training

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Running title: Training-induced responses to combined loadings

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ABSTRACT

Purpose: To examine acute responses and recovery of force and serum hormones to combined endurance and strength loadings utilizing different orders of exercises before and after training.

Methods: Physically active men were matched to an order sequence of endurance+strength (E+S, n=12) or strength+endurance (S+E, n=17). The subjects performed one experimental loading consisting of steady-state cycling and a leg press protocol before and after 24 weeks of order-specific combined training.

Results: No between-group difference in acute reductions of force was observed at week 0 (E+S -23%, $p<0.001$; S+E -22%, $p<0.01$) and 24 (E+S -25%, $p<0.001$; S+E -27%, $p<0.001$) and recovery in force was completed after 24h in both groups. Concentrations of growth hormone (22-kDa) increased post-acute loading at week 0 (E+S, +57 fold, $p<0.05$; S+E, +300 fold, $p<0.001$; between-groups $p<0.001$) and 24 (E+S, +80 fold, $p<0.01$; S+E, +340 fold, $p<0.05$; between-groups $p<0.05$). No significant acute responses in concentrations of testosterone were observed at week 0 or 24. However, at week 0 testosterone was reduced during recovery following the E+S loading only (24h -23%, $p<0.01$; 48h -21%, $p<0.001$; between-groups at 24h and 48h, $p<0.05$), but was no longer observed after training. 1RM strength improved similarly in E+S (13%, $p<0.001$) and S+E (17%, $p<0.001$).

Conclusions: This study showed an order effect (E+S vs. S+E) in concentrations of testosterone during 2 days of recovery at week 0, which was diminished after training at week 24. This initial difference in testosterone concentrations during recovery did not seem to be associated with strength development.

KEY WORDS: fatigue; testosterone; recovery; endurance cycling; concurrent training; combined training; training adaptations

LIST OF ABBREVIATIONS

C	-	cortisol
CK	-	creatine kinase
E	-	endurance
ECG	-	electrocardiogram
ES	-	effect size
E+S	-	endurance+strength
GH	-	growth hormone (22-kDa)
MVC_{max}	-	maximal isometric bilateral leg press force
S	-	strength
SD	-	standard deviation
S+E	-	strength+endurance
T	-	testosterone
TSH	-	thyroid stimulating hormone
$\dot{V}O_{2max}$	-	maximal oxygen consumption
1RM	-	one repetition maximum

INTRODUCTION

Acute responses to exercise loading create the biological foundation for the development of chronic adaptations (Kraemer and Ratamess 2005). While the magnitude of loading-induced stress can be quantified by temporary declines in performance and biological function, the anabolic and catabolic processes of tissue remodeling following exercise loadings are typically reflected by acute and chronic changes in hormonal concentrations (Hackney and Viru 2008). Due to the important biological functions for tissue growth and degradation, concentrations of testosterone (T), growth hormone (GH), thyroid stimulating hormone (TSH) and cortisol (C) are often utilized as indicators of loading induced tissue remodeling (Kraemer et al. 1990, Häkkinen and Pakarinen 1993, Hackney et al. 2012).

The magnitude of both endurance (E) and strength (S) loading-induced hormonal responses in men depend on the intensity and volume, as well as the exercise mode performed. Short bouts of high intensity endurance loadings may induce acute elevations in both anabolic (e.g. T, TSH, GH) and catabolic (e.g. cortisol) hormone concentrations (Pritzlaff et al. 1999, Hackney et al. 2012), while prolonged and physically demanding endurance performance (e.g. a marathon run) may in its final phases lead to decreases in testosterone and simultaneous increases in cortisol concentrations (Kuoppasalmi et al. 1980).

On the other hand, strength loading protocols utilizing heavy resistance, combined with short inter-set rest periods (i.e. hypertrophic strength loadings), result in acute increases in serum testosterone and GH, as well as cortisol concentrations (Kraemer et al. 1990). However, maximal strength loadings with high loads and low number of sets as well as explosive strength protocols utilizing maximal movement velocity typically require a prolonged inter-set recovery and may not be sufficiently physiologically demanding to induce as large increases in concentrations of anabolic or catabolic hormone concentrations (Kraemer et al. 1990; Häkkinen and Pakarinen 1993; Linnamo et al. 2005).

When combining endurance and strength loadings into one training session the question of exercise order arises (i.e. endurance+strength [E+S] vs. strength+endurance [S+E]). Previous studies have emphasized the sensitivity of strength performance to preceding endurance loadings (Leveritt and Abernethy 1999; Lepers et al. 2008), leading to reduced force production and possibly compromised long-term adaptations when compared to the

reverse loading order (Chtaha et al. 2008). Furthermore, it has been shown that force and hormone responses to combined loadings depend on the training status of the subjects and the specificity of the combined protocol performed (Cadore et al. 2012; Schumann et al. 2013; Taipale and Häkkinen 2013). In physically active men, a recent cross-sectional study showed reduced serum testosterone concentrations during a recovery period of (at least) 2 days when the strength loading was immediately preceded by endurance cycling (Schumann et al. 2013). However, the possible influence of prolonged training on acute force and hormone responses as well as the biological effects of acute loading-induced endocrine changes on long term strength development remains to be investigated.

Therefore, the purpose of the present study was to investigate acute responses and recovery of force and serum hormone concentrations (i.e. T, TSH, GH and C) to a combined endurance and strength loading protocol with different loading orders (E+S vs. S+E) performed before and after 24 weeks of combined training. A secondary purpose of this study was to examine whether loading order-induced differences in these acute responses are related to strength development.

In agreement with the above mentioned previous findings, it was postulated that a combined endurance and strength loading protocol typically utilized by physically active subjects (i.e. endurance cycling of moderate intensity and rather short duration and a mixed maximal, explosive and hypertrophic leg press protocol) may only lead to modest acute increases in anabolic and catabolic hormone concentrations (e.g. Häkkinen and Pakarinen 1993; Linnamo et al. 2005) but would still indicate loading order specific differences in hormonal responses (Schumann et al. 2013). Based on this assumption it was hypothesized that performing endurance cycling immediately before a strength loading protocol (E+S) would lead to less favorable hormonal responses when compared to the reverse loading order (S+E) and that this loading specific difference would be maintained after long-term training. Thus, it was further hypothesized that 1RM strength may be developed to a lesser extent in the E+S group compared to S+E training group.

METHODS

Subjects

Forty-two physically active men volunteered to participate in this study. The subjects were free of acute and chronic illness, disease and injury and reported not using medication that would contraindicate the performance of intense physical activity or affect endocrine metabolism and neuromuscular function. A standardized phone interview was conducted to initially assess subjects' health and activity status. The subjects reported to perform light physical activity such as walking, cycling or occasionally team sports for not more than 3 times per week but did not train systematically for endurance or strength training prior to inclusion into the study. Verbal and written instructions about the study procedures and possible risks were provided to the subjects before giving informed consent. In addition, a completed health questionnaire and resting ECG measurement were reviewed by a cardiologist prior to the first exercise testing and training. Following the pre-screening process subjects were matched according to age and physical performance at baseline to either of two training groups: Endurance+Strength (E+S n=21) or Strength+Endurance (S+E n=21). To be included in the data analysis, subjects were required to complete at least 90% of the supervised training sessions prescribed during a 24-week training period. Thus, out of the 42 originally recruited subjects, 13 subjects did not complete the study, mostly due to personal reasons (i.e. occupational changes) possibly attributed to the exceptional length of the study period. The demographic characteristics of the remaining 29 subjects (E+S n=12; S+E n=17) included in the data analysis were as follows (mean±SD): E+S age 30±5 years, height 179±6 cm, body mass 79±10 kg; S+E age 30±5 years, height 179±5 cm, body mass 75±9 kg. The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the ethics committee at the University of Jyväskylä.

Experimental design

To investigate the training adaptations in acute responses and recovery to combined endurance and strength loadings with different loading orders (i.e. endurance + strength [E+S] vs. strength + endurance [S+E]), a longitudinal research design was used and loading-specific responses and recovery patterns of force production and hormonal concentrations determined before and after the combined training of 24 weeks (Fig 1). As this study directly compared the order effect, no control group was included. Before the experimental loading, subjects were familiarized with the measurement procedures (day 1) and tested for baseline endurance (day 2) and strength (day 3) performance. Thereafter, all subjects performed one experimental session of combined

endurance and strength loadings in the order of the corresponding group (E+S or S+E) and returned to the laboratory for recovery measurements at 24h and 48h (Fig 1). To allow for sufficient recovery, all testing sessions (except for recovery measurements) were separated by at least 48h. Both the baseline and the experimental loading and recovery measurements were repeated after 24 weeks of combined training in the loading order specific to the corresponding group. Due to financial and time constraints, a cross over design was not possible and each group performed only one experimental loading both before and after the training (i.e. only E+S or S+E).

+++ *Figure 1 somewhere near here* +++

Strength and endurance loading

The strength and endurance loading protocols have been described in detail elsewhere (Schumann et al. 2013). Briefly, the strength loading (30min) was performed on a dynamic leg press device (David 210, David Health Solutions Ltd., Helsinki, Finland) and included sets aimed for explosive strength (3x10 repetitions at 40% of 1RM with 3 min rest between sets), maximal strength (1x3 repetitions at 75% of 1RM and 3x3 repetitions at 90% of 1RM with 3 min rest between sets) and muscle hypertrophy (1x10 repetitions at 75% of 1RM and 3x10 repetitions at 80-85% of 1RM with 2 min rest between sets). The loads were derived from subject's individually determined 1RM (at week 0 and 24, respectively) but additional load was added or assistance provided to achieve at least one set of a true repetition maximum during the maximal and hypertrophic sets (i.e. 3RM and 10RM, respectively). Based on both previous literature (Cadore et al. 2012) and a pilot study, the endurance loading was conducted on a cycle ergometer (Ergomedic 839E, Monark Exercise AB, Varberg, Sweden) over 30 minutes of steady-state cycling at 65% of subjects' individual maximal aerobic power (Watts), determined during an incremental ergometer test at week 0 and 24, respectively. Subjects were required to keep pedaling frequency constant at 70 rpm but for instances when the subjects failed to keep up the required frequency, intensity was reduced by 15 Watts every minute until the subject could complete the loading.

Baseline and loading measurements

To control the experimental conditions, subjects received both verbal and written instructions about the measurement preparation in order to minimize physical and mental stress and to allow for at least 7-8h of sleep on the day before as well as throughout the baseline and loading measurements. In addition, to assure the resting state of the subjects basal morning concentrations of serum hormones and creatine kinase (CK) were determined by drawing venous blood samples on the days of the experimental sessions (at week 0 and 24, respectively) after 12h of fasting, between 7:00 a.m. and 9:00 a.m.

Within the experimental loading sessions (at week 0 and 24, respectively), maximal isometric strength (horizontal bilateral isometric leg press) and concentrations of serum hormones (T, TSH, GH and C), creatine kinase (venous blood samples), and blood lactate (capillary blood) were determined. In order to obtain acute changes in these variables, force measurements and blood samplings were conducted at the following time points (Fig 1); prior to the start of the experimental loading (PRE), immediately following the first loading (MID, after the endurance or strength loading, respectively) as well as immediately after the completed combined session (POST). In addition, recovery of force as well as hormone (T, TSH and C) and CK concentrations were measured after 24h and 48h at ±1h from the end of each completed session. To control for circadian variations in force production and hormone concentrations, experimental loading and recovery measurements of each subject were performed at the same time of day with an accuracy of ±1h at week 0 and 24, respectively. The testing times of the experimental loadings at week 0 were (mean±SD): E+S 9:27 a.m. ± 1:38h; S+E 9:12 a.m. ± 2:25h. The corresponding recovery measurements in both groups were (mean±SD): at 24h in E+S 11:48 a.m. ± 1:45h; in S+E 11:29 a.m. ± 2:23h; at 48h in E+S, 11:48 a.m. ± 1:45h; in S+E, 11:25 a.m. ± 2:22h).

Isometric leg press: Maximal isometric bilateral leg press force (MVC_{max}) was measured on a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Finland) in a seated position at a hip and knee angle of 110 and 107 degrees, respectively (Häkkinen et al. 1998). On verbal command, subjects were instructed to produce maximal force as rapidly as possible with the entire foot against the force plate and maintain maximal tension for 3-4 seconds (as observed from the force trace by the researcher). During the execution of each maximum trial, subjects were required to grasp handles located by the

seat of the dynamometer, as well as to keep constant contact with the seat and the backrest and verbal encouragement was given to promote maximal effort. Prior to the start of the experimental loading session, as well as at both recovery measurements (at 24h and 48h), three trials separated by a resting period of 1 minute were conducted. If the maximum force during the last trial was greater than 5% compared to the previous trial, an additional attempt was performed. To assess acute force responses, at MID (after E or S in each experimental loading, respectively) and POST, **only two** maximal isometric trials were performed and **separated** by only 10-15 seconds. The best performance trial in terms of maximal force measured in Newtons, at PRE, MID, POST, 24h and 48h was used for statistical analysis. The force signal was low-pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK).

One repetition maximum: Subjects' one repetition maximum (1RM) of leg extensors was determined using a seated dynamic horizontal leg press (David 210, David Health Solutions, Helsinki, Finland). Prior to attempting 1RM, subjects completed a warm up consisting of 5 repetitions **at** 70% of the estimated maximal load, 2 repetitions at 80-85% and 1 repetition at 90-95% with 1 minute rest between the sets (i.e. 3 warm up sets). Following this warm up, no more than 5 trials were allowed to achieve 1RM. The starting knee angle for all subjects was (mean±SD) 58 ± 2 degrees. Subjects were instructed to grasp the handles located by the seat of the dynamometer and to keep constant contact with the seat and backrest during complete extension to 180 degrees knee angle. To promote maximal effort, verbal encouragement was given. The greatest weight that the subject could successfully lift (knees fully extended) at an accuracy of 1.25 kg was accepted as 1RM.

Maximal power output: Aerobic power and maximal oxygen consumption were determined during a graded cycle ergometer test (Ergometrics 800, Ergoline, Bitz, Germany). The initial load for all subjects was 50 Watts and was increased by 25 Watts every 2 minutes. Heart rate was monitored throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 seconds at each stage. Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). On each testing day, air flow calibration was performed using a manual flow calibrator and the gas analyzer was calibrated using a certified gas mixture of 16% O_2 and 4% CO_2. The $\dot{V}O_{2max}$ was taken as the highest 60-s $\dot{V}O_2$ value. To assure that $\dot{V}O_{2max}$ was achieved, other criteria such as heart rate, blood lactate and

respiratory exchange ratio (RER) were monitored throughout the test. Aerobic power (Watts) used for the determination of the endurance intensity during the experimental loadings was calculated using the equation: $W_{max}= W_{com}+(t/120)*25$, where W_{com} is the load of the last completed stage and t is the time of the last incomplete stage. Subjects' individual aerobic and anaerobic thresholds used to determine intensities for the endurance training were determined using deflection points obtained by plotting the curves of blood lactate, ventilation, oxygen consumption and production of carbon dioxide (Aunola and Rusko 1986).

Venous blood samples and blood lactate: Venous blood samples (10 ml) for the determination of serum hormone concentrations and CK were collected by a qualified lab technician, using sterile needles into serum tubes (Venosafe, Terum Medical Co., Leuven, Belgium). Whole blood was centrifuged at 3.500 rpm (Megafuge 1.0 R, Heraeus, Germany) for 10 minutes after which serum was removed and stored at -80°C until analysis (approximately 4-8 weeks). Analysis of total serum testosterone, TSH, GH (22-kDa) and cortisol were performed using chemical luminescence techniques (Immunlite 1000, Simens, New York, USA) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones were: T 0.5 $nmol \cdot l^{-1}$, TSH 0.004 $mlU \cdot l^{-1}$, GH 0.03 $mlU \cdot l^{-1}$ and C 5.5 $nmol \cdot l^{-1}$. The intra-assay coefficients of variation for T, TSH, GH and C were 8.7±2.7%, 7.1±4.6 %, 6.0±0.5% and 7.1±1.1%, respectively. The inter-assay coefficients of variation for T, TSH, GH and C were 10.6±3.2%, 11.1±4.3%, 5.8±0.3% and 7.9±1.2%, respectively. While being aware that loading induced changes in plasma volume shift may influence hormonal concentrations (Kargotich et al. 1998), we believe that the concentrations of hormones the receptors are exposed to are most critical for the initiation of tissue remodeling (Kraemer and Ratamess 2005). Therefore, plasma volume changes were estimated from changes in hematocrit and hemoglobin (Dill and Costill 1974) but were not used to correct obtained serum hormone concentrations.

Capillary blood samples for the determination of blood lactate concentrations were taken from the fingertip at the described time points. The amount of 20 µl of blood was inserted into pre-filled reaction capsules containing a hemolyzing agent and blood lactate concentrations were analyzed using a Biosen lactate analyzer (C_line Lab+, EKF, Magdeburg, Germany).

Training

Subjects were asked to maintain their habitual physical activity (light walking, cycling and occasional team sports) throughout the study period. In addition to training diaries completed during all prescribed training sessions, subjects were asked to record recreational physical activity in a standardized activity log.

The training was designed to reflect a program typically recommended for physically active populations (Thompson et al. 2010). The main objective was to improve both endurance and strength performance through a periodized program including both moderate and vigorous intensity aerobic loadings (Helgerud et al. 2007, Daussin et al. 2007) combined with hypertrophic and maximal strength loading protocols (Kraemer and Ratamess 2004). To assure the correct execution of the training prescribed, all training sessions were supervised by qualified instructors.

In order to familiarize the subjects with the equipment and exercises to be used during the consecutive 24 weeks of training, a 1-week preparatory period was conducted prior to the start of the experimental loading sessions and training. During the first 12 weeks of training, the subjects performed according to their corresponding training group either 2x [1E+1S] or 2x [1S+1E]) per week. During the second 12 weeks, the frequency was increased so that 2 combined training sessions were performed in every 1^{st} and 4^{th} week and 3 combined training sessions in every 2^{nd} and 3^{rd} week (i.e. 2x [1E+1S] or 2x [1S+1E] or 3x[1E+1S] or 3x [1S+1E], respectively).

The strength training program included exercises for all major muscle groups with special consideration to the lower extremities. Exercises for the lower body consisted of bilateral dynamic leg press, as well as both bilateral (weeks 1-7 and 13-18) and unilateral (weeks 8-12 and 19-24) dynamic knee extension and flexion. Additional exercises for the upper body included shoulder press and lat-pull down, as well as exercises commonly used to improve core stability. The overall duration of the strength loading within each combined training session was 30-60 min. During weeks 1-2, all exercises were conducted as a circuit using 2-4 sets of 15-20 repetitions at an intensity of 40-60% of 1RM. During the following 10 weeks of training, protocols aiming for muscle hypertrophy (2-5 x 8-10 repetitions at 80-85% of 1RM, 1.5-2 min rest between the sets) and maximal strength (2-5 x 3-5 repetitions at 85-95% of 1RM, 3-4min rest between the sets), as well as during the last 2 weeks protocols targeting explosive power (2 x 8-10 repetitions at 40% of 1RM, 3-4 min rest between the

sets) were incorporated into the training program. During the second 12-week period, the strength training program was further intensified by increasing both training volume and frequency while the major program structure was maintained. The strength training loads were controlled by the number of repetitions and execution velocity and increased progressively throughout the two 12-week periods.

Endurance training was performed on a cycle ergometer. The intensity was controlled by heart rate zones determined from subjects' individual aerobic and anaerobic threshold obtained during the baseline measurement at week 0 and 24. Subjects were asked to maintain a constant pedalling frequency at about 70-80 rpm during each training session, while the magnetic resistance of the ergometer was used to achieve the prescribed cycling intensity. The endurance program consisted of both steady-state and interval exercise sessions while the intensity was progressively increased from low (below the aerobic threshold) to high (above the anaerobic threshold) throughout both 12-week periods. The duration of cycling was between 30 and 60 min per combined session, leading to a total duration of 60-120 min for each combined training session (i.e. E+S and S+E, respectively).

Nutrition

To control nutritional intake, subjects received both verbal and written nutritional recommendations and were asked to maintain dietary intake constant throughout the 24 weeks of training. In preparation for all baseline and loading measurements, subjects were required to consume a light meal 2-3 h prior to the start of each test-session or experimental loading and asked to keep nutritional intake prior to the measurements similar at week 0 and 24. Furthermore, to control for hydration status during each experimental loading, subjects were instructed to begin each combined loading in a hydrated state and were allowed to ingest 2 dl of water during the combined loading at MID, immediately after the venous blood sample was taken.

Statistical analyses

Within- and between-group analyses were conducted in order to investigate 1) acute loading responses and recovery before the training intervention, 2) acute loading responses and recovery after 24 weeks of training and 3) training- or loading-induced changes in acute loading responses and recovery. Data are presented as

mean±SD and shown as relative changes from the pre-loading values unless indicated. All baseline and pre-loading data obtained before the training intervention were checked for normality. Concentrations of serum CK and GH were not normally distributed even after log transformation. Therefore, data of CK and GH were analyzed using non-parametric tests for all within- (Wilcoxon signed-rank test) and between-group (Mann-Whitney U-test) comparisons using Bonferroni adjustments by multiplying all pair-wise p-values with the number of comparisons. Within-group differences for all remaining variables before (week 0) and after (week 24) the training were analyzed with absolute values using repeated measurement analysis of co-variance (ANCOVA) with 5 levels (PRE, MID, POST, 24h and 48h). Training- or loading-induced within-group differences were analyzed by a paired t-test using relative changes (week 24 vs. week 0). Between-group differences were analyzed by an independent t-test using relative changes. The statistical significance for all tests was set at 0.05, where *=$p<0.05$, **=$p<0.01$ and ***=$p<0.001$ and effect size (ES) for both within and between-group comparisons is reported as Cohen's d (cliff's delta for CK and GH). Statistical analysis was conducted using IBM SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The training adherence was 99% in both the E+S and S+E groups. All subjects completed at least 90% of the prescribed training sessions. Baseline endurance and strength performance as well as basal concentrations of serum hormones and CK at week 0 and 24 are presented in Table 1.

+++ Table 1 somewhere near here +++

Both the E+S and S+E group significantly increased 1RM strength after 24 weeks of training (E+S +13±8%, $p<0.05$, ES=0.683; S+E +17±12%, $p<.0.05$, ES=0.998). No significant between-group difference in 1RM strength development was found.

Acute loading responses at week 0

Maximal force production

In E+S, MVC_{max} was significantly decreased at MID (-11±7%, $p<0.01$, ES=-0.773) and further decreased at POST (-23±12%, $p<0.001$, ES=-1.453) compared to PRE (Fig. 2a). In S+E, MVC_{max} significantly decreased at

MID (-20±13%, p<0.001, ES=-0.848) and remained reduced at POST (-22±9%, p<0.005, ES=-0.878) compared to PRE. The relative change at MID was significantly larger in S+E compared to E+S (-20±13% vs. 11±7%, p<0.05, ES=0.867). No significant between-group difference was found at POST. Both E+S and S+E significantly recovered from POST to 24h (E+S ES=1.161; S+E ES=0.753) and 48h (E+S ES=1.342; S+E ES=0.698), respectively, so that the MVC_{max} values obtained at 24h and 48h of recovery were not statistically different from PRE (p>0.05).

+++ Fig 2a and 2b somewhere near here +++

Serum hormone concentrations

Concentrations of serum T (Fig 3a) at MID were significantly increased in E+S only (+13±6%, p<0.05, ES=0.438) and did not statistically differ from PRE in either of the two groups at POST. The increase of serum T in S+E from MID to POST was significant (+17±18%, p<0.05, ES=0.517). A significant between-group difference was observed at MID (18%, p<0.05, ES=1.003) but not at POST. During recovery, concentrations of serum T decreased in E+S at 24h and 48h compared to PRE (at 24h -23±14%, p<0.01, ES=-0.834; at 48h -21±11%, p<0.001, ES=-0.884) but were not significantly different from PRE in S+E. The difference between E+S and S+E observed at 24h and 48h was significant (at 24h -23±14% vs. -1±32%, p<0.05, ES=0.891; at 48h -21±11% vs. -4±21%, p<0.05, ES=1.011).

+++ Fig 3a and 3b somewhere near here +++

Concentrations of serum TSH remained statistically unaltered during the two loadings at MID and POST. During recovery at 24h and 48h, serum TSH significantly decreased at 24h in E+S (-33±13%, p<0.001, ES=-1.317) and at 48h in S+E (-24±27%, p<0.01, ES=-0.582) compared to PRE. No significant between-group difference in acute responses or recovery was observed.

Concentrations of serum GH (Table 2) significantly increased in the two loadings at MID (E+S +250 fold p<0.01, ES=0.972; S+E +49 fold, p<0.01, ES=0.734) and POST (E+S +57 fold p<0.05, ES=0.888; S+E +300 fold, p<0.001, ES=0.953) compared to PRE. A significant between-group difference was observed at MID (p<0.05, ES=0.552) and POST (p<0.001, ES=0.719).

+++ Table 2 somewhere near here +++

Concentrations of serum C remained statistically unaltered during the two loadings at MID and POST (Fig. 4a). The increase from MID to POST in S+E was significant (+47±36%, $p<0.001$, ES=1.385). During recovery of 24h and 48h, concentrations of serum C significantly decreased in both E+S and S+E compared to PRE (E+S at 24h -22±26%, $p<0.05$, ES=-0.940; E+S at 48h -27±17%, $p<0.001$, ES=-1.093; S+E at 24h -26±26%, $p<0.01$, ES=-0.966; S+E at 48h -27±19%, $p<0.001$, ES=-0.926). No significant between-group difference in acute responses or recovery was observed.

+++ Fig 4a and 4b somewhere near here +++

Blood lactate and serum CK concentrations

Blood lactate concentrations (Table 2) significantly increased at MID (E+S +560±297%, $p<0.01$, ES=2.369; S+E +610±258%, $p<0.001$, ES=3.198) and POST (E+S +753±485%, $p<0.001$, ES=3.104; S+E +557±256%, $p<0.001$, ES=4.041) compared to PRE. Concentrations of serum CK (Table 2) significantly increased during both loadings at MID (E+S ES=0.236; S+E ES=0.215) and POST (E+S ES=0.320; S+E ES=0.368) compared to PRE. The largest relative increase of CK concentrations was observed during recovery at 24h and 48h (significant only at 48h in S+E +53±57%, $p<0.05$, ES=0.418) compared to PRE, while large standard deviations were observed.

Acute loading responses at week 24

Maximal force production

In E+S, MVC_{max} was significantly decreased at MID (-15±9%, $p<0.01$, ES=-0.604) and further decreased at POST (-25±11%, $p<0.001$, ES=-1.123) compared to PRE (Fig. 2b). In S+E, MVC_{max} significantly decreased at MID (-25±11%, $p<0.001$, ES=-1.259) and remained reduced at POST (-27±10%, $p<0.001$, ES=-1.160) compared to PRE. The decrease at MID was significantly larger in S+E compared to E+S (-25±11% vs. -15±9%, $p<0.05$, ES=1.045) while at POST no between-group difference was observed. Both E+S and S+E significantly recovered from POST to 24h (E+S ES=1.174; S+E ES=0.944) and 48h (E+S ES=1.240; S+E ES=0.910), so that the observed values at 24h and 48h did not statistically differ from PRE ($p>0.05$).

Serum hormone concentrations

Concentrations of serum T (Fig 3b) remained statistically unaltered during the two loadings at MID and POST. However, since the concentrations of serum T at MID somewhat increased in E+S (ES=0.634) but remained unaltered in S+E (ES=-0.072), the difference between the two loadings at MID was significant (between-group difference 25%, $p<0.01$, ES=1.196). Serum T significantly decreased from MID to POST in E+S (-13±11%, $p<0.05$, ES=-0.303) and significantly increased in S+E (+18±23%, $p<0.01$, ES=0.527). During recovery, concentrations of serum T were only slightly reduced at 24h and 48h compared to PRE in both E+S and S+E while the reduction in E+S at 48h was nearly significant (-18±20%, $p=0.052$, ES=-0.636) but no significant between-group difference was observed.

Concentrations of serum TSH remained statistically unaltered during the two loadings at MID and POST. During recovery, serum TSH concentrations significantly decreased at 24h in both loadings (E+S -22%, $p<0.05$, ES=-0.612; S+E -17%, $p<0.05$, ES=-0.597) and 48h in E+S only (-21%, $p<0.05$, ES=-0.692) compared to PRE. No significant between-group difference in acute responses or recovery was observed.

Concentrations of serum GH (Table 2) significantly increased at MID (E+S +330 fold, $p<0.01$, ES=0.972; S+E +53 fold, $p>0.05$, ES=0.637) and POST (E+S +80 fold, $p<0.01$, ES=0.847; S+E +340 fold, $p<0.001$, ES=0.990) compared to PRE. A significant between-group difference at MID ($p<0.001$, ES=0.740) and POST ($p<0.05$, ES=0.531) was observed.

Concentrations of serum C (Fig. 4b) remained significantly unaltered in E+S at MID and POST but were significantly increased in S+E from MID to POST (+42±50%, $p<0.01$, ES=1.382). The difference between E+S and S+E at MID was significant (+20±44% vs. -15±28%, <0.05, ES=0.960). During recovery at 24h and 48h, concentrations of serum C were slightly decreased in both E+S and S+E (at 48h E+S -20±23%, $p=0.057$, ES=-0.729; S+E -21±28%, $p<0.05$, ES=-0.932) compared to PRE but did not significantly differ between the two groups.

Blood lactate and serum CK concentrations

Concentrations of blood lactate (Table 2) significantly increased at in both loadings at MID (E+S +688±314%, p<0.001, ES=3.622; S+E +717±305%, p<0.001, ES=4.480) and POST (E+S +978±735%, p<0.001, ES=2.980; S+E +6161±224%, p<0.001, ES=3.998) compared to PRE. Concentrations of serum CK (Table 2) significantly increased in both loadings at MID (E+S +19±8%, p<0.05, ES=0.236; S+E +31±23%, p<0.01, ES=0.242) and POST (E+S +29±15%, p<0.05, ES=0.285; S+E +70±92%, p<0.001, ES=0.500) compared to PRE. The increase at POST was significantly larger in S+E compared to E+S (+70±92% vs. +29±15%, p<0.05, ES=0.469). Highest concentrations of serum CK were observed during recovery at 24h in both groups (E+S +155±60, p<0.05, ES=0.597; S+E +57±56, p<0.001, ES=0.422).

Differences in acute responses and recovery between the measurements at week 0 and 24

In S+E the reduction in MVC_{max} from PRE to MID (Fig. 2a) was significantly larger at week 24 compared to week 0 (-25±11% vs. -20±13%, p<0.05, ES=0.435).

No significant training or loading-induced changes were found for changes in serum T, TSH and GH concentrations in either of the two groups. In E+S, the relative change in serum C at MID (Fig. 4b) was significantly larger after the training intervention (+20±44 vs. +2±27%, p<0.05, ES=0.504).

Absolute values of CK in S+E (Table 2) during recovery at 24h and 48h were significantly lower at week 24 compared to week 0 (24h 173±124 $mlU \cdot l^{-1}$ vs. 290±170 $mlU \cdot l^{-1}$, p<0.01 ES=-0.570; 48h 123±61 $mlU \cdot l^{-1}$ vs. 221±129 $mlU \cdot l^{-1}$, p<0.01, ES=-0.566). In addition, the relative increase from PRE to 24h and 48h in S+E was significantly smaller at week 24 compared to week 0 (24h 157±56% vs. 200±81%, p<0.05, ES=-0.352; 48h 137±39% vs. 153±57%, p<0.05, ES=-0.398).

Plasma volume

No between-group differences in plasma volume shifts were observed at either week 0 or 24. Plasma volume shifts in the two groups ranged from -10% to -5% during loading and +1% to +7% during recovery, both compared to PRE.

DISCUSSION

The main findings of this study were: 1) Both loading protocols led to similar acute reductions in maximal force production at POST both before and after the prolonged combined training period. 2) The magnitude of reductions in maximal force production in the two groups at POST was similar before and after the training and recovery of force production was already completed at 24h after the two loading protocols at week 0 and 24. 3) Significant acute loading-induced hormone responses were found only in serum GH in both loadings before and after the training and serum T in E+S at MID before the training intervention only. 4) Concentrations of serum cortisol and TSH were reduced compared to pre-loading concentrations during recovery of (at least) 48h after both loading protocols and serum testosterone after the E+S loading only. Thus, a significant between-group difference (order effect) was found in concentrations of serum T during recovery at 24h and 48h. After training for 24 weeks, reductions of serum hormonal concentrations during recovery were no longer observed in either of the two groups. 5) Both training groups significantly improved 1RM strength after 24 weeks of training independent of the loading order.

Acute reductions in strength performance following strenuous exercise loading may result from both central and peripheral fatigue initiated by repetitive cycles of muscle contractions. In the present study, no significant between-group differences in the magnitude of acute reductions in maximal force production before or after the 24-week training period were observed. After the initial acute decrease in force production, strength performance returned to pre-loading levels already within 24h in both loading protocols at week 0 and 24. Since both repeated bouts of strength loadings and prolonged endurance cycling have been shown to result in decreased force production (Leveritt and Abernethy 1999; Moore et al. 2005; Schumann et al. 2013), the present findings are not surprising. Due to the nature of the present cycling and leg press protocol, the magnitude of loading-induced reductions in maximal force production, however, was relatively low (22-27%) and different results may possibly be observed by modifying the experimental loading performed.

Interestingly, at week 0 the endurance cycling in the E+S loading led to a reduction in MVC_{max} of 11% while in the S+E protocol endurance cycling performed after strength loading did not further reduce maximal force, demonstrating a plateau in fatigue as observed previously during prolonged performance of strength loadings only (Häkkinen and Pakarinen 1993; Ahtiainen et al. 2003a). Hence, while strength loading produces

neuromuscular fatigue when performed both before and after an endurance loading, cycling may only induce fatigue when performed in an unfatigued state. Even though steady-state cycling and both maximal and hypertrophic strength protocols mainly recruit different fiber types (Kraemer et al. 1995) and the number and size of motor units recruited depends on the intensity and activity performed (Henneman et al. 1965), some overlapping may occur between both types of loadings. Although muscle activation was not measured in the present study, it is likely that the strength loading activated high threshold motor units characterized by a high fatigability (Henneman et al. 1965), while the subsequent cycling only led to additional recruitment of fatigue-resistant slow twitch fibers, apparently not increasing the magnitude of overall fatigue. The underlying mechanisms for the present finding, however, may also be metabolic in nature and were not examined in detail.

The magnitude of acute reductions in maximal force at POST in both loading groups after 24 weeks of training was similar to that observed at week 0. In addition, no within group differences in the recovery of force production were observed before or after the training period. However, the reduction of maximal force in S+E at MID (i.e. after S) was significantly larger post-training compared to the corresponding change observed before the training intervention. Although not reflected in blood lactate concentrations, these results indicate an improved fatigue-resistance as previously shown in acute responses to strength loadings after periods of heavy resistance training only (Izquierdo et al. 2009, 2011; Walker et al. 2010). As increased fatigue-resistance allows subjects to sustain a larger magnitude of both mechanical and metabolic stress, the present findings would suggest strength loadings performed immediately before endurance cycling to be more favourable over the reverse loading order. However, these positive adaptations were not reflected in 1RM strength development after 24 weeks of training in this study. Therefore, the role of exercise order with regard to chronic neuromuscular adaptations needs further investigation, for example by modifying the frequency, volume and type of training and loading protocols.

Acute reductions in force production in response to endurance or strength loadings are typically accompanied by loading-induced changes in hormonal concentrations. Hypertrophic type strength loadings characterized by short rest periods as well as endurance exercise of short duration and high intensity may lead to acute increases in serum testosterone, growth hormone and cortisol concentrations (Kraemer et al. 1990; Häkkinen and

Pakarinen 1993; Stokes et al. 2013). Similarly, serum TSH as a precursor of thyroid hormones T_3 and T_4 may also significantly increase following both endurance and strength loadings (Hackney et al. 2012). In agreement with our hypothesis, significant acute hormone responses to the present two combined loading sessions, however, were only found in GH both before and after training and in T in E+S at MID only before the training. Since the highest concentrations of GH at both week 0 and week 24 were found in E+S at MID (i.e. after E) and S+E at POST, it appears that the present steady-state cycling at moderate- to high-intensity induced large increases in serum 22-kDa GH concentrations. The strength loading consisting of mixed explosive, maximal and hypertrophic leg press protocols, on the other hand, may not have been sufficiently metabolically demanding to stimulate GH responses (Häkkinen and Pakarinen 1993). Whether the present endurance and strength loading induced significant changes in other GH aggregates or variants (Kraemer et al. 1990) has not been examined.

When interpreting these results one must bear in mind that the intensity and volume of the present combined loading was purposefully chosen to 1) account for the capabilities of relatively untrained subjects and 2) to represent the overall periodized training program by combining moderate to high intensity steady-state cycling of a relatively short duration with a mixture of explosive, maximal and hypertrophic leg press protocols. In fact, only 2 out of the total 11 sets of the strength loading design were conducted using a purely hypertrophic protocol. In agreement with previous studies, and indicated by the low concentrations of blood lactate in this study, the present combined loading did not produce sufficient physiological stress to stimulate increases in serum testosterone, TSH and cortisol concentrations (Kraemer et al. 1990: Häkkinen and Pakarinen 1993; Linnamo et al. 2005; McCaulley et al. 2009).

However, even though no significant changes in serum concentrations of testosterone, TSH and cortisol in immediate response to the two loading protocols were observed, serum cortisol concentrations were significantly reduced during recovery at 24h and 48h at week 0, independent of the loading protocol. Furthermore, a significant reduction in serum TSH concentrations was observed at 24h in E+S and 48h in S+E. As shown previously, prolonged endurance performance may lead to reduced concentrations of serum cortisol for at least 24h in endurance trained subjects (Daly et al. 2005) and may induce a temporal non-pathological

hypothyroidism lasting for 12h to 72h (Moore et al. 2005; Hackney et al. 2012). Although in-line with previous investigations, the decreased concentrations of cortisol and TSH in the present study appeared not to be loading specific. These findings may, therefore, indicate that the concentrations of these hormones are not sensitive enough to reflect differences in the order of combined endurance and strength loadings.

Interestingly, a significant decrease in concentrations of serum testosterone during recovery at 24h and 48h at week 0 was observed in the present E+S group only. Therefore, in line with our hypothesis, the present study showed a significant between-group difference (order effect) before the training. Previous studies have demonstrated reduced concentrations of testosterone during recovery of (at least) 48h in strength athletes following intensive and voluminous strength loadings only (Häkkinen and Pakarinen 1993), in endurance athletes following an intermittent endurance loading during recovery of 12h (Hackney et al. 2012) and in recreational endurance athletes during recovery of 48h following a combined loading session (Taipale and Häkkinen 2013). The present findings may, thus, indicate the E+S loading protocol conducted before the training period to be physiologically more demanding for physically active men, leading to a requirement for prolonged recovery.

The detailed mechanisms for the present decreased basal hormonal concentrations during recovery are not yet conclusively understood. Loading or training induced changes in serum hormone concentrations may be associated with adaptations within the endocrine system but temporary fluctuations in circulating blood hormone levels can also result from 1) increased or decreased secretion, 2) increased or reduced hepatic clearance, 3) alterations in plasma volume or fluid shift or 4) increased or reduced degradation rates (Kraemer and Ratamess 2005). While the biological functions of transiently reduced concentrations of cortisol vs. TSH and testosterone may differ due to the catabolic vs. anabolic nature of these hormones, reduced concentrations of hormones during recovery have generally been linked with both an up-regulation of androgen receptors accompanied by increased target tissue uptake or an inhibited production of these hormones in the releasing gland or at the hypothalamus level (Vingren et al. 2010). However, since the kinetics of androgen receptor regulation and its association with circulating testosterone concentrations following strenuous exercise sessions

has not yet been fully elucidated, the biological meaning of reduced concentrations of serum testosterone during recovery has to be further examined.

Interestingly, the present initial decreases in serum cortisol, TSH and testosterone at 24h and 48h of recovery at week 0 diminished after the 24 weeks of training. The magnitude of immediate acute responses in both catabolic and anabolic hormone concentrations within each loading group, however, was similar at week 24 compared to week 0. The latter finding is in agreement with previous studies investigating chronic adaptations in loading- induced hormone concentrations following strength loadings and training only (Kraemer et al. 1990; Häkkinen et al. 2000; Ahtiainen et al. 2003b). The diminished reductions of both catabolic and anabolic hormone concentrations during recovery, however, suggest adaptations within the endocrine system which were especially pronounced in the E+S training group. Notably, after the 24-week training period a significant trend for decreases in serum testosterone at 48h of recovery in the E+S loading protocol was found and serum cortisol was significantly reduced in S+E and nearly significantly reduced in E+S at the same time point. Therefore, the present results may also indicate that the time course of hormonal concentrations to return to baseline levels after the training period was prolonged. It would have, thus, been interesting to measure the concentrations of these hormones after recovery of 72h.

Accumulated concentrations of anabolic hormones dramatically increase the likelihood for androgen receptor interactions and repeated loading-induced acute increases in these hormones during training have been shown to be associated with positive adaptations in muscle hypertrophy and strength development during pure strength training (Häkkinen et al. 2000). It is, therefore, reasonable to assume that reduced concentrations of anabolic and catabolic hormones during recovery may also impact on long-term strength development. However, although in the present study order-specific differences in hormonal concentrations between the E+S and S+E loading protocol at week 0 were found, both training programs led to similar increases in 1RM strength after 24 weeks of order-specific combined endurance and strength training. While few authors have questioned the relationship of loading-induced testosterone concentrations with chronic training adaptations (West et al. 2010), several studies have shown significant correlations between both basal and loading-induced concentrations of circulating testosterone and chronic development of muscle mass and strength during strength training only

both in men (McCall et al. 1999; Ahtiainen et al. 2003b; Kvorning et al. 2006) and women (Häkkinen et al. 1992). In the present study, however, no correlations were found between basal or loading-induced concentrations of the hormones examined and improvements in 1RM strength during the combined endurance and strength training period.

In contrast to studies investigating endurance or strength training only, one must consider the role of possible acute and chronic interference (Wilson et al. 2012) when interpreting the present findings. Since the endurance part of the combined loading possibly reduced the anabolic effects of the strength loading, a combination of both endurance and strength may in fact dilute possible correlations between loading-induced hormonal concentrations and chronic strength development. Furthermore, it has to be acknowledged that the training frequency in the present study was rather low, allowing for at least 2 full days of rest between consecutive training sessions. Since differences in hormonal concentrations during recovery before training were monitored for 48h only, this would be in-line with the finding that both groups developed 1RM strength to a similar extent. Finally, the present design including experimental loadings before and after a comparably long training period of 6 months was not able to elucidate the exact timing of endocrine adaptations. It is possible that initial differences in serum testosterone concentrations during recovery were diminished already in an early phase of the training program (for example after a few weeks) and, thus, the possible impact on strength development after 24 weeks was not observed.

CONCLUSIONS

This study has demonstrated that the acute force and hormone responses to combined endurance+strength vs. strength+endurance loadings were similar when compared before and after combined training. While the recovery of force was mainly completed after 24h at pre and post-training in the two loading groups, the order effect was reflected by significantly reduced serum testosterone concentrations at 24h and 48h of recovery in the E+S but not S+E group before the training period. This initial loading-specific difference during recovery was diminished after 24 weeks of combined endurance and strength training and both groups developed 1RM strength to a similar extent. Therefore, the present findings indicate that despite an initial order effect, the order of combined training does not seem to influence long-term adaptations of strength development in physically

active young men. However, this study also showed that performing E+S loadings may, especially in the early phase of the training, lead to prolonged recovery needs which may have a negative impact on training outcomes especially when the training frequency is high. Therefore, the present findings are limited to the training volume and frequency performed and should be applied to physically active young men only.

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CONFLICTS OF INTEREST

The authors of this manuscript do not have any conflicts of interest.

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TABLES

Table 1 Baseline values of endurance and strength performance and blood markers. Physical performance data were obtained on separate days before the loading measurements at week 0 and week 24, respectively. Serum hormone and CK concentrations were obtained in the morning of each loading after fasting for 12 hours

Group / **Variable**	E+S week 0	E+S week 24	S+E week 0	S+E week 24
1RM (Kg)	158±30	177±27***	143±24	165±21***
Aerobic power (W)	274±36$^{\#}$	302±34***	247±36	285±38***
MVC_{max} (N)	2628±692	2943±801*	2357±549	2599±580*
Basal Testosterone (nmol·l^{-1})	13±3.1	18.9±4.8***	14.3±3.5	19.9±4.2**
Basal Cortisol (nmol·l^{-1})	529.9±114.5	574±98*	534.5±113.2	597.4±139.5
Basal TSH (nmol·l^{-1})	2.6±0.8$^{\#}$	2.2±1.3	2.0±0.6	1.5±0.7
Basal GH (mlU·l^{-1})	1.2±1.8	2.2±4.6	2.4±6.7	0.9±1.5
Basal CK (mlU·l^{-1})	166.7±98	132.3±78.8	158.4±116.5	103.6±51.6*

$^{\#}$significant different from S+E at corresponding time point, p<0.05; *,**,***, significant different from measurements at week 0 (p<0.05,0.01 and 0.001, respectively)

Table 2 Serum growth hormone, blood lactate and serum creatine kinase concentrations before, during and after the two combined loadings obtained before and after loading order-specific combined training

		Week 0			**Week 24**		
		GH (mlU·l^{-1})	**Lactate** (mmol·l^{-1})	**CK** (mlU·l^{-1})	**GH** (mlU·l^{-1})	**Lactate** (mmol·l^{-1})	**CK** (mlU·l^{-1})
E+S Loading	**PRE**	1.2±1.8	1.1±0.4	168.5±98.1	2.2±4.6	1±0.2	134.8±79.2
	MID	56.3±29**†	5.8±2.8**	197.3±104.8*	68.6±43.5**†††	6.2±2.0***	159.8±92.8*
	POST	13.7±8*†††	8.3±3.2***	209±97.8*	19.1±18.6**†	9.2±3.9***	170.7±93.5*
	24h			404.8±229.3			313.8±199.6*
	48h			276±127.6			242.9±198
S+E Loading	**PRE**	2.4±6.7	1.4±0.4	160.4±118.5	0.8±1.5	1.5±0.8	106.6±52.1
	MID	15±27.5**	8±2.3***	185.7±139.7***	7.7±12.3	9±2.3***	137.8±82.5**
	POST	54.4±32.3***	7.2±2.0***	214.3±155***	56.7±37.5***	7.9±2.1***	174.8±99.4***
	24h			290.4±170**			172.6±123.6***$^{\#}$
	48h			221.3±128.8			122.8±61.2$^{\#}$

†,†††significant different from S+E at corresponding time point, (p<0.05 and p<0.001, respectively); *,**,***, significant different from corresponding PRE values(p<0.05,0.01 and 0.001, respectively), $^{\#}$significant different from measurements of week 0

FIGURE CAPTIONS

Fig 1 Experimental design for the examination of acute force and hormone responses and recovery to combined endurance and strength loadings conducted before and after the loading order-specific combined training. Baseline measurements consisted of tests for endurance and strength performance as well as the determination of serum hormone and CK concentrations

Fig 2 Acute responses and recovery of maximal isometric leg press force (MVC_{max}) before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; # significant different from corresponding time point at week 24 (p<0.05)

Fig 3 Serum testosterone concentrations during loading and recovery before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; † refers to a significant trend p<0.06; within the bar compared to PRE, outside the bar as indicated

Fig 4 Serum cortsiol concentrations during loading and recovery before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; # significant different from corresponding time point at week 24 (p<0.05); † refers to a significant trend p<0.06 compared to PRE