

# Neuromuscular Adaptations to Different Modes of Combined Strength and Endurance Training

## Authors

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## Key words

- concurrent training
- order effect
- voluntary activation

## Abstract

The present study investigated neuromuscular adaptations between same-session combined strength and endurance training with 2 loading orders and different day combined training over 24 weeks. 56 subjects were divided into different day (DD) combined strength and endurance training (4–6 d·wk<sup>-1</sup>) and same-session combined training: endurance preceding strength (E+S) or vice versa (S+E) (2–3 d·wk<sup>-1</sup>). Dynamic and isometric strength, EMG, voluntary activation, muscle cross-sectional area and endurance performance were measured. All groups increased dynamic one-repetition maximum ( $p < 0.001$ ; DD 13±7%, E+S 12±9% and S+E 17±12%) and isometric force ( $p < 0.05–0.01$ ), muscle cross-sectional area ( $p < 0.001$ ) and maxi-

mal power output during cycling ( $p < 0.001$ ). DD and S+E increased voluntary activation during training ( $p < 0.05–0.01$ ). In E+S no increase in voluntary activation was detected after 12 or 24 weeks. E+S also showed unchanged and S+E increased maximum EMG after 24 weeks during maximal isometric muscle actions. A high correlation ( $p < 0.001$ ,  $r = 0.83$ ) between the individual changes in voluntary activation and maximal knee extension force was found for E+S during weeks 13–24. Neural adaptations showed indications of being compromised and highly individual relating to changes in isometric strength when E+S-training was performed, while gains in one-repetition maximum, endurance performance and hypertrophy did not differ between the training modes.

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## Bibliography

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## Abbreviations

CSA	Cross-sectional area
DD	different day strength and endurance training
EMG	Electromyography
E+S	Same-session combined strength and endurance training, endurance preceding strength
ITT	Interpolated twitch technique
QF	Quadriceps femoris
S+E	Same-session combined strength and endurance training, strength preceding endurance
VA	Voluntary activation
VL	Vastus lateralis

## Introduction

Strength and endurance training are known to result in different neuromuscular adaptations. While strength training results in large gains in

maximal strength and muscle hypertrophy (e.g. [28]), endurance training leads only to minor changes in strength performance, but produces gains in endurance performance variables such as maximal oxygen consumption and maximal aerobic power output [22, 38, 49]. Previous research indicates that adaptations in strength, but not endurance, may be compromised when strength and endurance are trained for concurrently, in comparison to strength training alone (e.g. [24, 29]). This phenomenon has been observed with both different day (e.g. [6, 29]) and same day combined strength and endurance training (e.g. [32]). The compromises in strength training adaptations with concurrent training in previously untrained subjects were originally documented by Robert Hickson [24], when untrained subjects embarked on a training regimen with an overall remarkably high volume and frequency strength and endurance training. As a result of the strenuous training program, attenuations in strength gains were observed already after 5–6 weeks of combined training [24]. The same interference

phenomenon has also been clearly observed in well-trained endurance athletes both in terms of maximal and explosive strength [43]. Conversely, when the total training volume of the concurrent training design is reduced both in a single session as well as over time, increases in maximal strength, maximal voluntary neural activation and muscle hypertrophy of the trained muscles have been found to be more likely to occur [29,34,45]. While the time-saving aspect rationalizes the idea of combining the 2 modes in the same training session, it raises the question of the possible differences between disparate intra-session exercise orders (“the order effect”). Considering that the first loading may result in residual fatigue compromising the second one, it can be thought to accumulatively suppress the favorable adaptations over a prolonged period of training [11,44,45]. Fatigue-induced acute adjustments in the nervous system following an endurance training session may include reduced force output of a particular muscle group and thus altered muscle recruitment [48], decreased rate of force development [8,33] and reduced neural input to the muscle, ultimately resulting in decreased efficiency of the muscle contractile mechanisms [40]. Strength loading leads to acute decreases in neuromuscular performance [50] and, similarly, acute decreases in maximal force have also been noted following endurance exercise [46,47]. Attention has previously been directed towards molecular adaptations as an explanation for possible difficulties in optimizing performance when training concurrently for strength and endurance [4,15], whereas neural adaptations and differences between different modes of concurrent training have not been comprehensively investigated. Results from elderly populations point to an order effect in favor of strength preceding endurance with regard to training-induced neuromuscular adaptations [11,12], although age-related declines in the same parameters [30] may complicate the interpretation. Furthermore, numerous different research designs make the comparison difficult, thus obscuring how adaptations following different day and same-session combined strength and endurance protocols relate to each other. To the best of our knowledge, a long-term comparison of different day combined strength and endurance training and same-session combined training with different exercise orders has not been conducted. The purpose of this study was to examine possible differences or limitations in neuromuscular adaptations between same-session combined training with different loading orders and combined strength and endurance training performed on different days. Specifically, this object was achieved by monitoring adaptations in strength and force, voluntary activation, surface electromyography, muscle cross-sectional area and maximal power output during cycling over the course of a 24-week training intervention.

## Methods

### Subjects

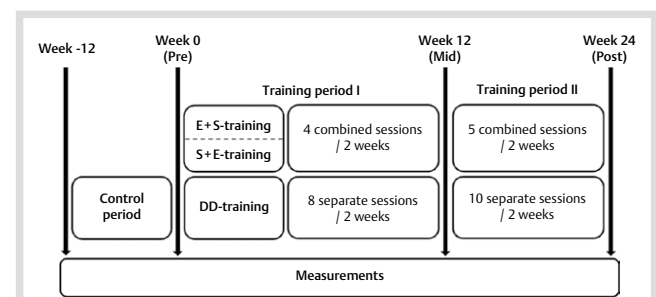
70 healthy men aged 18–40 from the Jyväskylä, Finland region were recruited to participate in the study. Recruitment was conducted by several public announcements. Requirements for participation included the subjects to 1) be recreationally active (i.e. physically active but without systematic strength or endurance training for at least one year prior to the study), 2) have a body mass index of less than  $30 \text{ kg/m}^2$ , 3) abstained from smoking for a minimum of one year prior to the start of the study, 4) be free of chronic illnesses and 5) have no injuries or diseases

of the locomotor system. All subject candidates were interviewed regarding their general health and attended a health screening that included resting ECG analyzed and approved by a cardiologist as a part of the pre-screening process. The selected subjects received detailed information about the study design, measurements and procedures and were required to give written informed consent prior to participation. The study received ethical approval from the ethics committee of the University of Jyväskylä and was conducted in accordance with the Declaration of Helsinki as well as the ethical standards of the International Journal of Sports Medicine [23].

### Study design

All subjects were initially familiarized with the training- and measurement protocols and equipment of the current study before proceeding to basal measurements of maximal strength, muscle cross-sectional area and endurance. The study spanned a 24-week period for the experimental groups and 12 weeks for the control period. The measurement protocols were repeated after the first 12 weeks as well as after the completion of the entire 24-week training period. The overview of the study design is presented in **Fig. 1**.

The strength and endurance measurements as well as measurement of muscle cross-sectional area were separated from each other by a minimum of 2 days. The measurements always took place at the same time of day ( $\pm 1 \text{ h}$ ) to minimize circadian fluctuation. Subjects were instructed to abstain from caffeine 12h and alcohol 24h prior to the measurements as well as to consume a light snack 2h before but allowing at least 4h between a main meal and the measurement session. The last session of both training periods was separated from the following measurements by a minimum of 2 and a maximum of 4 days. After the basal measurements of ultrasound and strength and endurance performance, each subject was assigned to one of the 3 training groups for the entire duration of the study: different day strength and endurance training (DD), same-session combined strength and endurance training with strength preceding endurance (S+E) or endurance preceding strength (E+S). Assignment was done by pairwise matching physical characteristics. The control period lasted for 12 weeks and took place prior to the start of the study. During the control period a group of subjects ( $n=24$ ) were asked to act as a control group. They were asked to refrain from strenuous physical strength and endurance activities, but were allowed to do light exercise and



**Fig. 1** Overview of the study protocol. Different day training (DD,  $n=21$ ) = Weeks 0–12:  $2 \text{ d} \cdot \text{wk}^{-1}$  [1 strength, S] and  $2 \text{ d} \cdot \text{wk}^{-1}$  [1 endurance, E], weeks 13–24:  $2\text{--}3 \text{ d} \cdot \text{wk}^{-1}$  [1S] and  $2\text{--}3 \text{ d} \cdot \text{wk}^{-1}$  [1E]. Same session combined training = endurance before strength (E+S,  $n=17$ ) and strength before endurance (S+E,  $n=18$ ), weeks 0–12:  $2 \text{ d} \cdot \text{wk}^{-1}$  [1E+1S] or [1S+1E], respectively; weeks 13–24:  $2\text{--}3 \text{ d} \cdot \text{wk}^{-1}$  [1E+1S] or [1S+1E], respectively.

maintain daily activities. After the control period of 3 months, 21 subjects continued the study to form the DD group. The entire 24-week program was completed by 56 subjects. 14 subjects did not finish the study due to minor injuries or medical issues, occupational changes or personal choices to drop out due to the demanding training program. • **Table 1** contains the data from subjects who completed the entire 24-week program.

### Familiarization session

To minimize a possible learning effect to the tested variables, subjects participated in a familiarization session a few days prior to their first actual strength measurement. During the session, subjects were familiarized with the testing procedures, equipment and execution of the tested variables. For the isometric leg press and the isometric knee extension, knee angles of 107° (180° representing full extension) were utilized [27]. A starting knee angle of below 60° (58° ± 2°) was utilized for the dynamic leg press. All knee angles were measured with a hand-held goniometer in the same measurement devices as the actual testing later took place. The greater trochanter of the femur and lateral malleolus of the ankle of the right limb were used as anatomical reference points. To ensure the device was set up in the same way throughout the study, the device settings were stored on both paper and in a computer file. To further reduce the sources of error, subjects were asked to wear the same shoes for all measurement sessions throughout the study. Small marks were tattooed to the right limb with a lancet and permanent ink to ensure repeatable electromyographic (EMG) measurements throughout the study [25].

### Neuromuscular measurements

**Dynamic strength:** One-repetition maximum (1 RM) in bilateral leg press was measured using a David 210 weight stack horizontal leg press device (David Health Solutions Ltd., Helsinki, Finland). The subjects were seated with a starting knee angle below 60° (58° ± 2°). As a preparation for the 1 RM trials, subjects performed 3 warm-up sets (5 × 70–75% estimated 1 RM, 3 × 80–85% estimated 1 RM, 2 × 90–95% estimated 1 RM) with 1 min of rest between sets. When verbally instructed, subjects performed a dynamic action to a full leg extension (knee angle 180°). Upon successful completion, the load was increased. Subjects were allowed a minimum of 3 and a maximum of 5 maximal trials, after which the trial with the highest load was accepted as the 1 RM.

**Maximal isometric bilateral leg press force:** Maximal bilateral isometric leg press force was measured at a knee angle of 107° on a horizontal leg press device designed and manufactured by the Department of Biology of Physical Activity (University of Jyväskylä, Jyväskylä, Finland). When verbally instructed, subjects performed an isometric bilateral leg press action as rapidly as possible with the aim of reaching their maximum at the beginning of the trial and maintaining it for approximately 3 s. Subjects were allowed a minimum of 3 and a maximum of 5

maximal trials. A fourth and fifth trial was allowed, if the difference from the third trial to the previous 2 exceeded 5%. Force signals were passed in real-time to an analog to-digital converter (Micro 1401, Cambridge Electronic Design, Cambridge, UK) and transferred to a computer. Force signals were recorded with Signal 2.16 software (Cambridge Electronic Design, Cambridge, UK) and sampled at 2000 Hz and processed with a low-pass filter of 20 Hz. The trial with the highest exerted maximal force was used for further analysis. Trials were analyzed by a customized, automated script (Signal 2.16 software, Cambridge Electronic Design, Cambridge, UK).

### Maximal unilateral isometric knee extension force and interpolated twitch technique:

Maximal unilateral isometric knee extension force was recorded and electrical stimulation performed on a device designed and manufactured by the Department of Biology of Physical Activity (University of Jyväskylä, Jyväskylä, Finland). Subjects were seated upright with a knee joint angle of the right limb at 107°. The left limb was lifted onto a chair and positioned parallel to the floor. Subjects were secured to the knee extension device by a seatbelt at the hip and a pad strapped over the right knee. The ankle was strapped to the device 2 cm above the right lateral malleolus with a Velcro strap, which was connected to a strain gauge. Subjects were instructed to increase force gradually, reaching maximum voluntary force in approximately 3 s and maintaining the reached force level for a duration of approximately 4 s. Subjects performed 3 maximal isometric knee extension trials, the one with the highest voluntarily achieved force prior to the electrical muscle stimulation being accepted as the trial to be analyzed.

To assess voluntary activation (VA) of the quadriceps femoris (QF) muscle group, electrical muscle stimulation was delivered to the QF during the maximal unilateral knee extension using the interpolated twitch technique (ITT). 4 galvanically paired self-adhesive electrodes (6.98 cm Vtrodes, Mettler Electronics Corp, USA) were placed on the proximal and intermediate regions of the quadriceps muscle belly of the right limb. Rectangular single pulses of 1 ms were delivered during rest with stimulation intensity being increased in 5 mA increments with a constant-current stimulator (400V, Model DS7AH, Digitimer, UK) until a plateau in the stimulation-induced force was noted. To ensure maximal effect for the knee extension trials an additional 25% of stimulation intensity was then added to the current which was observed to produce maximum twitch force during rest. The supra-maximal single-pulse electrical stimulation was delivered to the muscle at 3 separate times during each trial: 3 s before voluntary knee extension (at rest), during the plateau of maximal voluntarily exerted force (super-imposed twitch) during knee extension and 5 s after the end of the contraction [35]. The voluntary activation percentage was calculated as  $VA\% = (1 - (P_{ts}/P_t)) \times 100$  [7], i.e. amplitude of twitch elicited by the electrical stimulation on top of the voluntary contraction ( $P_{ts}$ ) divided by the following control twitch ( $P_t$ ) delivered to the resting muscle 5 s after the maximal voluntary

Group	n	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )
control period	24	29.2 (±6.0)	180 (±6.0)	79.6 (±10.0)	24.5 (±2.9)
DD	21	29.2 (±6.0)	180 (±6.0)	80.5 (±11.1)	24.8 (±3.2)
E+S	17	29.4 (±6.1)	178 (±6.0)	79.7 (±11.9)	25.1 (±3.1)
S+E	18	29.8 (±4.4)	179 (±5.0)	75.2 (±8.5)	23.5 (±2.1)
total of training groups	56	29.4 (±5.8)	179 (±5.8)	78.8 (±10.4)	24.5 (±2.8)

**Table 1** Subject characteristics by group. DD = different day training; E + S = same session training, endurance preceding strength; S + E = same session training, strength preceding endurance

contraction. The stimulation procedure was always carried out by the same member of staff. Force signals were recorded with Signal 4.04 software (Cambridge Electronic Design, Cambridge, UK), sampled at 2000 Hz and processed with a low-pass filter of 20 Hz. Maximal force was manually analyzed on Signal version 4.04 (Cambridge Electronic Design, Cambridge, UK).

**Electromyography (EMG):** Muscle activity of the vastus lateralis (VL) muscle of the right leg was monitored through surface electromyography. Placement of 2 adhesive electrodes (bipolar configuration Al/AgCl electrodes with an inter-electrode resistance <5 k $\Omega$ , Blue Sensor N ECG Electrodes, Ambu A/S, Denmark) was defined according to the SENIAM guidelines as two-thirds of the distance along the line between the anterior spina iliaca superior to the lateral side of the patella, on the muscle belly. This point was subcutaneously tattooed onto the right limb during the familiarization session, and during the actual measurements the area over and around the tattoo was prepared for better signal conduction by shaving, abrasion, and wiping with rubbing alcohol. The electrodes were placed as close as possible to each side of the tattoo, aligned with the estimated pennation angle of the VL. The activity of the VL muscle was recorded during the isometric muscle actions and electrical muscle stimulation.

The raw EMG signals from the maximal isometric bilateral leg press was amplified by a factor of 1 000 and sampled at 3 000 Hz. The signals were passed from a portable transmitter to a receiver box (Telemetry 2400R, Noraxon, Scottsdale, AZ, USA) from which the signal was passed on to a desktop computer via an analog-to-digital converter (Micro 1401, Cambridge Electronic Design, Cambridge, UK). Analysis of the isometric EMG and conversion into integrated EMG (iEMG, mV·s) was performed using a customized, automated script (Signal 2.16, Cambridge Electronic Design, Cambridge, UK). Average maximum iEMG (mV·s) of the VL was determined during the 500–1 500 ms time period after the onset of the contraction, representing the peak force phase for isometric bilateral leg press.

The raw EMG signals from the unilateral isometric knee extension with super-imposed electrical twitch were band-pass filtered (20–350 Hz) and manually converted to root mean square (rmsEMG, mV) during a 500 ms time frame of the peak force phase immediately preceding the super-imposed twitch using Signal 4.04 software (Cambridge Electronic Design, Cambridge, UK).

The neuromuscular measurements were performed in the following order: isometric force, electrical stimulation and dynamic strength.

### Cross-sectional area

Cross-sectional area (CSA) of the VL muscle was measured using a B-mode axial-plane ultrasound (SSD-a10, Aloka, Tokyo, Japan) and employing a panoramic imaging technique [1] where the 10 MHz linear-array probe was moved sagittally across the thigh, starting from the lateral side and moving over the thigh medially. Images were taken of the right limb at 50% of the femur length, defined as the midpoint between the greater trochanter and the joint space on the lateral side of the knee. The midpoint was measured with the subject lying on the left side while the anatomical landmarks were palpated, marked and the distance measured with a measuring tape. The midpoint was marked subcutaneously with ink to ensure that the measurement was conducted at the same point throughout the study. During the actual measurement, subjects were lying in a supine position with legs fixed with a Styrofoam knee support to pre-

vent movement during the measurement. To ensure that the probe was moved in a straight line over the thigh, lines perpendicular to the measurement table were drawn across the thigh with a marker pen before every measurement session with the help of a specially crafted device. 3 clear images from every measurement point were saved for analysis, and the mean of the 2 values closest to each other were used as the final value in the statistical analyses. Images were analyzed with ImageJ -software (National Institute of Health, USA, version 1.44). The CSA was measured by manually marking the outlines of the muscles onto the image. The measurements were conducted by 2 members of staff trained for the task. The measurement for a given subject was always conducted and the images analyzed by the same person.

### Maximal power output during cycling

A maximal endurance loading was conducted on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) utilizing a graded exercise protocol. The initial load for each subject was 50 W, with 25 W increments applied every 2 min until volitional exhaustion. Maximal power output was calculated using the following equation:  $W_{\max} = W_{\text{com}} + (t/120) * 25$ , where  $W_{\text{com}}$  was defined as the load of the last completed stage and  $t$  the time of the last incomplete stage. The aerobic and anaerobic thresholds were determined individually for each subject by the points of deflection in the curves of ventilation, oxygen consumption, production of carbon dioxide and blood lactate [5].

At the end of each stage the Borg Rating of Perceived Exertion (RPE)-scale was used to monitor the subjective level of exhaustion. Heart rate was measured throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland).

### Training

No supervised training was performed during the control period. However, light to moderate intensity physical activity 1–2 times per week for 30–60 min at a time was allowed. Additionally, maintenance of normal daily activities was encouraged. The 24-week training period was divided into two 12-week periods and was preceded by a preparatory phase with familiarization of the training procedures, equipment, loads and management of training programs and logs. During training period I, all same-session combined subjects completed 2 weekly “double” sessions of either [1E+1S] or [1S+1E], depending on the group to which they were assigned. During training period II the training frequency was increased to 5 training sessions over 2 weeks (5 sessions of [1E+1S] or [1S+1E]). A 5 to 10-min break was allowed between the 2 training modes (S or E). The DD group adhered to the same training program, but trained S and E on alternating days, thus completing 4 training sessions per week during training period I and 10 sessions over 2 weeks during training period II. All training sessions were supervised by the project staff to ensure completion and adherence to the training program. In addition to the supervised training sessions the maintenance of normal daily activity was encouraged, and the instructions for the subjects regarding additional physical activities were similar to those given for the control period.

Strength training was performed for all major muscle groups focusing on knee extensors, hip extensor and knee flexors. The training program was designed to be progressive and periodized, starting with circuit training and progressing through hypertrophy-inducing training towards maximal strength train-

**Table 2** Strength training for the lower extremities during the 24-week training intervention.

	Training period I				Training period II		
	Weeks 1–3	Weeks 4–7	Weeks 8–12	Weeks 13–14	Weeks 15–16	Weeks 17–20	Weeks 21–24
<b>load</b>	40–80%	70–85%	80–95%	40–60%	65–80%	80–85%	80–95%
<b>sets</b>	2–3	2–3	3–5	3	2–3	2–4	2–5
<b>reps</b>	10–20	10–15	3–10	12–20	10–12	8–10	3–10
<b>rest</b>	None (circuit)	1.5–2 min	1–3 min	None (circuit)	1.5–2 min	1.5–2 min	2.5–3 min
<b>exercises:</b>							
<b>LP</b>	Bilateral	Bilateral	Bilateral*	Bilateral	Bilateral	Bilateral	Bilateral*
<b>KE</b>	Bilateral	Bilateral	Unilateral	Bilateral	Bilateral	Bilateral	Unilateral
<b>KF</b>	Bilateral	Bilateral	Unilateral	Bilateral	Bilateral	Bilateral	Unilateral

\* Explosive repetitions with a load of 40% in 3 training sessions (out of 4) during weeks 11–12 and 3 sessions (out of 5) during weeks 23–24, Load = % of estimated 1 RM, Reps = repetitions per set, Rest = rest between sets and exercises, LP = leg press, KE = knee extension, KF = knee flexion

**Table 3** Endurance training (by cycling) during the 24-week training intervention.

	Training period I							
	Weeks 1–3	Weeks 4–7		Weeks 8–9		Weeks 10–11		Week 12
		Session I	Session II	Session I	Session II	Session I	Session II	
<b>intensity</b>	< AT	<AT and >AT	< AT	<AT and ~AnT	<AT and >AT	>AT and >AnT	<AT, >AT and >AnT	>AT – >AnT
<b>mode</b>	Continuous	Continuous	Continuous	Interval	Continuous	Interval	Interval	Interval
<b>duration</b>	30 min	30 min	45 min	45 min	50 min	45 min	45–50 min	35–45 min
	Training period II							
	Weeks 13–14	Weeks 15–16		Weeks 17–20		Weeks 21–23	Week 24	
		Session I	Session II	Session I	Session II			
<b>intensity</b>	< AT and >AT	~AT, <AnT	~ AT	<AT and ~AnT	~AT and >AT	>AT – >AnT	<AT and >AnT	
<b>mode</b>	Continuous	Continuous	Continuous	Interval	Continuous	Interval	Interval	
<b>duration</b>	40–45 min	30–35 min	30–45 min	25–45 min	30–50 min	35–50 min	25 min	

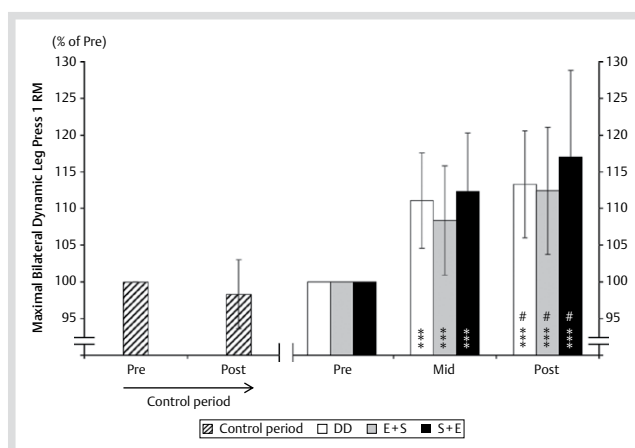
AT = aerobic threshold, AnT = anaerobic threshold, ≤5–10 bpm below, ≥5–10 bpm above, ~at the threshold (±5 bpm)

ing (Table 2). The periodization for the exercises of the extensors and flexors of the arms followed a similar pattern. In addition, exercises for the trunk were included. All lower body exercises were performed with weight-stack devices, while some of the upper body exercises were performed with dumbbells.

All endurance training sessions were carried out indoors on a cycle ergometer equipped with magnetic resistance. Training intensity was controlled by heart rate zones corresponding to the threshold values of aerobic (AT) and anaerobic thresholds (AnT), which were determined after the maximal cycle ergometer endurance test conducted during the basal measurements. During weeks 1–7 and 13–16 the training consisted of 30–45 min continuous cycling near the AT and progressed to interval training at and above AnT from weeks 8 and 17 onwards (Table 3). The thresholds were recalculated from the endurance test performed after 12 weeks of training and applied during training period II.

### Statistical analysis

Results are presented as means ± standard deviations. Data was analyzed with IBM SPSS Statistics v.20 software (IBM Corporation, Armonk, New York, USA). Repeated measures analysis of covariance (ANCOVA) with baseline values as covariates was used to assess training effects between groups. One-way ANOVA was used to assess differences in relative changes over time when time × group interactions were present. Within-group changes were analyzed with repeated measures ANOVA. Bonferroni post hoc procedures were applied when appropriate. The reported correlations are bivariate Pearson correlation coefficients ( $r$ ). Significances were set at \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Reported effect sizes (ES) are Cohen's  $d$ .

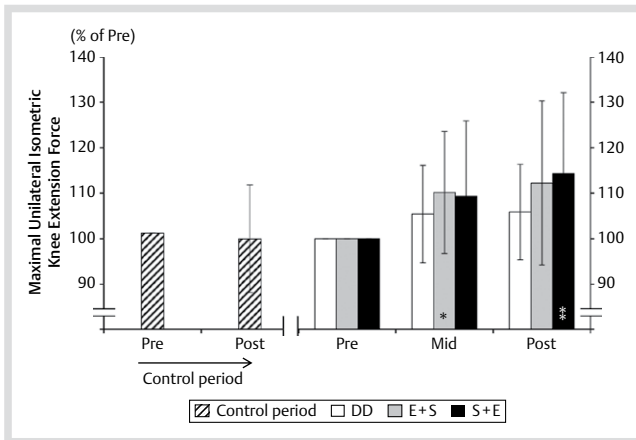


**Fig. 2** Relative changes in maximal bilateral dynamic leg press 1RM for all experimental groups and during the control period. \* = significant from pre; # = significant from mid. \*\*\*  $p < 0.001$  and #  $p < 0.05$ . (DD,  $n = 21$ ; E + S,  $n = 17$ ; S + E,  $n = 18$ ; control period,  $n = 24$ ).

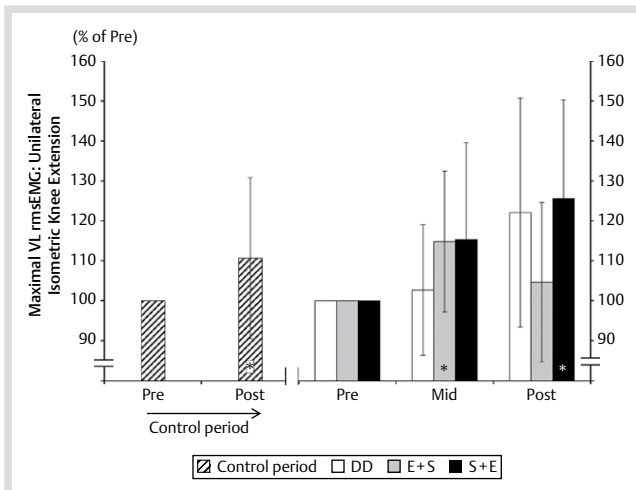
## Results

### Maximal strength

All groups significantly ( $p < 0.001$ ) increased bilateral concentric 1 RM during weeks 0–24 (DD by 13% from  $142 \pm 23$  kg ( $p < 0.001$ ,  $ES = 0.775$ ); E + S by 12%  $159 \pm 30$  kg,  $p < 0.001$ ,  $ES = 0.631$ ; S + E by 17% from  $143 \pm 23$  kg,  $p < 0.001$ ,  $ES = 1.03$ ) (Fig. 2). A significant ( $p < 0.05$ ) difference was observed between DD and E + S at Pre, but no significant between-group differences were observed for any of the experimental groups after training. No significant



**Fig. 3** Relative changes in maximal unilateral isometric knee extension force for all experimental groups and during the control period. \* = significant from pre. \* p<0.05 \*\* p<0.01. (DD, n=21; E+S, n=15; S+E, n=18; control period, n=24).

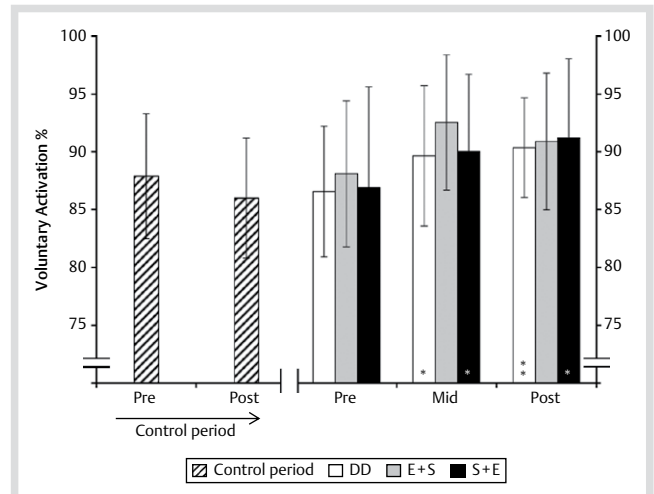


**Fig. 4** Relative change in maximal VL rmsEMG during a 500ms epoch of maximal force phase during unilateral knee extension for all experimental groups and during the control period. \* = significant from pre. \* p<0.05. (DD, n=16; E+S, n=14; S+E, n=15; control period, n=20).

change was observed in 1-RM strength during the control period.

All groups increased maximal bilateral isometric leg press force during weeks 0–12 (DD by 7% from 2332±588N, p<0.001, ES=0.263; E+S by 11% from 2626±653N, p<0.05, ES=0.431; S+E by 9% from 2338±540N, p<0.05, ES=0.338) and during weeks 0–24 (DD by 11%, p<0.001, ES=0.413; E+S by 12% p<0.05, ES=0.408; S+E by 13%, p<0.05, ES=0.481). There were no significant between-group differences in changes for the experimental groups at any time point. No significant change was observed in leg press force during the control period.

Changes in isometric knee extension force between weeks 0–24 were not statistically significant for the DD and E+S groups (Fig. 3). The S+E group increased knee extension force by 14% during 0–24 weeks (from 560±91N, p<0.01, ES=0.787). There were no significant between-group differences in changes for the experimental groups at any time point. No significant change was observed in knee extension force during the control period.



**Fig. 5** Voluntary activation percentage for all experimental groups and during the control period. \* = significant from pre. \* p<0.05 and \*\* p<0.01. (DD, n=17; E+S, n=15; S+E, n=17; control period, n=21).

**EMG and voluntary activation**

The DD and E+S groups increased average maximal VL iEMG during bilateral isometric leg press (at 500–1500ms) during weeks 0–12 (DD by 26%, p<0.001, ES=0.824; E+S by 24% p<0.01, ES=0.467). The DD and S+E groups increased maximal VL iEMG significantly during weeks 0–24 (DD by 31%, p<0.001, ES=0.376; S+E by 42%, p<0.001, ES=0.648). The change during 0–24 for E+S was not significant. There were no significant between-group differences in changes for the experimental groups at any time point. No significant change was observed in average maximal VL iEMG during the control period.

No significant change in maximal VL rmsEMG was observed in the DD or E+S groups at week 24 (Fig. 4). The S+E group experienced a significant (p<0.01, ES=0.708) increase of 26% during weeks 0–24. A significant (p<0.05) increase of 11% in maximal VL rmsEMG during unilateral isometric knee extension was observed during the control period.

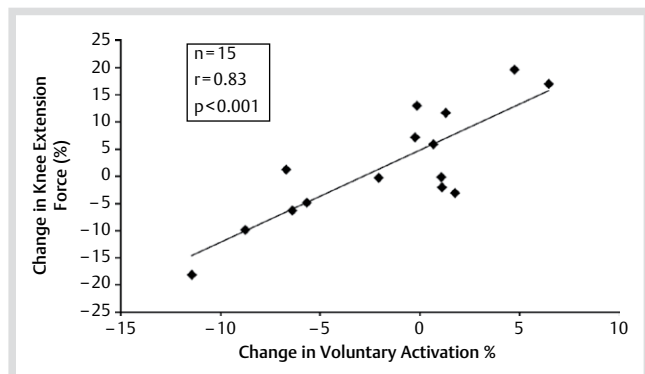
The DD and S+E groups increased voluntary activation during weeks 0–12 and 0–24 (DD by 3.8% from 86.6±5.7 to 90.4±4.3, p<0.01, ES=0.829; S+E by 4.3% from 86.9±8.8 to 91.2±6.9, p<0.01, ES=0.489) (Fig. 5). No significant change was observed in voluntary activation during the control period.

In the DD group a significant correlation between the individual changes in voluntary activation percentage and changes in knee extension force was found between weeks 13 and 24 (r=0.57, p<0.05). A significant correlation was found for the E+S group between weeks 0 and 12 (r=0.70, p<0.01), weeks 0–24 (r=0.70, p<0.01) as well as weeks 13–24 (r=0.83, p<0.001) (Fig. 6). In the S+E group a significant (r=0.50, p<0.05) correlation between the 2 variables was observed between weeks 0 and 12.

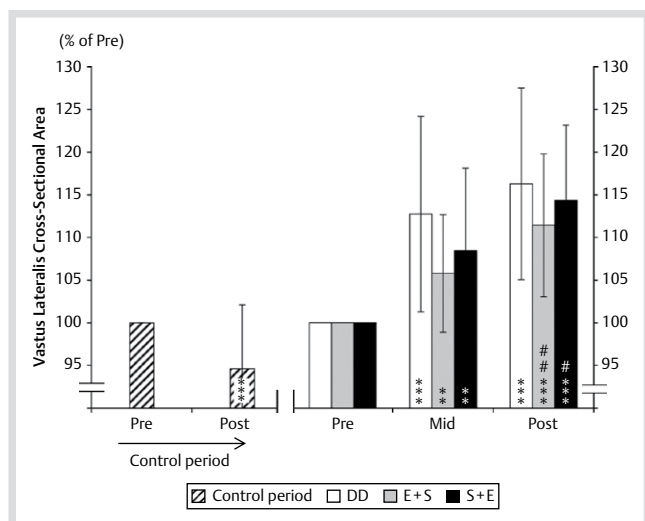
**Cross-sectional area of VL**

All groups increased VL cross-sectional area during weeks 0–24 (DD by 16% from 22.3±3.7cm<sup>2</sup>, p<0.001, ES=0.928; E+S by 11% from 26.9±4.3cm<sup>2</sup>, p<0.001, ES=0.668; S+E by 14% from 26.2±4.0cm<sup>2</sup>, p<0.001, ES=0.884) (Fig. 7). A significant (p<0.05) difference was observed between DD and E+S pre-training, but no significant between-group differences were observed for any of the experimental groups after training. A significant (p<0.001, ES=0.341) decrease in VL cross-sectional

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**Fig. 6** Correlation between the individual change in the voluntary activation percentage and the relative changes in maximal knee extension force in the E+S group during weeks 13–24.

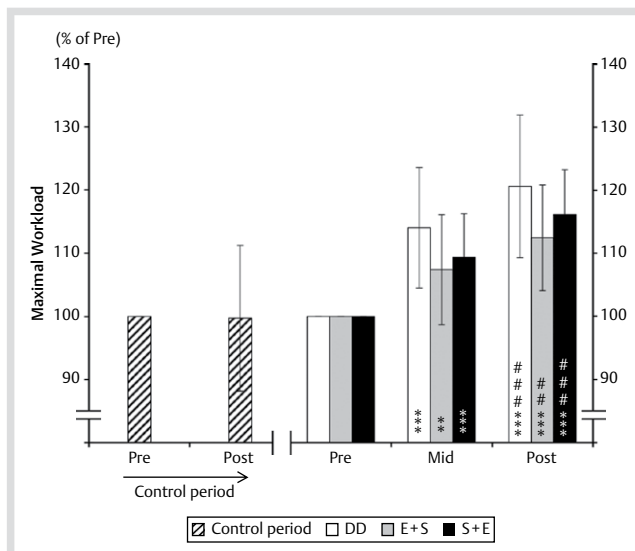


**Fig. 7** The relative change in VL cross-sectional area for all experimental groups and during the control period. \* = significant from pre # = significant from mid. \*\*  $p < 0.01$  \*\*\*  $p < 0.001$  and ##  $p < 0.01$ . (DD,  $n = 21$ ; E+S,  $n = 17$ ; S+E,  $n = 18$ ; control period,  $n = 24$ ).

area was observed during the control period ( $-5\%$  from  $23.5 \pm 3.5 \text{ cm}^2$ ).

### Maximal power output during cycling

All groups increased maximal power output during the cycle ergometer test during weeks 0–24 (DD by  $21 \pm 11\%$  from 233 W,  $p < 0.001$ ,  $ES = 1.56$ ; E+S by  $13 \pm 8\%$  from 266 W,  $p < 0.001$ ,  $ES = 0.809$ ; S+E by  $16 \pm 7\%$  from 245 W,  $p < 0.001$ ,  $ES = 1.07$ ). A significant ( $p < 0.05$ ) difference was observed between DD and E+S pre-training, but no significant between-group differences were observed for any of the experimental groups after training. No significant change in maximal power output was observed during the control period. RPE for the groups at the end of the last completed stage of the test were as follows: Ctrl pre  $19 \pm 1$  and post  $18 \pm 1$ ; pre DD, E+S, S+E  $18 \pm 1$ ,  $19 \pm 1$ ,  $19 \pm 1$  and post  $18 \pm 1$ ,  $19 \pm 1$ ,  $18 \pm 1$ . Maximal heart rate during the test was as follows: Ctrl pre  $196 \pm 7$  and post  $195 \pm 8$ ; pre DD, E+S, S+E  $195 \pm 8$ ,  $190 \pm 11$ ,  $193 \pm 8$ ; and post  $193 \pm 8$ ,  $189 \pm 10$ ,  $192 \pm 9$  (● Fig. 8).



**Fig. 8** The relative change in maximal power output during cycling ergometer test for all experimental groups and during the control period. \* = significant from pre # = significant from mid. \*\*  $p < 0.01$  \*\*\*  $p < 0.001$  ##  $p < 0.01$  and ###  $p < 0.001$ . (DD,  $n = 21$ ; E+S,  $n = 17$ ; S+E,  $n = 18$ ; control period,  $n = 24$ ).

### Discussion

The main finding of the present study was that gains in dynamic strength, hypertrophy and maximal power output during cycling were significant for all groups following the 24-week training intervention with no between-group differences. However, the study revealed indications for differing adaptations of voluntary activation level and muscle EMG between the combined strength and endurance training modalities during the 24-week training period. The statistically significant increase in voluntary activation percentage that was observed in the DD and S+E groups after 24 weeks of training was not present in the E+S group. Moreover, the individual changes in voluntary activation and maximal force development in isometric unilateral knee extension were correlated during the latter half of the training intervention for the E+S group.

The present findings in terms of training-induced gains in performance and muscle hypertrophy are for the most part consistent with findings from previous studies. In agreement with previously reported changes in muscle thickness [9, 11], the relative gains in VL CSA did not differ between the present groups (increases of 16%, 11%, 14% for DD, E+S and S+E, respectively). The increases in maximal power output reported in the present study (21%, 13%, 16% for DD, E+S and S+E, respectively) are of similar magnitudes to what has been reported previously [11] for elderly subjects already after 12 weeks of either E+S or S+E training with a 3-times-weekly regimen. In the corresponding time frame, the 3 groups in the present study performed one less endurance session weekly, improving maximal power output significantly (7–14%) and making further significant increases during weeks 13–24 with an increased training frequency. Similarly, the gains in maximal dynamic leg press did not differ statistically between the groups. The gains observed in 1 RM in the same-session combined groups (increases of 12% and 17%, for E+S and S+E, respectively) following 24 weeks of combined training can be considered comparable with earlier

studies, albeit somewhat different in the time course of adaptations [13,45]. With regards to different day strength and endurance training, the DD group demonstrated smaller (13%) gains in maximal dynamic leg press 1 RM in comparison to some earlier findings (22–25%) [29,45]. Sale [45] has suggested same-session combined training to be inferior to different day training in terms of gains in maximal dynamic strength, but this did not take place in the present study. The differences observed between results from earlier studies and the present study could be attributed to different training frequencies and volumes, acute variables of the training programs or even subject material.

Our findings with regards to the effects of same-session loading sequence on neural adaptations support in part speculations presented in earlier studies [9,11]. Cadore et al. [9] suggested that the E+S sequence in elderly subjects would hinder neural adaptations of the strength training from occurring, whereas no such compromise in adaptations was found for S+E. These earlier findings also suggest the E+S sequence to be unfavorable in terms of strength development in comparison to S+E. In the present study we found no between-group differences in either strength gains or maximal power output. However, the present S+E group demonstrated increased VL EMG and force in isometric actions during weeks 0–24, while the E+S group showed no significant increases in the same variables during this time period. Furthermore, the highly significant correlation ( $r=0.83$ ,  $p<0.001$ ) observed between individual changes in voluntary activation percentage and changes of knee-extension strength development during weeks 13–24 for the E+S group demonstrated that individuals who experienced reduced strength gains also decreased their voluntary activation percentage. These findings suggest that potential inhibition or interference in the nervous system could occur in the E+S group in a further prolonged period of combined training. This type of inhibition could be due to increased firing and inhibitory feedback from type III and IV afferents [3] or impairment in neuromuscular propagation [17] following the endurance loading during training. Ultimately, this could affect the subsequent strength loading and, thus, strength-training induced adaptations. However, we are unable to confirm these speculations using the present methodology.

It is worth noting that as increases in maximal force are not exclusively explained by neural adaptations but also rely on muscle hypertrophy [25,37,39], which could partly explain the improvements in strength for the E+S group despite the compromised adaptation in EMG activity and voluntary activation. The neural adaptations initially being responsible for improvements in force with hypertrophy following later [25,37,39] supports this assumption, as the E+S group experienced significant gains in VL CSA during both training periods. Furthermore, the stimuli for muscle hypertrophy that is produced by combined training has been suggested to override possible hypertrophy-blunting effects observed particularly after interval-type endurance training [15], which is reflected in the significant improvements of VL cross-sectional area for all groups in the present study. It is worth noting that even cycling alone has been found to induce a certain level of hypertrophy, as far as previously untrained subjects are concerned. The limited increase observed by Mikkola et al. [36] in the quadriceps femoris cross-sectional area was attributed to be the result of the repeated pedal push action, as the force produced was suggested to be high enough and the push-phase long enough for hypertrophy-inducing stimulus to occur. This could further clarify the

increases in VL cross-sectional area despite the training program not being exclusively focused on hypertrophy.

Recent findings [10,46] regarding the acute responses of combined strength and endurance loadings has raised concerns about the effects of possible residual fatigue that the first part of a combined strength and endurance loading may have on the subsequent part. Considering this, the obvious difference between the training programming of the present 3 groups was the timing of the endurance modality in relation to the strength exercises. The DD group consistently had a minimum of 24 h of recovery between the training modes, and S+E performed strength training in a recovered state. Thus, E+S was the only group which continuously performed the strength exercises possibly in a fatigued state. Therefore, despite the fact that no acute variables were measured in the present study it is reasonable to discuss the possible role of the first part of the loading on the second part to find possible underlying mechanisms for the compromised neural adaptations. Possible residual fatigue from a cycling endurance loading could be expected to compromise the quality of a subsequent lower-body strength loading session, as it is known that the quadriceps femoris muscle group contributes to continuous cycling with a significant percentage of its maximum activity even at relatively low workloads [19]. Additionally, impairments in maximal voluntary neuromuscular function following fatiguing cycling appear to be specific to the working muscles [18], and prolonged cycling exercise appears to limit the force-generating capacity to the muscles via both central and peripheral mechanisms, thus leading to a reduction in muscular capacity in the working muscles during subsequent activities [2,18,33]. Further clarification is needed as to whether fatigue-induced neural responses from the endurance component alone through inhibitory mechanisms can be regarded as responsible for disadvantageous neural adaptations following the present E+S training program, or whether the immediate addition of a strength-loading is the factor overloading the nervous system. A recent study [46] investigating the order effect in an acute setting gives an indication for the latter, as in the E+S sequence both the first and second part of the loading significantly contributed to the total fatigue in terms of acute decreases in maximal isometric force and rapid force production. In the S+E condition, however, the measured variables were not further affected by the endurance loading, indicating that the strength loading was responsible for inducing most of the total fatigue when performed first. However, bearing in mind that all groups in the present study experienced gains in maximal power output during cycling, it appears that the possible strenuousness of strength loading does not prevent adaptations in endurance performance.

Despite the present differences in neural adaptations there were no significant differences in strength and hypertrophic adaptations between the groups. This, in turn, could partly be attributed to the periodized nature of the training program. The present strength training did not exclusively consist of high-load, neurally demanding strength training and thus did not provide a maximum stimulus to the nervous system [26]. Had this been the case, the unfavorable adaptations in muscle activity and voluntary activation may have appeared more pronounced as the strength training for the E+S group would continuously have been performed in an already fatigued state. However, as the voluntary activation percentage did significantly increase over 24 weeks for DD and S+E approximately at the same magnitude as would be seen after pure strength train-



ing [31,42], the strength training protocol of the current study cannot be considered insufficient, but rather diluted by lighter strength-endurance type training. This, on the other hand, supports previous suggestions of endurance training playing the inhibitory part in compromised strength performance-related adaptations following combined training by inhibiting the force-generating properties of the neuromuscular system [16,24,29]. Furthermore, cycling as the choice of endurance training modality may not hinder lower-body strength development to the same extent as e.g. running, due to similar biomechanical patterns as multi-joint lower-body strength exercises [21].

### Methodological Considerations and Limitations

To investigate training-induced neural adaptations, the present study evaluated voluntary activation utilizing the ITT technique together with surface EMG. Increases or decreases in VA are not necessarily reflected similarly in the surface EMG-signal, thus meaning that EMG, as a representation of neural drive, may be an oversimplification [14,20]. In order to minimize possible additional inaccuracies in the EMG-recordings arising from technical, anatomical or physiological sites [14], all EMG recording-related procedures were carefully conducted, and the EMG locations were permanently marked subcutaneously. In the measurement setting of the present study, direct stimulation of the femoral nerve was found to be too challenging to perform to obtain highly repeatable results. Thus, we opted for direct electronic muscle stimulation of the QF, as this method has been suggested to be a valid alternative to nerve stimulation to assess activation level [41]. As the results of the ITT-measurements may be muscle and angle-specific and depend on the timing of the superimposed and resting twitches [20], this was counteracted by identical measurement settings throughout the study and always having the same member of the staff performing the ITT-measurements. It should be noted, that in the present study both VA and EMG were found to follow the same direction of training-induced adaptations in the E+S group during the latter half of the 24-week training period when compromised adaptations were observed. Nevertheless, the interpretation of these findings needs to be done with caution with regard to neural adaptations, as both the EMG and ITT-procedures have their methodological limitations.

In conclusion, the choice of a combined training mode may not be of great importance in terms of gains in strength and endurance performance or muscle hypertrophy in previously untrained subjects. However, it could be speculated that either a longer training period than the present 24-week intervention or higher training volume and/or frequency might result in more severe neural interference or limitations in the E+S training mode. Whether this would also be reflected in compromises in strength development needs to be further investigated.

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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