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**Author(s):** Schumann, Moritz; Eklund, Daniela; Taipale, Ritva; Nyman, Kai; Kraemer, William J; Häkkinen, Arja; Izquierdo, Mikel; Häkkinen, Keijo

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**Running head:** Acute responses to combined endurance and strength loadings

Acute neuromuscular and endocrine responses and recovery to single session combined endurance and strength loadings: 'Order effect' in untrained young men.

Schumann, Moritz<sup>1</sup>, Eklund, Daniela<sup>1</sup>, Taipale, Ritva S<sup>1</sup>, Nyman, Kai<sup>2</sup>, Kraemer, William J<sup>3</sup>, Häkkinen, Arja<sup>2,4</sup>, Izquierdo, Mikel<sup>5</sup> and Häkkinen, Keijo<sup>1</sup>

<sup>1</sup>Department of Biology of Physical Activity, University of Jyväskylä, Finland

<sup>2</sup>Central Hospital of central Finland, Jyväskylä, Finland

<sup>3</sup>Department of Kinesiology, University of Connecticut, Storrs, USA

<sup>4</sup>Department of Health Sciences, University of Jyväskylä, Finland

<sup>5</sup>Department of Health Sciences, Public University of Navarra, Navarra, Spain

**Address of corresponding author:**

Moritz Schumann

Department of Biology of Physical Activity

P.O. Box 35 (VIV)

40014 University of Jyväskylä

Finland

Tel.: +358408053757

Fax: +358142602071

moritz.schumann@jyu.fi

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## ABSTRACT

The purpose of this study was to investigate acute neuromuscular and endocrine responses and recovery to a single session of combined endurance and strength loading using two loading orders. Forty-two men were demographically matched to perform a single session of combined endurance+strength (E+S) or strength+endurance (S+E) loading. The strength loading was conducted on a leg press and included sets of power, maximal strength and hypertrophic loads with an overall duration of 30 minutes. The endurance loading was conducted on a bike ergometer and performed by continuous cycling over 30min at 65% of subject's individual maximal Watts. Both loading conditions led to significant acute reductions in maximal force production (E+S, -27%,  $p<0.001$ ; S+E, -22%,  $p<0.001$ ), rapid force produced in 500ms (E+S, -26%,  $p<0.001$ ; S+E, -18%,  $p<0.001$ ) and counter movement jump jumping height (E+S, -15%,  $p<0.001$ ; S+E, -12%,  $p<0.001$ ) while no significant differences between the two loadings were observed. Maximal and explosive force production recovered after 48h following both loading conditions. Whereas no significant acute responses were found in concentrations of serum testosterone (T) and thyroid releasing hormone (TSH) in the two loading conditions, concentrations of T were significantly reduced in E+S during recovery at 24h (-13%,  $p<0.05$ ) and 48h (-11%,  $p=0.068$ ) but not in S+E and TSH following both loading conditions (24h, E+S, -32%,  $p<0.001$ ; S+E, -25%,  $p<0.01$ ; 48h, E+S, -25%,  $p<0.001$ , S+E, -18%,  $p<0.01$ ). The present loading conditions showed that neuromuscular performance recovered already within 2 days, while endocrine function, observed particularly by decreased concentrations in serum testosterone following the E+S loading order, remained altered still after 48h of recovery. These results emphasize the different needs for recovery following two loading orders.

**Key words:** fatigue, serum hormones, testosterone, TSH, cortisol, maximal force, explosive strength

## INTRODUCTION

Both the neuromuscular and endocrine systems have been shown to play an integrative role in acute responses and long term adaptations to endurance and strength training. Research often shows that strength and/or power development are compromised when prolonged periods of strength training are combined with endurance training sessions performed on separate days (18, 20, 26) and on the same day (7). This phenomenon has commonly been described as the "interference effect" which is caused by high training volume and/ or frequency of combined endurance and strength training (19).

Several studies have shown that acute alterations and recovery of neuromuscular and endocrine function in response to a single bout of exercise are most critical in the development of long term adaptations (7, 27). Endurance and strength exercises performed on separate days elicit divergent acute neuromuscular and endocrine responses and recovery patterns which might in part explain the limitations in strength gains when both exercises are chronically combined.

Endurance performance places a relatively small demand on force production but may produce some neuromuscular fatigue (30). Resistance loadings, on the other hand, typically cause drastic decreases in maximal neural activation, maximal force production and force-time characteristics of the muscles loaded (14). Although short bouts of high intensity endurance exercise can lead to similar acute increases in steroid hormone concentrations as observed immediately after heavy strength loadings (35, 40), prolonged intense endurance performance produces a catabolic environment, dominated by dramatic increases of cortisol concentrations (8) and accompanied by decreases in testosterone concentrations, after e.g. a marathon run (22, 40).

Several authors have demonstrated the synergistic function of the neuromuscular and endocrine systems in acute response to exercise performance (1, 31). The time course of the recovery from exercise, however, might differ between neuromuscular and endocrine variables (15). While the recovery of neuromuscular performance following endurance or strength loadings usually takes place within 24h - 48h (16, 34), serum basal testosterone concentrations can remain decreased for much longer than 48h following heavy resistance loadings (15).

Acute neuromuscular and endocrine responses and recovery to endurance and strength loadings performed on separate days are recently relatively well studied, but only a few studies (e.g. 5, 13) have investigated acute neuroendocrine alterations following a single session of combined endurance and strength loading. Moreover, to the best of our knowledge, no studies are available investigating short and/ or long term recovery. Growing evidence suggests, however, that residual fatigue caused by an initial loading may reduce the quality of a subsequent loading, reflected in specific acute responses and recovery time courses (30), leading to compromised long term adaptations (7). Muscular force development during a strength loading conducted immediately following endurance exercise has been shown to be either decreased (10) or unaltered (13), and seems to depend on the intensity of the preceding endurance loading (9). Similarly, serum concentrations of steroid hormones (growth hormone) can be attenuated following a single combined endurance and strength loading session in physically active men (13) but have also been shown to be acutely increased (testosterone) in strength trained subjects performing a strength and endurance loading (5).

Considering the importance of acute neuromuscular and endocrine responses and recovery to a single session of combined endurance and strength loading for long term adaptations, it is of crucial relevance to investigate whether the first half of a loading session (i.e. endurance or strength exercise, respectively) alters acute neuromuscular responses and recovery of the subsequent loading (i.e. strength or endurance exercise, respectively). Moreover, it is of great importance to examine whether one loading order (e.g. endurance+strength) produces more favorable anabolic responses than the opposite order (e.g. strength+endurance). Thus, the purpose of the present study was to investigate acute neuromuscular and endocrine responses and recovery to a single session of combined endurance and strength loading using two different loading orders in moderately physically active young men.

## METHODS

### *Experimental Approach to the Problem*

A cross sectional design was used to investigate the acute effects and recovery in response to a single session of combined endurance and strength loadings with different exercise orders (i.e. endurance + strength [E+S] vs. strength + endurance [S+E]). While both groups performed the identical loadings in terms of volume

and intensity, specific responses and recovery patterns of neuromuscular performance and endocrine function originating from the different order were measured. Prior to the start of the measurements, all subjects were matched according to age, physical activity background and physical fitness and assigned to either of two groups (E+S, n=21 and S+E, n=21). Since this research project was part of a larger study including a longitudinal training intervention, all subjects performed only one loading order (E+S or S+E). All subjects proceeded to the laboratory for a total of 6 times. First, subjects were familiarized with the measurement procedures and measurement equipment was set up according to the individual needs of each subject. During the following two laboratory visits, maximal oxygen consumption ( $\dot{V}O_{2max}$ ) as well as maximal workload during a graded cycle ergometer test were determined. In addition, subject's one repetition maximum (1RM) and maximal voluntary isometric contraction ( $MVC_{max}$ ) during seated horizontal leg press were obtained. Thereafter, all subjects performed one single session of combined endurance and strength loading in the order of the corresponding group (E+S or S+E) and returned to the laboratory for recovery measurements at 24h and 48h after the loading session. The duration between each baseline measurement session was at least 3 days. The duration between the last baseline measurement session and the loading was a minimum of 4 days. Subjects were asked to continue their normal activities of daily living throughout the measurement period.

### *Subjects*

The subjects for the present study were recruited by newspaper ads from the city of Jyväskylä. After subjects expressed their interest in the research, forty-two moderately physically active young men were selected by phone questionnaires to participate in the study. All included subjects were free of acute and chronic illness, disease or injury and reported not using medication that would contraindicate the performance of intense physical activity or interfere with neuromuscular function and endocrine metabolism. Furthermore, all included subjects reported to perform physical activity such as light walking, cycling or occasionally sport games (e.g. soccer or floorball) for not more than 3 times per week but did not conduct systematic endurance and/or strength training. Prior to the participation in the study, subjects were informed about the study procedures and possible risks both verbally and in written form before signing an informed consent. Prior to the testing, subjects were required to complete a health questionnaire and conduct a resting ECG measurement which both have been reviewed by a cardiologist. The demographic characteristics of the subjects were as follows (mean±SD):

Age  $29.2 \pm 4.9$  years, height  $178.3 \pm 5.2$  cm, body mass  $75.9 \pm 8.6$  kg. The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the University of Jyväskylä.

### *Procedures*

All baseline and loading measurements were conducted during October and November of 2011. The measurements within the combined loading included the recording of maximal isometric strength (horizontal bilateral isometric leg press) and power (counter movement jump on a force plate) as well as the determination of serum hormone concentrations, creatine kinase (both from venous blood samples), and blood lactate (capillary blood). These measurements within the loading were conducted at the following time points (Figure 1): Prior to the start of the combined session (PRE), immediately following the first exercise (MID, after endurance or strength loading, respectively) and right after the completed combined session (POST). The follow-up measurements including the same measurements were conducted after recovery of 24h and 48h at  $\pm 1$ h from the end of the complete loading. The acute loading measurements of both groups (E+S and S+E) were conducted between 7:00 and 15:00 (mean $\pm$ SD, E+S, 9:01 a.m. $\pm$ 1:57h; S+E, 8:47 a.m. $\pm$ 2:20 h). The corresponding follow-up measurements were conducted at the time between 9:00 a.m. and 16:00 p.m. (mean $\pm$ SD; 24h, E+S, 11:14 a.m. $\pm$ 2:05 h; S+E, 11:03 a.m. $\pm$ 2:19; 48 h, E+S, 11:13 a.m. $\pm$ 2:06 h; S+E, 11:00 a.m. $\pm$ 2:18 h).

Figure 1 somewhere near here

At PRE, 24h and 48h the blood sample was drawn first and then the maximal strength and power tests were conducted. At MID and POST, the blood sample was taken after the maximal strength and power tests. Blood lactate concentrations were measured by collecting capillary blood from the finger tip in both loading orders at the following time points: Pre-endurance, after 10 minutes (Endurance 10), after 20 minutes (Endurance 20), Post-endurance, Pre-strength and Post-strength. To account for changes in the hydration status of subjects, body mass was determined before the start of a loading and immediately after the completion of the loading. Subjects were required to begin the combined loading in a hydrated state and were allowed to ingest 2 dl of water at MID, right after the venous blood sample was taken.

In addition to the measurements of acute loading responses and recovery, basal morning levels of serum hormone concentrations and creatine kinase (CK) were recorded by drawing venous blood samples on the day of the combined loading after 12h of fasting, between 7:00 a.m. and 9:00 a.m. In order to control the experimental conditions, subjects were asked to minimize physical and mental stress on the day before as well as throughout the 3 days of the loading measurements. Furthermore, subjects were asked to have at least 7-8h of sleep and to keep their nutritional intake similar on all three measurement days.

### *Strength and endurance loading*

*Strength loading:* The strength loading focused primarily on leg extensors and was performed on a dynamic leg press device (David 210, David Health Solutions Ltd., Helsinki, Finland). The strength loading included sets of explosive power, maximal strength and sets of hypertrophic loads with an overall duration of 30 minutes. The starting knee angle for all exercises was similar to the knee angle used for the determination of the one repetition maximum (1RM) during the baseline measurements (<60 degrees).

Subjects first performed 3 sets of 10 repetitions with a load of 40% of 1RM. During these sets, subjects were instructed to fully extend their legs while producing force as rapidly and explosively as possible. Next, subjects performed a set of 3 repetitions at 75% of 1RM followed by 3 sets of 3 repetitions with a load of 90% of 1RM (maximal strength). The resting period between all explosive and maximal sets was 3 minutes. The strength loading was concluded by performing 4 sets of 10 repetitions with a load of 75% and 80-85% of 1RM (first and last set 75%, second and third set 80-85%) with a resting period of 2 min between the sets, a protocol typically used during hypertrophic strength training. The initial loads were calculated from each subject's pre-determined 1RM and, in order to standardize the loading conditions, during the maximal and hypertrophic sets additional load was added to achieve at least one set of a true repetition maximum (i.e. 3RM and 10RM, respectively). During these maximal sets, failure was allowed and in case the subjects were not able to perform the required amount of repetitions, assistance was provided so that both loading groups performed identical loadings.

*Endurance loading:* The endurance loading was conducted on a bike ergometer (Ergonomic 839E, Monark Exercise AB, Vansbro, Sweden). Subjects performed 30 minutes of continuous cycling at 65% of subject's



individual maximal Watts (achieved during the baseline measurement). Subjects were required to keep the pedaling frequency constant at 70 rpm. In case the subjects were not able to keep up the required frequency, intensity was reduced by 15 Watts. If the subject was not able to return back to a frequency of 70 rpm within 1 minute, intensity was reduced by another 15 Watts. This procedure was repeated until the subjects were able to keep up the initial frequency.

### *Measurements*

*One repetition maximum:* Subject's one repetition maximum (1RM) of leg extensors was determined using a seated horizontal leg press (David 210, David Health Solutions Ltd., Helsinki, Finland). Prior to attempting 1RM, subjects completed a warm up consisting of 3 sets using 5 repetitions with 70% of the estimated maximal capacity, 2 repetitions at 80-85% and 1 repetition at 90-95% with 1 minute rest between the sets. Following this warm up, no more than 5 attempts were allowed to reach 1RM. The starting knee angle for all subjects was (mean $\pm$ SD) 58.4 $\pm$ 1.9 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 the complete extension to 180 degrees. To promote maximal effort, verbal encouragement was given. The greatest weight that the subject could successfully lift (knees fully extended) at the accuracy of 1.25 kg was accepted as 1RM.

*Isometric leg extension:* Maximal isometric bilateral leg extension force (MVC<sub>max</sub>) was measured on a horizontal dynamometer (16, Department of Biology of Physical Activity, University of Jyväskylä, Finland) in a seated position at a knee angle of 107 degrees. Subjects were instructed to produce maximal force as rapidly as possible with the entire foot against the force plate for a duration of 3-4 seconds. Subjects were instructed to keep a constant pre-tension of about 200 N prior to the maximal contraction. During the execution of each maximum trial, subjects were required to grasp the handles located by the seat of the dynamometer as well as to keep constant contact with the seat and the backrest. Verbal encouragement was given to promote maximal effort. Prior to the start of the loading as well as at both follow-up measurements (24h and 48h), three trials with a resting period of 1 minute were conducted. At MID (after E or S, respectively) and POST, only two subsequent trials separated by a resting period of 15 seconds were conducted. The force signal was low pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge,

UK). In addition to maximal force, rapid force produced in 500 ms ( $MVC_{500}$ ) and maximal rate of force development at 10 ms ( $RFD_{10}$ ) were calculated from the force curve.

*Counter Movement Jump:* Maximal dynamic power was determined by the countermovement jumping height on a force plate (Department of Biology of Physical Activity, Jyväskylä, Finland). Subjects were asked to stand with their feet hip width apart and their hands on their hips and were instructed to perform a quick and explosive countermovement jump after a self selected start. Force data was collected, low pass filtered and analyzed by computer software (Signal 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). Jumping height was then calculated from flight time using this equation:  $(9.81 \cdot h^2/8) \cdot 100$  (4).

*Maximal workload:* Maximal workload and maximal oxygen consumption were determined using a graded protocol on the bike ergometer (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 continuously throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland). Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Before each test, air flow calibration was performed using a manual flow calibrator and the gas analyzer was calibrated using a certified gas mixture of 16% O<sub>2</sub> and 4% CO<sub>2</sub>. The  $\dot{V}O_{2max}$  was taken as the highest 30-s  $\dot{V}O_2$  value. To assure that  $\dot{V}O_{2max}$  was reached, other criteria such as heart rate, blood lactate and respiratory exchange ratio (RER) were monitored throughout the test. Maximal workload was calculated using the equation:  $W_{max} = W_{com} + (t/120) \cdot 25$ , where  $W_{com}$  is the load of the last completed stage and t is the time of the last incomplete stage (5).

*Venous blood samples:* 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, Hanau, Germany) for 10 minutes after which serum was removed and stored at -80°C until analysis. Analysis of total serum testosterone (T), serum cortisol (C), serum growth hormone (GH) and serum thyroid stimulating hormone (TSH) were performed using chemical luminescence techniques (Immunlite 1000, Simens, NY, USA) and hormone specific immunoassay kits (Siemens, New York, NY, USA). The sensitivity for serum hormones were: T, 0.5 nmol·l<sup>-1</sup>; C, 5.5 nmol·l<sup>-1</sup>; GH, 0.03 mIU·l<sup>-1</sup> and TSH, 0.004 mIU·l<sup>-1</sup>. The intra-assay coefficients of

variation were: T, 8.5%, C, 4.6%, GH, 5.3% and TSH, 3.9 %. The inter-assay coefficients of variation were T, 14.2%; C, 6.1%; GH, 5.6% and TSH, 8.9%.

*Blood lactate concentrations:* Capillary blood samples were taken from the finger tip at described time points. Blood lactate concentrations were analyzed using a Biosen lactate analyzer (S\_line Lab+, EKF, Magdeburg, Germany).

### *Statistical analysis*

Conventional statistical methods were used for the calculation of means and standard-deviations. Before applying further statistical methods, data of both loading groups were checked for normality. Within group differences for normally distributed variables were analyzed using repeated measures of ANOVA with 5 levels (PRE, MID, POST, 24h and 48h). Within group differences for not normally distributed variables were analyzed using the Wilcoxon Signed Rank Test and p-values were corrected for Bonferroni by multiplying all pair-wise p-values with the number of comparisons conducted for each variable. Between loading comparison for normally distributed variables were conducted by using an independent sample T-test. If either of the two compared groups was not normally distributed, a non-parametric Mann-Whitney U test was conducted. The statistical significance for all tests was set for a baseline of  $p < 0.05$  where \*= $p < 0.05$ , \*\*= $p < 0.01$  and \*\*\*= $p < 0.001$ . Statistical analysis was conducted with IBM SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

*Baseline measurements:* Obtained values of the baseline measurements for strength (1RM,  $CMJ_{base}$ ,  $MVC_{max_{base}}$ ,  $MVC_{500_{base}}$  and  $RFD_{10_{base}}$ ) and endurance ( $\dot{V}O_{2max}$  and  $Work_{max}$ , Table 1) and pre-loading values of the same variables (Table 2) did not differ significantly between the two loading groups ( $p > 0.05$ ). No significant differences were found in  $MVC_{max}$ ,  $MVC_{500}$ ,  $RFD_{10}$  and CMJ between baseline and pre-loading values ( $p > 0.05$ ). Fasting concentrations of serum hormones and CK (Table 3) did not differ significantly between both loading groups ( $p > 0.05$ ). No significant differences in concentrations of serum hormones and CK measured in the fasting state and at pre-loading were found in the E+S loading group ( $p > 0.05$ ). In the S+E loading group, concentrations of serum testosterone and cortisol were significantly lower at pre-loading

compared to the fasting state (C,  $472.3 \pm 138$  vs.  $526.3 \pm 108$  nmol·l<sup>-1</sup>,  $p < 0.05$ ; T,  $12.8 \pm 4.5$  vs.  $14.0 \pm 3.8$  nmol·l<sup>-1</sup>,  $p < 0.05$ ).

Tables 1, 2 and 3 somewhere near here

*Within loading measurements:*

Both loading groups showed similar reductions in bodyweight from PRE to POST (E+S, -1%; S+E, -1%,  $p > 0.05$ ).

*Strength and power:* Both the E+S and S+E loading groups induced significant acute reductions in MVC<sub>max</sub> (Figure 2a) at MID (E+S, -14%,  $p < 0.001$ ; S+E, -21%,  $p < 0.001$ ) and POST (E+S, -27%,  $p < 0.001$ ; S+E, -22%,  $p < 0.001$ ) compared to PRE. The relative change in S+E at MID was somewhat larger than that of E+S ( $p = 0.056$ ). The reduction from MID to POST was significant in E+S (-13%,  $p < 0.001$ ) but not in S+E. MVC<sub>max</sub> recovered in both loadings significantly from POST to 24h (E+S, +22%,  $p < 0.001$ ; S+E, +21%,  $p < 0.001$ ) so that at 24h and 48h no significant differences compared to the pre-loading values were found. MVC<sub>500</sub> (Figure 2b) was significantly reduced in both loading conditions at POST (E+S, -26%,  $p < 0.001$ ; S+E, -18%,  $p < 0.001$ ) compared to PRE. MVC<sub>500</sub> recovered in both loadings significantly from POST to 24h (E+S, +17%,  $p < 0.001$ , S+E +13%,  $p < 0.05$ ) but the values remained significantly reduced compared to PRE in E+S (-9%,  $p < 0.01$ ). RFD<sub>10</sub> was reduced significantly at MID only in E+S (-21%,  $p < 0.01$ ) and POST in both loadings (E+S, -32%,  $p < 0.001$ ; S+E, -23%,  $p < 0.05$ ). RFD<sub>10</sub> recovered from POST to 24h following both loading conditions (significant in E+S only, +30%,  $p < 0.01$ ) so that both groups returned back to pre-loading values at 24h. CMJ jumping height (Figure 2c) was significantly reduced at MID only in S+E (-11%,  $p < 0.001$ ). The difference between E+S and S+E at MID was, thus, significant (difference 7%,  $p < 0.01$ ). Both loading conditions led to significant decreases in CMJ at POST (E+S, -15%,  $p < 0.001$ ; S+E, -12%,  $p < 0.001$ ) and recovered significantly from POST to 24h (E+S, +14%,  $p < 0.001$ , S+E, +8%,  $p < 0.05$ ). No differences were found between the CMJ measures at 24h and 48h and pre-loading values in either of the loading conditions ( $p > 0.05$ ).

Figure 2 somewhere near here

*Serum hormone concentrations:* Total serum testosterone concentration (Figure 3a) significantly increased in E+S at MID (+16%,  $p<0.001$ ) and remained slightly increased at POST (+7%,  $p>0.05$ ) compared to PRE. In S+E, serum T concentration did not change at MID, but was slightly increased at POST (+16% compared to MID,  $p<0.01$ ; +10% compared to PRE,  $p>0.05$ ). The difference between E+S and S+E at MID was significant (difference of 20%,  $p<0.001$ ). Serum T concentrations significantly decreased in E+S at 24h of recovery compared to POST (-20%,  $p<0.05$ ) and PRE (-13%,  $p<0.05$ ) and remained reduced at 48h (-18% compared to POST,  $p<0.05$ ; -11% compared to PRE,  $p=0.068$ ). Serum T concentrations in S+E were not significantly different from pre-loading values at 24h and 48h of recovery. Serum growth hormone concentrations (GH, Figure 3b) significantly increased in both loadings at MID (E+S, +242 folds,  $p<0.001$ ; S+E, +41 folds,  $p<0.001$ ) and increased further in S+E at POST (+7 folds compared to MID,  $p<0.001$ ; +272 folds compared to PRE,  $p<0.001$ ). In E+S, GH concentration at POST was reduced compared to MID (-4 folds,  $p<0.001$ ) but remained elevated compared to PRE (+66 folds,  $p<0.001$ ). The differences between the loadings at MID and POST were significant ( $p<0.001$ ). No significant acute changes were observed for concentrations of serum TSH (Figure 3c) in either of the loadings at MID and POST. Serum TSH concentrations were significantly reduced at 24h compared to POST (E+S, -36%,  $p<0.001$ ; S+E, -22%,  $p<0.05$ ) and PRE (E+S, -32%,  $p<0.001$ ; S+E, -25%,  $p<0.01$ ) and remained reduced at 48h in E+S compared to POST (-19%,  $p<0.001$ ) and PRE (E+S, -25%,  $p<0.001$ ) and S+E compared to PRE (-18%,  $p<0.01$ ). Concentration of serum cortisol (Figure 3d) did not change in E+S at MID and POST but was increased in S+E at POST compared to MID (+46%,  $p<0.01$ ). Serum C was significant decreased at 24h of recovery compared to POST in S+E only (-54%,  $p<0.05$ ) and compared to PRE in both loading conditions (E+S, -21%,  $p<0.01$ ; S+E, -26%,  $p<0.001$ ) and remained reduced at 48h (E+S, -22%,  $p<0.001$ ; S+E, -29%,  $p<0.001$ ) compared to PRE.

Figure 3a, 3b, 3c and 3d somewhere near here

*Creatine kinase (CK):* Both loading conditions led to an increase of CK (Figure 4) at MID (E+S, +20%,  $p<0.001$ ; S+E, +16%,  $p<0.001$ ) which further increased at POST compared to MID (E+S, +12%,  $p<0.001$ ; S+E, +18%,  $p<0.001$ ) and PRE (E+S, +32%,  $p<0.001$ ; S+E, +34%,  $p<0.01$ ). The highest concentrations of CK were observed in both loadings at 24h of recovery (compared to PRE, E+S, +185%,  $p<0.001$ ; S+E, +95%,

$p < 0.001$ ). The values remained significantly increased at 48h of recovery compared to PRE (E+S, +95%,  $p < 0.001$ ; S+E, +50%,  $p < 0.01$ ).

Figure 4 somewhere near here

*Blood lactate concentrations:* Blood lactate concentrations measured prior to the start of the endurance loading were significantly higher in S+E compared to E+S (mean $\pm$ SD,  $5.77 \pm 2.09$  mmol $\cdot$ l $^{-1}$  vs.  $1.58 \pm 0.49$  mmol $\cdot$ l $^{-1}$ ,  $p < 0.001$ , Figure 5a). This difference was smaller after 10 minutes (mean $\pm$ SD,  $7.82 \pm 1.96$  mmol $\cdot$ l $^{-1}$  vs.  $5.69 \pm 1.98$  mmol $\cdot$ l $^{-1}$ ,  $p < 0.001$ ) and further diminished after 20 minutes (mean $\pm$ SD,  $7.60 \pm 1.78$  mmol $\cdot$ l $^{-1}$  vs.  $6.21 \pm 2.52$  mmol $\cdot$ l $^{-1}$ ,  $p < 0.05$ ) and was not significant at the end of the endurance loading (mean $\pm$ SD,  $7.19 \pm 1.99$  mmol $\cdot$ l $^{-1}$  vs.  $6.28 \pm 2.56$  mmol $\cdot$ l $^{-1}$ ,  $p > 0.05$ ). Blood lactate concentrations obtained prior to the strength loading were significantly higher in E+S compared to S+E (mean $\pm$ SD,  $4.48 \pm 1.60$  mmol $\cdot$ l $^{-1}$  vs.  $1.81 \pm 0.61$  mmol $\cdot$ l $^{-1}$ ,  $p < 0.001$ ) whereas no significant difference was found at post-strength loading (mean $\pm$ SD,  $8.06 \pm 2.62$  mmol $\cdot$ l $^{-1}$  vs.  $7.45 \pm 2.29$  mmol $\cdot$ l $^{-1}$ ,  $p > 0.05$ , Figure 5b).

Figure 5a and 5b somewhere near here

## DISCUSSION

The present study investigated acute neuromuscular and endocrine responses and recovery to a single session combined endurance and strength loading with two different exercise orders in moderately physically active men. The primary findings indicated that both loading orders led to similar significant reductions in maximal isometric force production, rapid force production produced in 500ms, rate of force development and power performance. While these acute reductions in neuromuscular performance were recovered already at 24h, the present results showed that the time course of recovery of both neuromuscular performance and endocrine function differed. This was primarily shown by decreased concentrations of serum T and TSH still observed after 48h of recovery, particularly following the E+S loading order. These findings suggest that continuous cycling at moderate to high intensity performed immediately prior to a strength loading session consisting of various leg press protocols may considerably influence endocrine function during recovery.

Reductions in maximal and explosive neuromuscular performance have commonly been shown following both endurance (12, 28, 39) and strength (1, 24, 32) loadings. In the present study, the observed reductions of  $MVC_{max}$  in S+E at MID were larger than the observed reductions in E+S obtained at the same time point. Similarly, the reductions in  $MVC_{500}$  and CMJ jumping height were somewhat larger at MID in S+E compared to E+S.

Typically, a strength training session with maximal loads leads to acute fatigue in the neuromuscular system, observed as dramatic decreases in maximal force production of the exercised muscles (16). Kraemer & Häkkinen (24) indicated that the magnitude of fatigue induced decrements in neuromuscular performance are related to the overall volume, intensity and recovery between the sets, while maximal loads combined with short resting intervals are likely to lead to the highest acute reductions in maximal force and power production.

Similarly to strength loading induced reductions in force production, endurance performance may also produce neuromuscular fatigue. The magnitude of endurance exercise induced impaired neuromuscular performance seems to be specific to the intensity of endurance exercise performed (12). Furthermore, it has been suggested that the exercise mode of the endurance loading performed has an impact on the acute alterations in neuromuscular function while both differences (39) and similarities (29) between continuous and intermittent cycling protocols have been found. Since the largest reductions in neuromuscular performance have been obtained following prolonged repeated cycles of stretch shortening exercises, observed in e.g. marathon running (33), the exercise mode seems to play a key role with regard to the magnitude of neuromuscular fatigue produced.

Possible reasons for reduced neuromuscular function in acute response to both strength and endurance exercises may generally be related to fatigue originating centrally, peripherally or from both. Changes in the contractile properties of the quadriceps muscle, shown by alterations of the M-wave and isometric muscular twitch (28), impaired calcium release from the sarcoplasmic reticulum (2) and ultra structural lesions of muscle tissue (33) have been shown to affect force production during fatigue. However, since in the present study no deeper analysis of neuromuscular function was conducted (e.g. electromyographic, muscle and nerve stimulation) and the loading did not involve high impact stretch shortening type of exercise, as e.g.

expressed by the low values of creatine kinase, the reasons for the impaired neuromuscular performance following both strength and endurance exercise remain speculative. It is also possible that blood lactate accumulation and the resulting lowered blood pH observed in the present study have inhibited the rate of cross-bridge binding as previously shown by Sahlin (37) which then may have inhibited force production.

In the present study, the observed difference in reduction of neuromuscular performance between loading conditions in the present study at MID disappeared after both combined loadings were completed. However, the decrements in explosive and maximal force production at POST were somewhat higher in E+S compared to S+E and, in fact, significantly reduced compared to MID, whereas neuromuscular performance remained decreased following the opposite loading order. It appears that higher reductions in  $MVC_{max}$ ,  $MVC_{500}$ ,  $RFD_{10}$  and CMJ were caused by the strength loading rather than the endurance cycling but caution must be paid when applying these findings to loading protocols other than the protocol used in the present study. Cycling does not involve stretch shortening mechanics and, thus, may not lead to comparable ultra structural muscle tissue lesions as observed after e.g. long distance running (33). This, in turn, might explain that in the present study only small differences in acute changes of neuromuscular performance between both loading conditions were found.

The present study did not show differences in the level of recovery in neuromuscular performance between E+S and S+E. Following both loading conditions, pre-loading values of  $MVC_{max}$ ,  $MVC_{500}$ ,  $RFD_{10}$  and CMJ were obtained mainly already after 24h of recovery. Decreases in force production for up to 2-3 days have been reported by e.g. Häkkinen (14) and Ahtiainen et al. (1) following extremely strenuous bouts of bilateral dynamic leg press with an acute decrease in maximal force down to 60% of the maximal force. Following endurance exercises such as a marathon run, maximal force has been shown to recover already after 24h while explosive force production may remain reduced for at least 2 days (33, 34). Relative acute decreases of maximal and explosive force in the present study were comparably small and, thus, neuromuscular recovery was mainly completed already after 24h, with no noteworthy differences between both groups.

Changes in neuromuscular performance are commonly accompanied by alterations in endocrine function. Moderate to high intensity of both strength and endurance loadings elicit remarkable acute responses in both



anabolic and catabolic hormonal concentrations (15, 22, 25, 40). The most prominent findings of the present study were the significant increase of serum testosterone at MID in E+S and the decrease of serum testosterone concentrations following the E+S loading and TSH and cortisol following both loading conditions at 24h and 48h of recovery.

Testosterone as an anabolic steroid plays a major role in energy metabolism and has been identified to promote tissue repair and muscle growth. Elevated concentrations of anabolic steroids in response to an exercise training session have been typically associated with beneficial effects on maximal strength and muscle growth (25). The highest concentrations of serum testosterone are generally observed following heavy resistance loadings with short resting intervals which are characterized by high metabolic stress (15, 25). While endurance loadings of shorter durations have been shown to acutely increase concentrations of anabolic hormones, high intensity endurance exercise of prolonged duration may lead to reductions in concentrations of testosterone (40).

Since in the present study significant increases in serum testosterone were found at MID in E+S, elevations in testosterone concentrations might have been induced by the endurance loading. The present endurance protocol consisted of continuous cycling of moderate to high intensity. As the subjects were moderately physically active and remarkable changes in neuromuscular fatigue were observed in E+S at MID, the present data suggest that the cycling performance required not only cardiorespiratory ability but, to a great extent, continuous muscular effort, produced especially by the quadriceps muscles leading to increases in serum testosterone concentrations. The strength loading, on the other hand, consisted of various protocols and particularly the neural type of resistance loadings with resting periods of 3 minutes may not be sufficient enough to induce noticeable changes in serum testosterone concentrations (31). This may explain the observed difference between the loading groups at MID.

Serum testosterone concentrations observed at POST did not significantly differ from the pre-loading values in the loading groups which is in contrast to findings of Cadore et al. (5) who found testosterone levels to be increased following an S+E loading in young strength trained men. In the present study, however, serum testosterone remained unaltered in S+E but significantly decreased in E+S at 24h and 48h. Interestingly,

whereas neuromuscular performance in the present study was mainly recovered already after 24h in both loading groups, serum testosterone responses remained reduced following the E+S loading for up to two days.

Similarly to serum testosterone concentrations, TSH concentrations were significantly decreased during 24h and 48h of recovery following both loading conditions. Thyroid hormones and in particular thyroid stimulating hormone (TSH), play a major role in thermoregulation and energy metabolism but have also been shown to indirectly promote protein metabolism and tissue remodeling (38). Both endurance and strength exercise performed separately may induce acute elevations of TSH concentrations (3, 6) and the magnitude of this alteration seems to be directly related to the exercise intensity (6). Since in the present study no acute alterations in concentrations of TSH were observed in either of the loading conditions, these findings may suggest that the present combined loadings were not intensive enough to acutely stimulate thyroid function. However, the observed decrease of TSH concentration during recovery, which was somewhat larger in E+S compared to S+E, underline the possibility of delayed alterations in endocrine function particularly following the E+S loading order.

The obtained results of neuromuscular performance and changes in serum testosterone and TSH concentrations generally provide evidence for differences in the time course of recovery between the neuromuscular performance and endocrine function. A similar phenomenon has been observed in strength athletes following an excessively strenuous strength loading session (15). The present data suggest that in moderately physically active men, continuous cycling immediately followed by a combined neural and hypertrophic strength loading session develops a state of endocrine imbalance during recovery which is mainly reflected by decreased concentrations of serum testosterone and TSH. While the main cause of this observation remains speculative, it is plausible that reduced concentrations of T and TSH, especially following the E+S loading order, indicate up regulation of receptors and, thus, enhanced utilization. That would make the E+S loading order more demanding when compared the S+E loading, leading to prolonged needs of recovery. However, in the present study androgen receptor content was not measured and, thus, the mechanisms behind these observations remain speculative. Furthermore, whether this decrease was caused by increased needs of testosterone for tissue remodeling and/or repair or, on the other hand, a possibly impaired endocrine function particularly of the pituitary gland or hypothalamus cannot be explained by the present data. When

considering the present results, one must also bear in mind that hormonal concentrations follow a circadian rhythm and in particular in the morning hours considerable changes in testosterone concentrations are generally observed (23).

The present study did not lead to significant acute changes in concentrations of serum cortisol compared to pre-loading values in either of the loading conditions. During recovery, however, the concentrations of serum cortisol significantly decreased and remained low for at least 48h following both loading conditions. Previous research has shown that serum cortisol concentrations are likely to return to baseline levels already within 2 hours after a heavy strength training session (17). Following endurance exercise, however, concentrations of cortisol have been shown to be significantly decreased after 24h of recovery (8). While diurnal variations of cortisol may potentially account for observed decreased concentrations during recovery (36), other mechanisms such as increased receptor binding or a suppressed function of the adrenal gland during recovery are possible and would underline the evidence for altered or impaired endocrine function following a combined endurance and strength loading session.

While the present loading conditions led to only moderate or no increases in anabolic testosterone and TSH and catabolic cortisol concentrations, somewhat surprising was the magnitude of observed increases of growth hormone concentrations in both loadings which reached peak values considerably higher than reported in previous studies obtained immediately following endurance (35) and strength loadings only (31). Whereas some studies have shown that the magnitude of changes in growth hormone concentrations seems to depend on intensity and volume of the exercise performed (35) other external factors such as sleep and nutrition (11) may influence acute changes in growth hormone concentrations. Moreover, the release of growth hormone generally occurs in a pulsatile pattern and additional factors such as hypoxia and breath holding may have an influence on growth hormone release and might have accounted for the observed growth hormone concentrations in the present subjects.

Interestingly, the present loading induced significant increases in serum growth hormone concentrations in E+S were significantly higher at MID and significantly lower at POST when compared to S+E. The present results may, thus, suggest that cycling performed first in a combined exercise session may blunt growth hormone responses caused by strength exercises performed in the second half of the training session, a

phenomenon also observed by Goto et al. (13). However, while previous studies suggested growth hormone release to be possibly suppressed by the accumulation of free fatty acids (13) or insulin like growth factor-1 (21), the present data do not provide a thorough insight in possible mechanisms behind this observation.

## CONCLUSIONS

This study showed that the present two combined endurance and strength loadings with different exercise orders led to similar acute responses in maximal and explosive force production and endocrine function in moderately physically active young men. However, while a similar recovery of neuromuscular performance in both loading groups already after 24h was observed, serum hormonal concentrations of testosterone, thyroid stimulating hormone and cortisol remained decreased during recovery for at least 48h, particularly when endurance cycling was immediately followed by a strength loading session. The present loading conditions consisting of continuous cycling and various bilateral leg press protocols, thus, led to different recovery time courses between the neuromuscular performance and endocrine function. It needs to be, however, considered, that these results are limited to the present loading conditions of using various leg press protocols and continuous cycling, a training regimen typically used for inexperienced subjects. Thus, great care must be taken when evaluating the practical applications of these findings for other types of loading conditions as well as for athletic populations.

## PRACTICAL APPLICATIONS

The present findings generally indicate that a complete recovery following a single session combined endurance and strength loading may take longer than indicated by the measures of strength performance only. Since endocrine function may be altered for at least 48h following the present strenuous exercises, the measures of neuromuscular performance may not be sufficient to detect a true recovery status. When comparing the two loading orders, the present results showed that following the E+S loading the serum testosterone concentrations were significantly reduced during the recovery for up to 48h, while the serum testosterone concentrations following the S+E loading were not different from the concentrations measured at baseline. The present results, thus, indicate that the present E+S loading seemed to require a longer recovery when compared to the S+E loading which may become important when performing single session combined endurance and strength training. However, additional research is necessary in order to investigate the

relevance of the present findings with regard to prolonged training adaptations and athletic populations.

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## FIGURE LEGENDS

Fig 1. Experimental design for the determination of neuromuscular and endocrine responses and recovery to two single session combined endurance and strength loading conditions. Basal measurements included measurement familiarization and the determination of endurance ( $Work_{max}$ ,  $\dot{V}O_{2max}$ ) and strength ( $MVC_{max}$ ,  $MVC_{500}$ , RFD10 and 1RM) performance and were conducted on three separate days prior to the experimental loading.

Fig 2. Acute responses and recovery of  $MVC_{max}$  (a),  $MVC_{500}$  (b) and countermovement jumping height (c) following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 3a. Acute changes and recovery of serum concentrations of testosterone following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 3b. Acute changes of serum concentrations of growth hormones following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 3c. Acute changes and recovery of serum concentrations of TSH following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 3d. Acute changes and recovery of serum concentrations of cortisol following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 4. Acute changes and recovery of serum concentrations of creatine kinase following the two combined endurance and strength loading conditions. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 5a. Blood lactate concentrations obtained before (Endurance 0), after 10min (Endurance 10), after 20min (Endurance 20) and immediately after (Endurance 30) the 30min endurance exercise of both loading conditions.

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig 5b. Blood lactate concentrations obtained pre and post to the 30min strength exercise of both loading conditions.

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

## TABLES

Table 1. Physical characteristics of all subjects in absolute values. Values were obtained at separate days prior to the loading measurements.

Group	MVC <sub>max</sub> <sub>base</sub>	MVC <sub>500</sub> <sub>base</sub>	RFD <sub>10</sub> <sub>base</sub>	CMJ	1RM	$\dot{V}O_{2max}$	Work <sub>max</sub>
	(N)	(N)	(N·s <sup>-1</sup> )	(cm)	(kg)	(ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	(W)
Loading E+S (mean±SD)	2695±664	1680±418	16288±6803	31.3±5.3	155.3±27.1	43.2±5.9	254.9±39.3
Loading S+E (mean±SD)	2345±537	1492±428	13150±5552	30.3±4.9	142.2±26.6	43.9±6.8	248.6±36.8

Table 2. Maximal and explosive strength values of all subjects in absolute values. Values were obtained on the same day immediately before the start of the loading.

Group	MVC <sub>max</sub>	MVC <sub>500</sub>	RFD <sub>10</sub>	CMJ
	(N)	(N)	(N·s <sup>-1</sup> )	(cm)
Loading E+S (mean±SD)	2619±569	1701±351	17137±5610	29.2±5.3
Loading S+E (mean±SD)	2334±635	1529±359	14438±3374	29.5±5.1

Table 3. Morning concentrations of serum hormones of all subjects in absolute values. Values were obtained after 12h of fasting in the morning of the same day as the loading.

Group	Serum T	Serum C	Serum GH	Serum TSH	CK
	(nmol·l <sup>-1</sup> )	(nmol·l <sup>-1</sup> )	(mIU·l <sup>-1</sup> )	(mIU·l <sup>-1</sup> )	(mIU·l <sup>-1</sup> )
Loading E+S (mean±SD)	14.0±4.6	526.14±104.2	0.76±1.4	2.71±1.1	141.76±81.4
Loading S+E (mean±SD)	13.99±3.8	526.25±108.1	1.87±5.8	2.54±1.4	136.10±108.9

## FIGURES

Figure 1

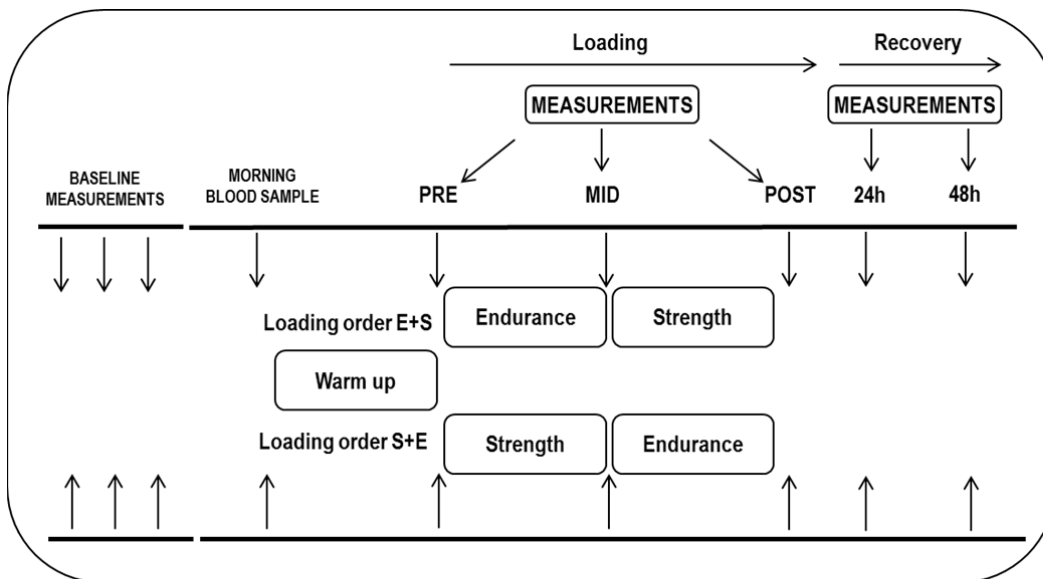


Figure 2

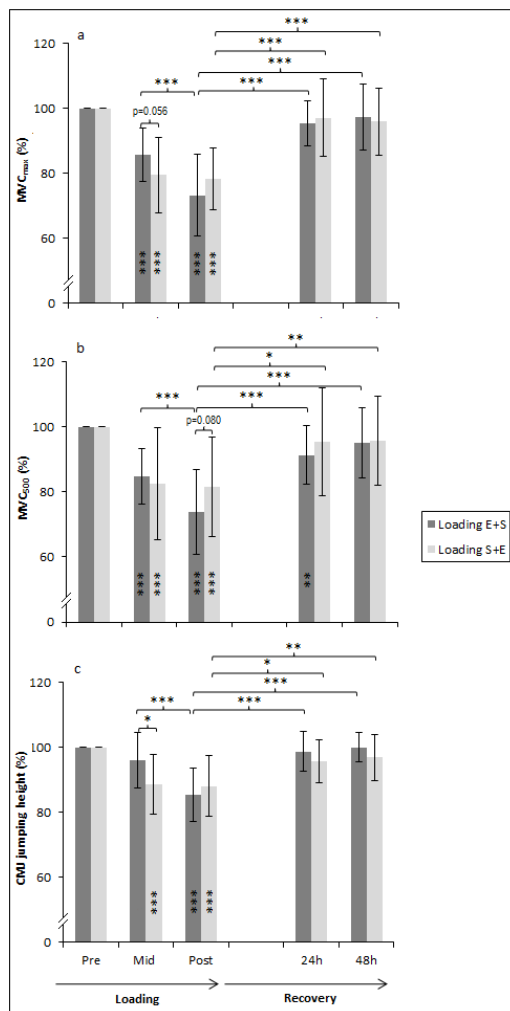


Figure 3a

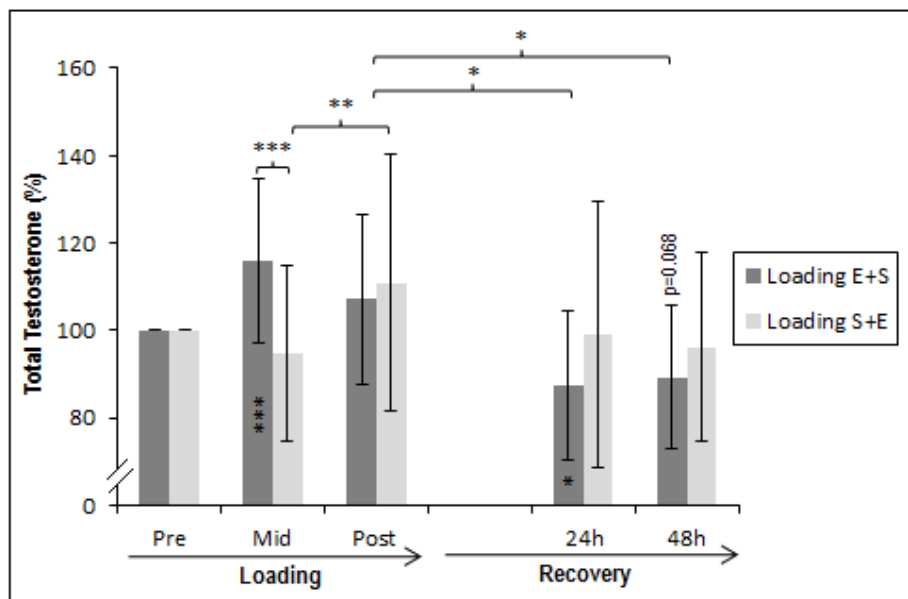


Figure 3b

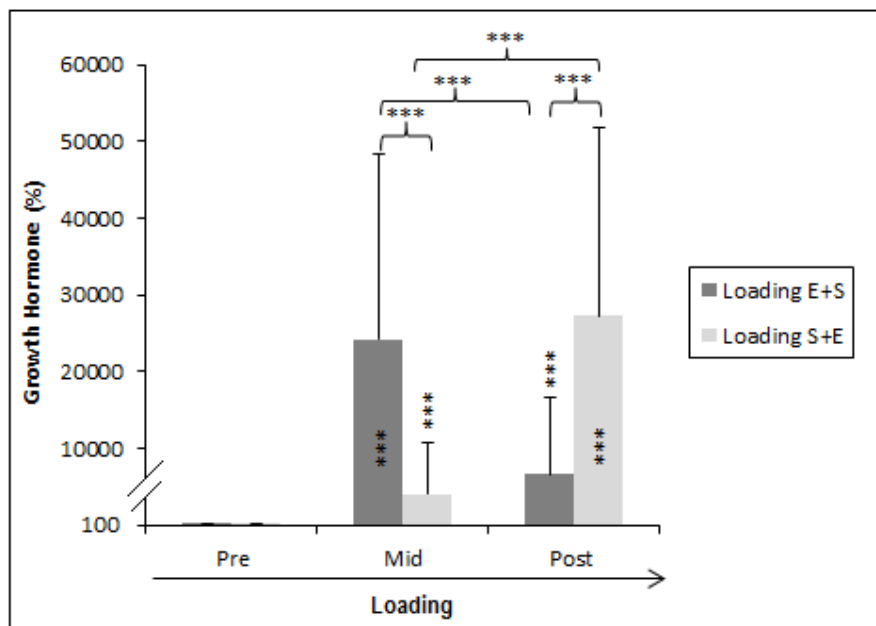


Figure 3c

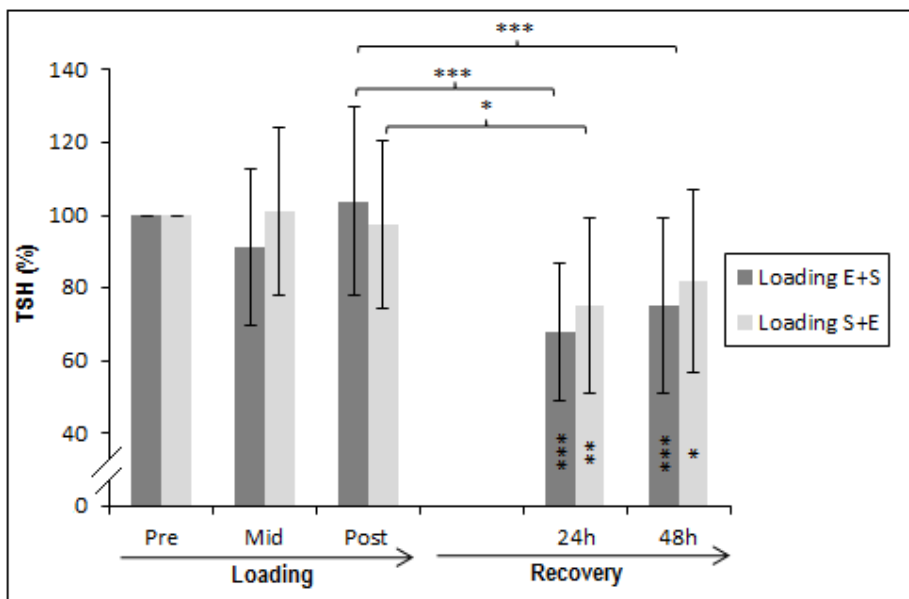


Figure 3d

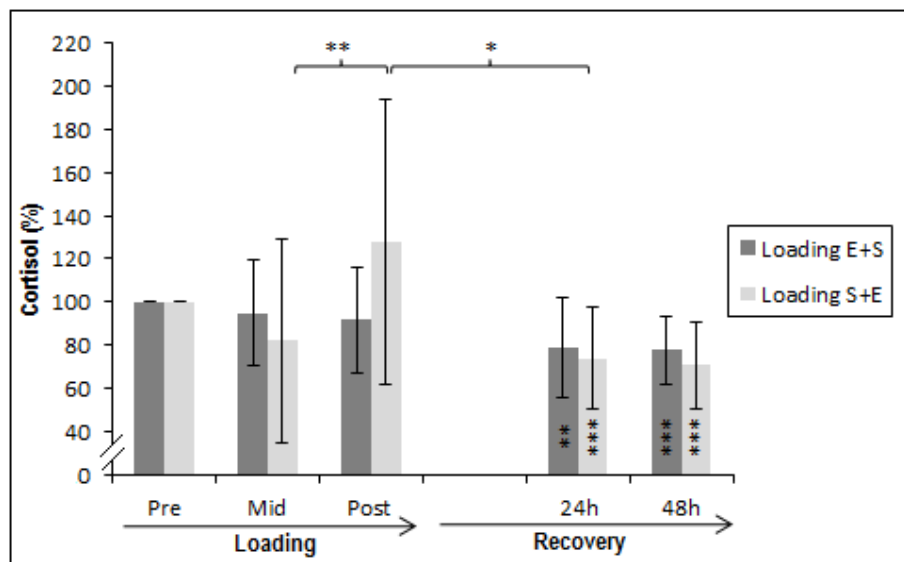


Figure 4

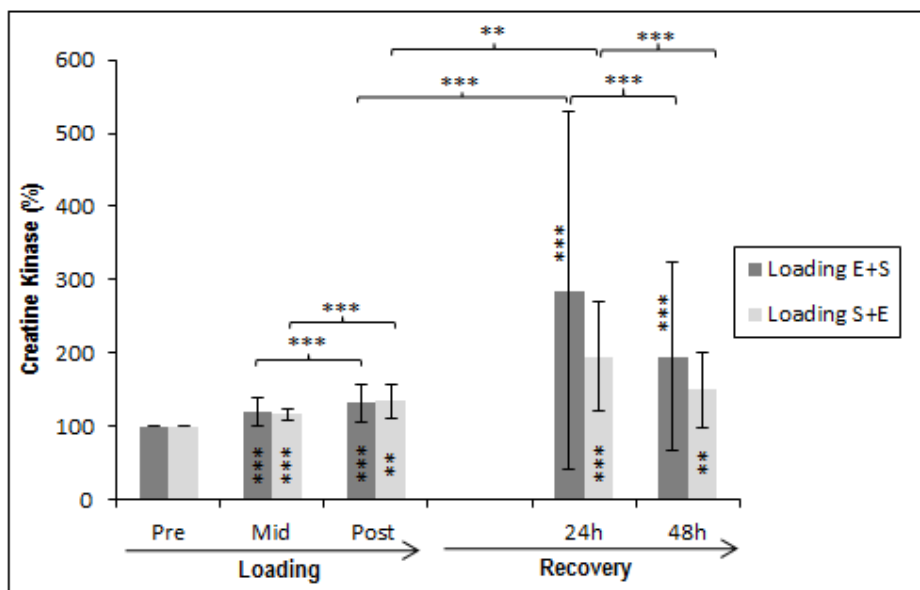


Figure 5a

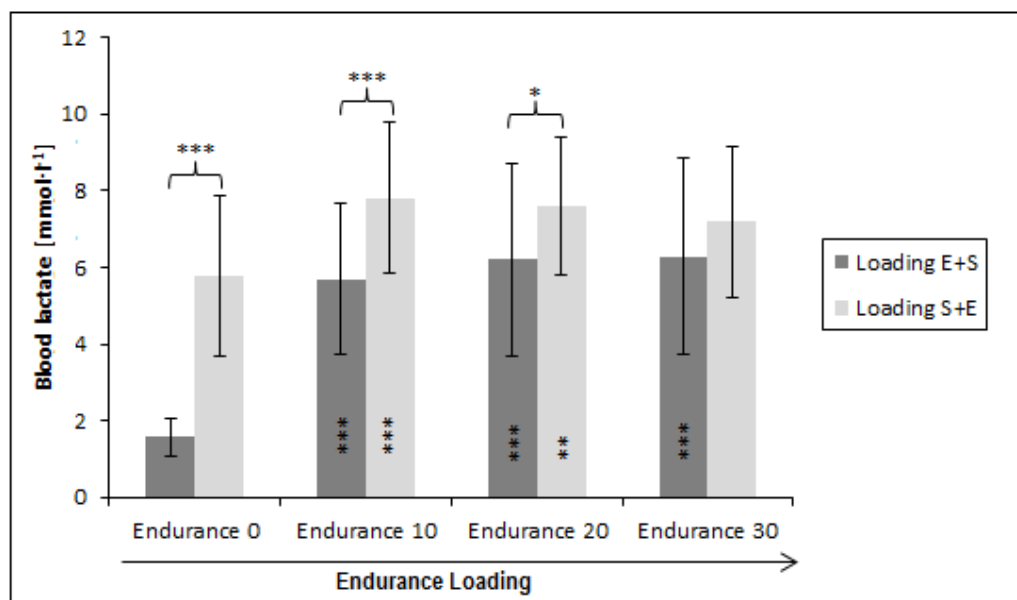




Figure 5b

