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**Neuromuscular adaptations to combined strength and endurance training:
order and time-of-day**

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1 **Abstract**

2 The present study examined the effects of 24 weeks of morning vs. evening same-session
3 combined strength (S) and endurance (E) training on neuromuscular and endurance performance.
4 Fifty-one men were assigned to the morning (m) or evening (e) training group where S preceded
5 E or vice versa (SE_m , ES_m , SE_e and ES_e) or to the control group. Isometric force, voluntary
6 activation, EMG and peak wattage during the maximal cycling test were measured. Training
7 time did not significantly affect the adaptations. Therefore, data are presented for SE_{m+e}
8 (SE_m+SE_e) and ES_{m+e} (ES_m+ES_e). In the morning no order specific gains were observed in
9 neuromuscular performance. In the evening, the changes in isometric force (SE_{m+e} $15.9\pm 16.7\%$,
10 $p=0.001$; ES_{m+e} $4.1\pm 12.2\%$, $p=0.615$) and EMG (SE_{m+e} $38.3\pm 31.7\%$, $p=0.001$; ES_{m+e}
11 $14.67\pm 36.44\%$, $p=0.486$) were larger ($p=0.014$) in SE_{m+e} than in ES_{m+e} and in voluntary
12 activation larger ($p=0.026$) in SE_{m+e} compared to controls. Peak wattage increased in the
13 morning (SE_{m+e} $15.9\pm 9.2\%$, ES_{m+e} $22.0\pm 7.0\%$; $p<0.001$) and evening (SE_{m+e} $16.3\pm 7.2\%$, ES_{m+e}
14 $21.0\pm 9.0\%$; $p<0.001$) but were larger ($p<0.05$) in ES_{m+e} . The current training program led to
15 greater neuromuscular adaptations when SE-training was performed in the evening, whereas the
16 ES-training provided more optimal conditions for endurance performance adaptations both in the
17 morning and evening.

18 **Keywords:** diurnal rhythms; EMG; voluntary activation; concurrent training; muscle force

1 **Introduction**

2 Maximal neuromuscular performance has been shown to fluctuate with time-of-day, with 5-15%
3 higher strength values observed in the evening [8,13,24,36,46] compared to the morning.
4 However, in the case of endurance performance, the effect of diurnal rhythms seems to dissipate
5 [11,15,17], although some studies have demonstrated that tolerance of high-intensity endurance
6 exercise (e.g. performed as cycling) is higher in the evening [2,6]. It has been proposed that these
7 fluctuations in strength and endurance performance may also affect the chronic adaptations to
8 exercise training [11]. Previous strength training interventions have found that changes in
9 maximal strength performance might be largest at the time-of-day when the training is regularly
10 performed [10,45,47]. Therefore, it has been suggested that strength training in the morning
11 hours may blunt the typical diurnal fluctuations [45,47]. However, the absolute increases in
12 maximum strength have been found to be similar between the morning and evening strength
13 training groups [47]. Literature regarding the time-of-day-effect on endurance training
14 adaptations has not been equally consistent. While some studies have suggested that similarly to
15 strength training, adaptations to endurance training are time-of-day-specific [31], other studies
16 do not demonstrate this interaction [30].

17 American College of Sports Medicine guidelines for general health and fitness [21] suggest
18 engaging in both endurance and strength exercise. However, combining these two exercise
19 modes within the same training program may lead to an “interference effect” [29,49] due to a
20 divergent influence of the two training regimes on the neural and muscular adaptations [48].
21 Although some recent studies have suggested that the interference effect can be avoided when
22 more than eight hours separate strength and endurance training [22], performing these two
23 training modes in close proximity may possibly interfere with the training adaptations. Lepers et

1 al. [37] have suggested that strength training adaptations may possibly be interfered by prior
2 endurance training, due to the acute residual fatigue developed in the neuromuscular system.
3 Therefore, one possible factor responsible for the interference effect is the intra-session sequence
4 of strength and endurance exercises [38]. E.g., in elderly men, same-session combined training
5 has been shown to lead to greater improvements in strength performance in the group which
6 always started the session with strength training (order-effect) [7]. However, age-induced
7 functional and physiological changes in the neuromuscular system [42] may have influenced the
8 training adaptations. In previously untrained young participants, the intra-session exercise
9 sequence does not seem to influence the strength improvements [9,44], although neural
10 adaptations have shown indications of being compromised and highly individual when
11 endurance training constantly precedes strength training over a period of several months [19].
12 Maximal endurance performance development has been shown mostly not to be impaired by the
13 order of performing strength and endurance training [14,16,19].

14 To the best of our knowledge, time-of-day-specific adaptations to prolonged combined strength
15 and endurance training have not been studied. The purpose of the present study was to examine
16 how the strength and endurance training order and time-of-day (morning vs. evening) affect the
17 adaptations in neuromuscular and endurance performance after 24 weeks of time-of-day-specific
18 same-session combined strength and endurance training. To investigate the time-of-day and
19 order specific adaptation, we hypothesized that performing endurance training regularly before
20 strength training would limit neuromuscular adaptations, whereas the intra-session order of
21 strength and endurance training would not influence the adaptations in endurance performance.
22 In addition, we hypothesized that the adaptations in strength and endurance performance would
23 show some time-of-day dependency.

1 **Methods**

2 Participants

3 Fifty-one recreationally physically active, healthy men (age 32.3 ± 5.6 years, 1.81 ± 0.06 m,
4 80.8 ± 10.9 kg) participated in the study. Participants had no history of previous strength or
5 endurance training over the past year. They had no medical contraindications or musculoskeletal
6 issues that could put them at risk during testing or training or compromise their ability to adapt.
7 Before involvement in the study, each participant was screened via a health questionnaire and
8 resting ECG by a physician. Participants' chronotype was assessed before the study based on the
9 Munich Chronotype Questionnaire [43]. None of the participants belonged to an extreme
10 morning or evening chronotype or were involved in shift or night work. None of the participants
11 reported the use of medications that would affect the diurnal rhythms or sleep cycle. All
12 participants were informed of the procedures, risks and benefits of the study, and they provided
13 written consent before participation. The study was conducted in accordance with the ethical
14 standards of the journal [25], complied with the Declaration of Helsinki and was approved by the
15 Ethics Committee in the University of Jyväskylä.

16 Participants were divided into four training groups matched for anthropometrics and physical
17 performance following baseline testing [35]: (i) training in the morning (m) and performing
18 endurance (E) training always before strength (S) training (ES_m , $n=9$), (ii) training in the
19 morning with strength always preceding endurance training (SE_m , $n=9$), (iii) training in the
20 evening (e) and performing endurance before strength training (ES_e , $n=11$), (iv) training in the
21 evening with strength always preceding endurance training (SE_e , $n=12$). The controls ($n=10$)
22 were asked to maintain their pre-experimental physical activity level throughout the study. All

1 participants were instructed to continue their normal dietary intake and habitual physical
2 activities throughout the intervention period but to avoid any additional strength and/or
3 endurance training.

4 Study design and measurements

5 The study design is described more in detail in Kääsmaa et al. [35]. The 24-week combined
6 strength and endurance training period consisted of two 12-week periods and the measurements
7 were carried out before (Pre), during (Mid) and after (Post) the intervention. Strength and
8 endurance measurements took place both in the morning (between 6:30 ± 30 min and 9:30 ± 30
9 min) and in the evening (between 16:30 ± 30 min and 19:30 ± 30 min) independent of the group
10 assignment. Within individuals, the tests were always carried out in the same order and at the
11 same time-of-day (±1h) at all three measurement points with 36 hours separating the
12 performance tests. For the measurements after 12 and 24 weeks of training, the last training
13 session and the first measurement were always separated by a minimum of two and maximum of
14 four days. The participants were asked to follow their usual sleeping habits on the night
15 preceding each testing session and to refrain from exercise training for two days before the
16 testing. They were asked to avoid alcohol for 24 hours and caffeine for 12 hours before the
17 physical performance tests.

18 Neuromuscular performance

19 Before the start of the measurements a familiarization testing session was carried out for all
20 participants on a non-training-specific time-of-day. During the familiarization session
21 participants were familiarized with the testing procedures and set-up for the equipment were
22 recorded for each participant. Also the placement of electromyographic (EMG) electrodes was

1 marked with indelible ink tattoos according to the SENIAM guidelines [28] to ensure repeatable
2 electrode positioning [32].

3 Maximal unilateral isometric knee extension force (MVC_{KE}) was measured using a device
4 designed and manufactured by the Department of Biology of Physical Activity (University of
5 Jyväskylä, Finland). The participant was seated in the device with a knee angle of 107° for the
6 right leg and the left leg rested in the horizontal position on a chair [33]. Hip and knee angles
7 were firmly secured by a seatbelt at the hip, pad strapped over the right knee and an adhesive
8 fabric strap above the right ankle. Participants were asked to perform three maximal trials by
9 increasing force gradually over 3 seconds. The trial with the highest force was used for further
10 analysis. The force signal was sampled at 2000 Hz and low-pass filtered (20 Hz). Maximal force
11 was manually analyzed using Signal 4.04 (Cambridge Electronic Design, UK).

12 To assess the voluntary activation percentage (VA%) of the quadriceps femoris muscle, the
13 interpolated twitch technique [39] was used to stimulate the right quadriceps muscles during the
14 isometric knee extension action. Four galvanically paired self-adhesive electrodes (7 cm
15 PolarTrode; Polar Frost USA; Anaheim, CA; USA) were placed on the proximal and mid-
16 regions of the quadriceps muscle belly of the right leg. The current of single 1-ms rectangular
17 pulses were increased progressively using a constant-current stimulator (Model DS7AH,
18 Digitimer Ltd, UK) in 5mA steps until a plateau in the passive twitch response was observed. To
19 ensure maximal effect for the knee extension trials, 25% of the stimulation current was added.
20 This supramaximal single-pulse stimulation was delivered to the muscle at rest 3 seconds before
21 the voluntary knee extension, during the plateau of voluntary peak knee extension force and 5
22 seconds after the cessation of contraction. VA% was calculated according to the formula by
23 Bellemare & Bigland-Ritchie [4]:

1
$$VA\% = [1-(P_{ts}/P_t)] \cdot 100,$$

2 where P_{ts} is the amplitude of the twitch elicited by the electrical stimulation on top of the
3 maximal voluntary contraction and P_t is the amplitude of the twitch delivered to the passive
4 muscle 5 seconds after the voluntary contraction.

5 Muscle activity was recorded through surface electromyography (EMG) during MVC_{KE} from the
6 vastus lateralis (VL) muscle of the right leg. EMG was collected from the maximum force level
7 over the 500 ms time period, immediately before the superimposed twitch. EMG was amplified
8 by a factor of 1000 (NeuroLog Systems NL844, Digitimer Ltd, UK) and sampled at a frequency
9 of 2000 Hz. The raw EMG signal was band-pass filtered (20-350 Hz) and converted to root mean
10 square (rmsEMG) on Signal 4.04 software (Cambridge Electronic Design, UK).

11 Endurance performance

12 Peak wattage (W_{peak}) was measured during the graded maximal aerobic cycling test to
13 volitional exhaustion on a mechanically braked cycle ergometer (Ergomedic 839E, Monark
14 Exercise AB, Sweden). The exercise intensity was increased by 25 W every two minutes starting
15 with 50 W. Pedaling frequency was sustained at 70 rpm throughout the test. The participants
16 were encouraged by the testing personnel to continue cycling until volitional exhaustion. Peak
17 wattage achieved during the cycling test was calculated with the following formula:

18
$$W_{peak} = W_{com} + (t/120) \cdot 25,$$

19 where W_{com} is the last cycling power completed and is the time in seconds the non-completed
20 power was maintained [34].

21

1 Training programs

2 The training program has been described in detail previously [35]. To summarize, training during
3 the intervention consisted of two 12-week progressive same-session combined strength and
4 endurance training periods either in the morning or in the evening. During the first 12 weeks
5 (wks 1-12) participants trained two times per week [2x(1S+1E) or 2x(1E+1S)] and during the
6 second 12-week training period (wks 13-24) all participants performed 5 training sessions in 2
7 weeks [5x(1S+1E) or 5x (1E+1S)]. The morning training groups (SE_m and ES_m) performed all
8 training sessions between 6:30-10:00. The evening training groups (SE_e and ES_e) performed all
9 training sessions between 16:30-20:00. Strength and endurance training was always performed in
10 a row with a maximum of 5-10 min break in between the two training modes. The training
11 programs were identical for the SE and ES group independent of the training time, only differing
12 in the sequence of training modes. All training sessions were supervised.

13 Strength training. Strength training consisted of hypertrophic and maximal strength exercises for
14 the whole body with the main focus being on the knee extensors and flexors as well as hip
15 extensors. Strength training was periodized to improve muscular endurance in the first 4 weeks,
16 which was performed as circuit training (intensity of 40-70% of 1RM, 2-3 sets, 10-20
17 repetitions). The subsequent 4 weeks (weeks 5-8) were designed to produce muscle hypertrophy
18 (intensity of 70-85% of 1 RM, 3-4 sets, 10-15 repetitions and 1.5-2 min of rest), followed by 4
19 weeks (weeks 9-12) of mixed hypertrophic and maximal strength training (intensity of 75-95%
20 of 1 RM, 3-5 sets, 3-8 repetitions and 2-3 min of rest). The same periodization was repeated
21 during the second 12 weeks of training with intensities adjusted for each subject to match the
22 current strength level.

1 Endurance training. Endurance training was carried out on cycle ergometers. Training intensities
2 were based on the maximum heart rate (HR_{max}) determined during the graded, training-time-
3 specific, maximal, incremental cycling test. During the first 12 weeks interval training session,
4 which consisted of 4x4 min high-intensity intervals (85-100% of HR_{max}) and separated by 4-min
5 active recovery periods (70% of HR_{max}) as well as continuous (65-80% of HR_{max}) training
6 session were performed once a week, respectively. During the second 12 weeks (wks 13-24),
7 when the training frequency increased, one additional high-intensity interval training session was
8 added.

9 Statistical analyses

10 Results are presented as means \pm standard deviation. Statistical analyzes were performed using
11 the Statistical Package for Social Sciences (SPSS version 22, Chicago, IL). Normality of the data
12 was checked using the Shapiro-Wilk test. EMG and VA% data were log transformed but
13 remained non-normally distributed even after log transformation. Morning and evening
14 differences at wk 0, 12 and 24 performance variables were checked by using paired samples T-
15 tests. Within-group changes over time in the morning and in the evening were examined with
16 repeated measures general linear models, where Time, with 3 levels (wk 0, wk 12, wk 14) was
17 set as the only factor. One-way analysis of variance (ANOVA) was used to assess time \times group
18 interactions in relative changes over time. Bonferroni post hoc procedures were applied when
19 appropriate. For the non-normally distributed data the paired-samples Wilcoxon signed rank test,
20 Friedman test and Kruskal-Wallis ANOVA were used respectively for within-group and
21 between-group differences. A Bonferroni adjustment was applied by multiplying the pairwise p
22 values with the number of comparisons. To analyze associations between different variables in
23 neuromuscular performance, Spearman correlation coefficients (r) were calculated. Statistical

1 significance was accepted a criterion alpha of $p < 0.05$. P-values ≤ 0.06 were accepted as a trend.
2 Effect sizes (es) for both within-group and between-group comparisons are presented as Cohen's
3 d for the normally distributed data and for non-normally distributed data effect sizes are
4 calculated based on the following equation:

$$5 \quad \text{es} = Z/\sqrt{n},$$

6 where Z is the z-score and n is the number of observations on which Z is based.
7

8 **Results**

9 No between-group differences were found in any variables at the baseline. None of the
10 neuromuscular or endurance performance variables showed significant morning to evening
11 differences in any group at any measurement time point. Time-of-day of training did not have
12 significant effect to the training adaptations and therefore, most of the data from the SE_m and SE_e
13 groups are combined and presented as SE_{m+e} and data from ES_m and ES_e presented as ES_{m+e} .

14 Maximal unilateral isometric knee extension force

15 In the morning isometric MVC_{KE} increased significantly in the SE_{m+e} ($p=0.028$; $es=0.439$) but
16 not in ES_{m+e} ($p=0.104$; $es=0.430$) (Fig 1a; Table 1). There were no statistically significant
17 between-group differences in changes for the experimental groups during the intervention in the
18 morning. In the evening MVC_{KE} increased in SE_{m+e} during the first 12 weeks ($p=0.002$;
19 $es=0.525$) and by week 24 ($p=0.001$; $es=0.636$), but not in ES_{m+e} ($p=0.615$; $es=0.235$). The
20 increases in SE_{m+e} were significantly larger than the changes in ES_{m+e} , during weeks 0-12
21 ($p=0.017$; $es=0.904$) ($SE_e > ES_e$; $p=0.039$) and 0-24 ($p=0.033$; $es=0.806$) (Fig 1b). Changes in

1 SE_{m+e} were larger than in C during first 12 weeks ($p=0.024$) and after 24 weeks of training
2 ($p=0.004$).

3 EMG and voluntary activation

4 In the morning both SE_{m+e} and ES_{m+e} increased VL rmsEMG by week 24 (SE_{m+e} : $p<0.001$,
5 $es=0.590$; ES_{m+e} : $p=0.037$, $es=0.461$) (Fig 2a; Table 1). In the evening only SE_{m+e} significantly
6 increased VL rmsEMG activity during weeks 0-12 ($p=0.002$; $es=0.584$) and 0-24 ($p=0.001$;
7 $es=0.602$), whereas the changes in ES_{m+e} were not significant ($p=0.486$; $es=0.258$). These
8 increases in the evening were significantly larger in SE_{m+e} compared to insignificant changes in
9 the ES_{m+e} group during the first 12 weeks ($p=0.004$; $es=0.512$) and after 24 weeks of training ($p=0.014$;
10 $es=0.473$) (Fig 2b).

11 VA% remained statistically unaltered in the SE_{m+e} and ES_{m+e} group after 24 weeks of training in
12 the morning (SE_{m+e} : $p=0.093$, $es=0.052$; ES_{m+e} : $p=0.801$, $es=0.084$) and in the evening (SE_{m+e} :
13 $p=0.444$, $es=0.394$; ES_{m+e} : $p=0.846$, $es=0.076$) (Table 1). In the evening, at week 24, the
14 $2.1\pm 4.5\%$ increase in VA% in the SE_{m+e} was significantly larger ($p=0.026$; $es=0.535$) than the -
15 $2.1\pm 3.5\%$ ($es=-0.035$) change in the control group (Fig 3).

16 In the SE_{m+e} and ES_{m+e} groups, a significant correlation between the individual changes in VA%
17 and changes in MVC_{KE} in the morning was found between weeks 0-12 (SE_{m+e} $r=0.625$, $p=0.013$
18 (Fig 4); ES_{m+e} $r=0.635$, $p=0.005$) and in the SE_{m+e} group during weeks 0-24 ($r=0.521$, $p=0.046$).
19 Individual changes in the morning in VA% and changes in VL rmsEMG were correlated in the
20 SE_{m+e} group during weeks 0-12 ($r=0.685$, $p=0.003$) and during weeks 0-24 ($r=0.479$, $p=0.050$).
21 In SE_{m+e} changes in MVC_{KE} and VL rmsEMG were correlated during weeks 0-12 and 0-24 both

1 in the morning (wks 0-12 $r=0.509$, $p=0.018$ (Fig 5); wks 0-24 $r=0.479$, $p=0.028$) and in the
2 evening (wks 0-12 $r=0.462$, $p=0.035$; wks 0-24 $r=0.481$, $p=0.027$).

3 Maximal power output during cycling

4 W_{peak} during the cycle ergometer test increased in SE_{m+e} and ES_{m+e} throughout the 24-week
5 training period in the morning (SE_{m+e} : $p<0.001$, $es=0.910$; ES_{m+e} : $p<0.001$, $es=1.560$) and in the
6 evening (SE_{m+e} : $p<0.001$, $es=0.997$; ES_{m+e} : $p<0.001$, $es=1.406$) (Table 1). In the morning the
7 increase of $22.0\pm 7.0\%$ in ES_{m+e} was significantly larger compared to $15.9\pm 9.2\%$ in SE_{m+e} during
8 weeks 0-24 ($p=0.022$; $es=0.746$) ($ES_e > SE_e$; $p=0.020$) (Fig 6). In the evening the increase of
9 $8.5\pm 5.7\%$ in ES_{m+e} was significantly larger compared to the $5.0\pm 3.8\%$ in SE_{m+e} during weeks 13-
10 24 ($p=0.027$; $es=0.723$).

11

12 **Discussion**

13 The main results of the present study suggest that the order of strength and endurance training
14 may influence the magnitude of adaptations in neuromuscular and endurance performance (order
15 effect), whereas time-of-day of the training does not seem to affect the results. Larger gains in
16 neuromuscular performance were observed in the evening, when strength training was performed
17 before endurance. Endurance performance development seemed to favor the order of endurance
18 training constantly preceding strength, both in the morning and in the evening.

19 Neuromuscular performance

20 In the present study no order effect in maximal isometric force development was observed during
21 the training period in the morning. However, in the evening maximal isometric knee extension

1 force increased significantly more in the SE order compared to the ES. Previous combined
2 training studies, which have not controlled the time-of-day-effect have shown that intra-session
3 exercise sequence does not seem to influence maximal strength performance development in
4 young previously untrained participants [9,44].Eklund et al. [19] have, however, shown that
5 neural adaptations might be compromised when endurance training constantly precedes strength
6 training. In elderly men, same-session combined training has been shown to lead to greater
7 improvements in strength performance when the combined training session always started with
8 strength training [7]. This possible interference by prior endurance training has been attributed
9 both to impeded molecular adaptations [3,12,26] and to acute fatigue developed in the
10 neuromuscular system [37]. Failure in force production has been associated with changes in
11 contractile as well as neural properties of working muscles [37]. Consequently, when the
12 neuromuscular system cannot produce an optimal contraction due to previous fatigue,
13 improvements in muscle strength may be possibly reduced [5]. This could be a possible
14 mechanism why isometric strength performance was compromised in the group which started
15 with endurance training.

16 Analogous to isometric force, the morning increases in rmsEMG were similar between the two
17 orders, whereas the evening changes in rmsEMG were significantly larger in the SE_{m+e} group
18 compared to the ones in ES_{m+e} . The present correlations revealed that in the SE_{m+e} group
19 individual changes in maximal isometric knee extension force development were positively
20 related to the changes in VL rmsEMG, demonstrating that the individuals who increased
21 rmsEMG experienced concomitant increases in maximal knee extension force. Although Eklund
22 et al. [19] did not observe any between-group differences, participants who constantly performed
23 strength before endurance training demonstrated increased force and EMG activity during

1 isometric actions by the end of the 24 weeks of same-session combined training, while the
2 reverse order produced no significant increases. Similarly to the present study, these results are
3 suggesting that performing endurance training before strength may potentially inhibit neural
4 adaptations and, thereby, hinder adaptations in neuromuscular performance such as maximal
5 isometric force. However, it is worth of pointing out that the EMG data was not normalized to
6 maximum M-wave. Although, this is a limitation of the present study, we took great care to
7 minimize the methodological and physiological errors during the EMG-recordings by
8 standardizing the measurement procedure and permanently marking EMG electrode positions
9 subcutaneously.

10 In addition to rmsEMG, neuromuscular activation in the present study was measured by using
11 the twitch interpolation technique to quantify the level of voluntary muscle activation. Although
12 no significant within-group changes were observed in VA% in the evening, the SE order led to
13 significantly larger changes in VA% compared to the control group. Previously, Eklund et al.
14 [19] observed enhanced voluntary activation after combined training only in the group which
15 performed strength before endurance training. Whereas no significant correlations were observed
16 in the evening, a significant correlation between the improvements in voluntary activation level
17 and knee extension force was found in the morning in both SE_{m+e} and ES_{m+e} groups. However,
18 the level of adaptations varied widely among individuals in both orders, as demonstrated by large
19 standard deviations. The significant correlations observed between individual changes in VA%
20 and changes of rmsEMG over the 24-week training period were observed only by adhering to the
21 SE order. Previously, Eklund et al. [19] have shown that combined training for longer than 12
22 weeks may potentially inhibit adaptations in the nervous system when endurance is regularly
23 performed before strength training. It has been suggested that already small increases in VA%

1 represent a physiologically significant improvement in muscle activation [27], therefore, it is
2 possible the statistically insignificant changes in the present study still affected strength
3 performance adaptations.

4 Not only neuromuscular adaptations but also muscle hypertrophy contributes to training-induced
5 increases in maximal contractile force. However, in concordance with previous studies [40], the
6 previous report by our research team [35] showed no significant differences between SE and ES
7 orders in hypertrophy development. Therefore, it is likely that in the present study
8 neuromuscular adaptations rather than morphological changes were responsible for the order-
9 specific gains in isometric strength performance. In addition, all experimental groups in the
10 present study followed training programmes which were carefully matched for modes,
11 frequencies, intensities and durations of strength and endurance training. Therefore, differences
12 in improvements in neuromuscular performance might be explained by the sequence of training.
13 However, when interpreting the results it needs to be remembered that the present training
14 program consisted of dynamic exercises and that dynamic tests may be more suitable than
15 isometric ones to evaluate the training adaptations [1]. This may help to partly explain the
16 differences between the present study and previous study by our research group [35] which did
17 not find order effect in dynamic strength performance.

18 The present results suggested that, unlike after strength training only [45,47], prolonged
19 combined strength and endurance training in the morning or in the evening do not lead to time-
20 of-day-specific adaptations in neuromuscular performance. The assumption that adaptations to
21 exercise training depend on the training time-of-day is based on the fact that various
22 physiological variables (e.g. body temperature, contractile state of the muscle, neural input) have
23 been shown to fluctuate relative to the time-of-day [11]. In the present study neuromuscular

1 performance did not show any morning to evening fluctuation at any time point. Although this
2 finding is in contrast to most of the previous time-of-day-specific studies [46], it is possible that
3 the lack of diurnal variation in neuromuscular performance in the present study may have in part
4 masked the time-of-day specific training adaptations in strength performance.

5 Although the time-of-day of the training did not influence the training adaptations, between-
6 group differences in neuromuscular performance were found only in the evening testing time.
7 Therefore, it is possible that in previous combined training studies testing along the day may
8 have masked the presence of the order effect. The design and results of the present study allows
9 us to suggest that in addition to the training mode, duration and frequency [22], also the time-of-
10 day when the measurements are performed may be an important factor influencing the order
11 effect.

12 Endurance performance

13 Peak wattage increased in all training groups over the 24-week combined training period. During
14 the first 12 weeks the increases were similar in both training orders, after which greater
15 improvements were observed in the ES group, compared to the opposite order. With respect to
16 peak wattage, previous studies that have investigated the effects of simultaneous strength and
17 aerobic training on endurance performance, mostly demonstrate that strength and endurance
18 training order does not interfere with the development of endurance performance [7,14,19]. The
19 endurance training intensity might be one factor to explain the differences between the studies.
20 In the previous study by our laboratory [44], which did not observe any order effect in endurance
21 performance, a similar endurance training program as in the present study was used, except a
22 smaller amount of high intensity interval training sessions were included. In addition, in the

1 present study the between-group differences were observed only after the training intensity and
2 frequency were increased during the second training period (wks 13-24). This is supported by
3 Nelson et al. [41] who also noted suppressed adaptation in endurance performance only after the
4 training period was prolonged over 11 weeks. Cycling has been shown to be biomechanically
5 similar to many strength exercises [23], therefore, fatigue from strength training in close
6 proximity with intensive cycling exercise may cause interference in optimizing physiological
7 adaptations to endurance training [18,41], especially when performed over a prolonged period of
8 time. Therefore, it is possible that the increased training intensity, the larger amount of interval
9 training sessions as well as the increased training frequency and total training volume during the
10 second training period may have led to suppressed endurance performance adaptations, when
11 constantly performing strength before endurance training. However, it needs to be remembered
12 that, although, W_{peak} is a commonly used measure which has been shown to accurately predict
13 cycling performance [20], it has, in addition to cardiorespiratory factors also a neuromuscular
14 component. However, the physiological mechanisms behind cardiorespiratory adaptations were
15 out of scope of the present report.

16 The present study suggest that time-of-day-specific combined strength and endurance training
17 will not lead to time-of-day-specific training adaptation in endurance performance when
18 measured as peak wattage produced during the maximal cycling test. Previous literature
19 regarding time-specific endurance training is limited and equivocal, as some of the studies have
20 shown that adaptations to endurance training are time-of-day-specific [31], while others disagree
21 [30]. Similarly to strength performance, in the present study endurance performance did not vary
22 with the time-of-day. This is in accordance with Deschenes et al. [17], who showed that although
23 some physiological variables such as blood pressure, plasma lactate and rectal temperature may

1 fluctuate with time-of-day, while other important variables such as oxygen uptake and
2 pulmonary ventilation fail to demonstrate significant diurnal fluctuation. It is possible that the
3 effects of time-of-day on endurance performance are not explained just by one or two variables
4 but represent the effect of a combination of factors and, therefore, the lack of diurnal variation in
5 endurance performance may have in part masked the time-of-day specific adaptations in the
6 present study.

7

8 Conclusions

9 The present same-session combined training protocol led to adaptations specific to the strength
10 and endurance training order. The magnitude of adaptations in physical performance was similar
11 after morning and evening combined training, however, the time-of-day of neuromuscular testing
12 influenced the present results. In the evening, improvements in maximal strength performance
13 seemed to be accompanied by increased neuromuscular activity in the group that performed
14 strength training constantly before endurance training, while the reversed order may not be
15 optimal conditions for neuromuscular performance adaptations. On the other hand, performing
16 endurance training (by cycling) regularly before strength training may help to avoid possible
17 fatigue caused by strength training and, thereby, lead to greater endurance performance
18 adaptations both in the morning and in the evening, especially when the training period is
19 prolonged and the training intensity and/or frequency increased. Therefore, individuals who wish
20 to perform strength and endurance in close proximity to each other over prolonged training
21 periods are advised to choose the training order based on individual goals.

22

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

2

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6

1 **Figure legend**

2 **Figure 1.** Relative changes in maximal unilateral knee extension force after 12 and 24 weeks of
3 combined training; *sign. within-group increase or as indicated, $* < 0.05$, $** < 0.01$; # sign.
4 different from the control-group, $(\#) < 0.06$, $\# < 0.05$, $### < 0.001$. ES_{m+e} = combined morning and
5 evening endurance before strength training group; SE_{m+e} = combined morning and evening
6 strength before endurance training group

7 **Figure 2.** Relative changes in maximal VL rms EMG during unilateral knee extension after 12
8 and 24 weeks of combined training; *sign. within-group increase or as indicated, $* < 0.05$,
9 $** < 0.01$, $*** < 0.001$; # sign. different from the control-group, $\# < 0.05$. ES_{m+e} = combined
10 morning and evening endurance before strength training group; SE_{m+e} = combined morning and
11 evening strength before endurance training group

12 **Figure 3.** Relative changes in maximal voluntary activation during unilateral knee extension
13 after 12 and 24 weeks of combined training; # sign. different from the control-group, $\# < 0.05$.
14 ES_{m+e} = combined morning and evening endurance before strength training group; SE_{m+e} =
15 combined morning and evening strength before endurance training group

16 **Figure 4.** Correlations between the individual change in the voluntary activation % and the
17 relative changes in maximal knee extension force in the morning in SE_{m+e} group during weeks 0-
18 12. SE_m = morning strength before endurance training group. SE_e = evening strength before
19 endurance training group

20 **Figure 5.** Correlations between the relative change in the maximal VL rmsEMG during maximal
21 knee extension and the relative changes in maximal knee extension force in the morning in SE_{m+e}
22 group during weeks 0-12. SE_m = morning strength before endurance training group. SE_e =
23 evening strength before endurance training group

24 **Figure 6.** Relative changes in maximal power output during cycling after 12 and 24 weeks of
25 combined training; *sign. within-group increase or as indicated, $* < 0.05$, $*** < 0.001$; # sign.
26 different from the control-group, $(\#) < 0.06$, $\# < 0.05$, $## < 0.01$, $### < 0.001$. ES_{m+e} = combined
27 morning and evening endurance before strength training group; SE_{m+e} = combined morning and
28 evening strength before endurance training group

29

30 **Table legend**

31 **Table 1.** Absolute values \pm SD of isometric knee extension force (MVCKE), rmsEMG of vastus
32 lateralis, voluntary activation % (VA%) and peak wattage (W_{peak}) at pre-, mid- and post-
33 measurements in the morning and in the evening.

a) Morning measurements

b) Evening measurements

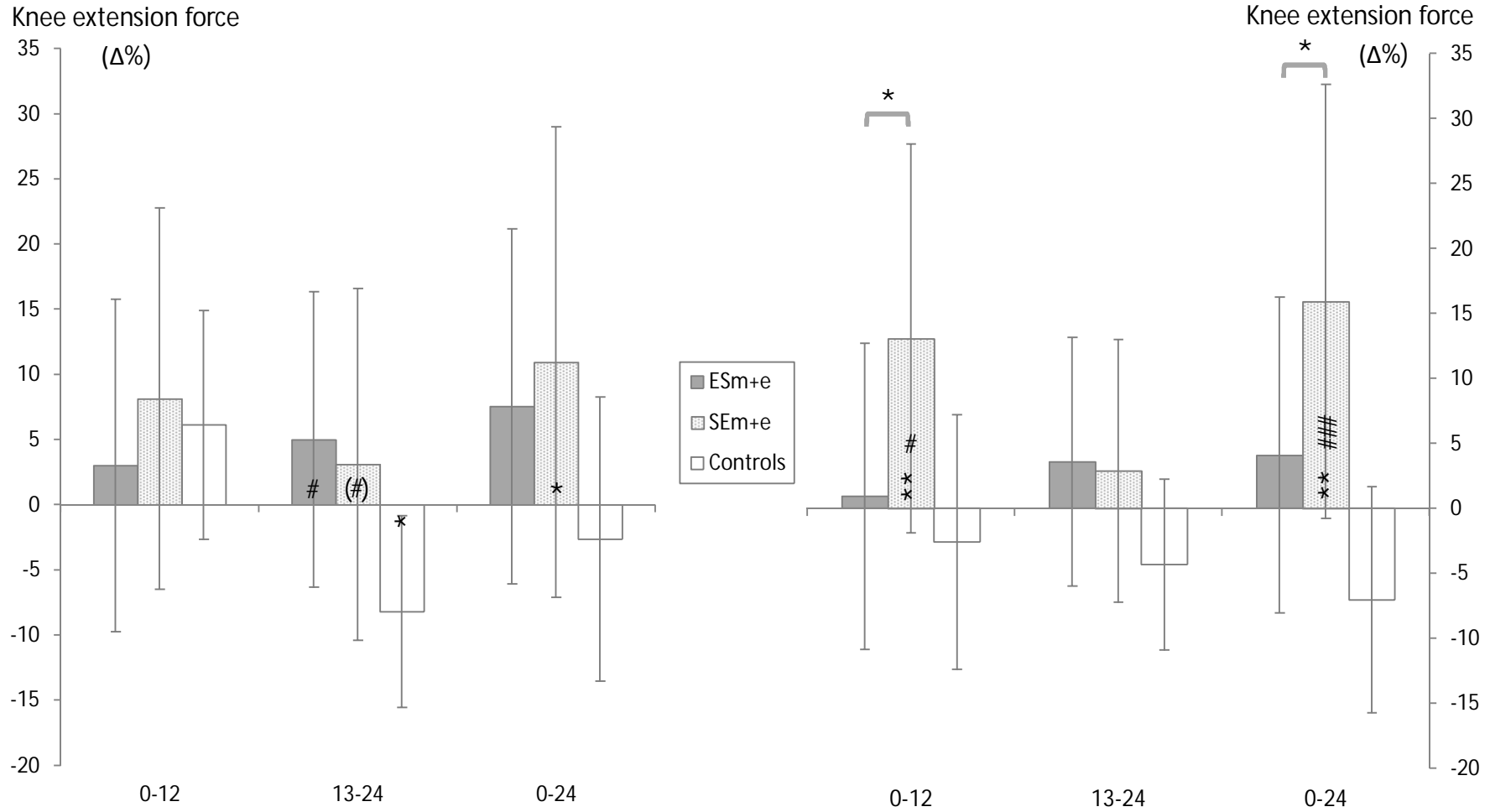
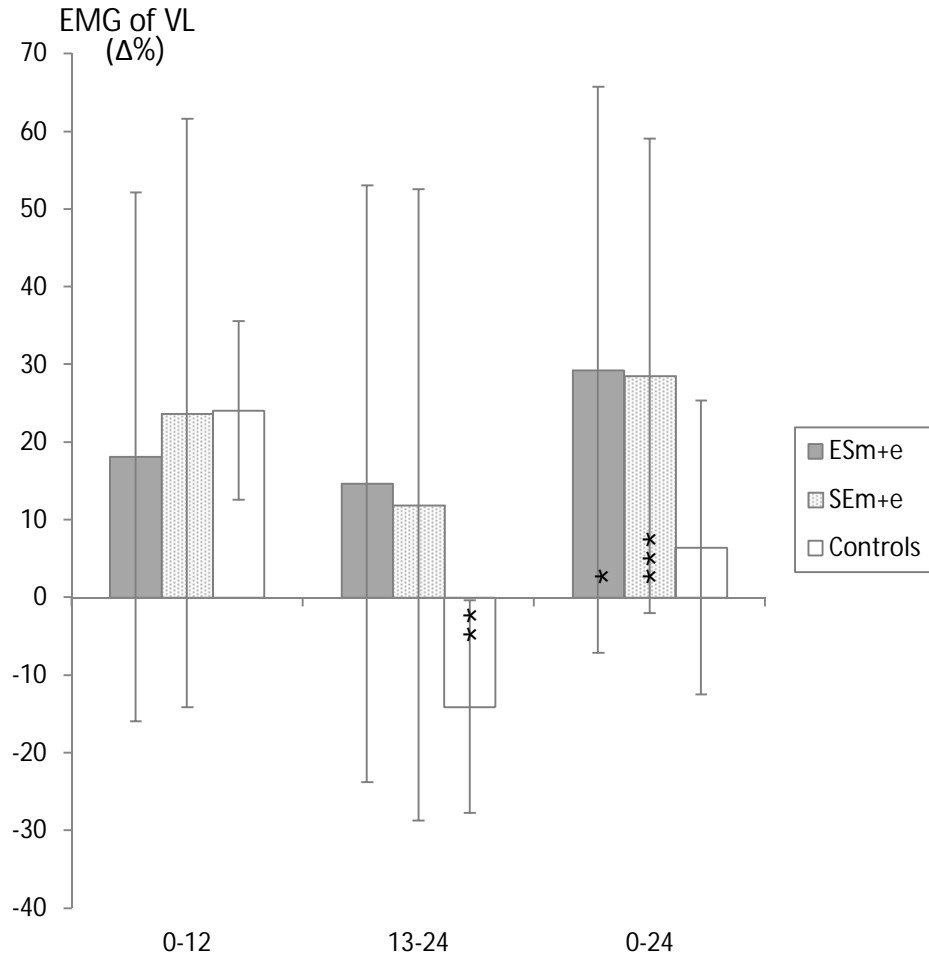


Fig. 1

a) Morning measurements



b) Evening measurements

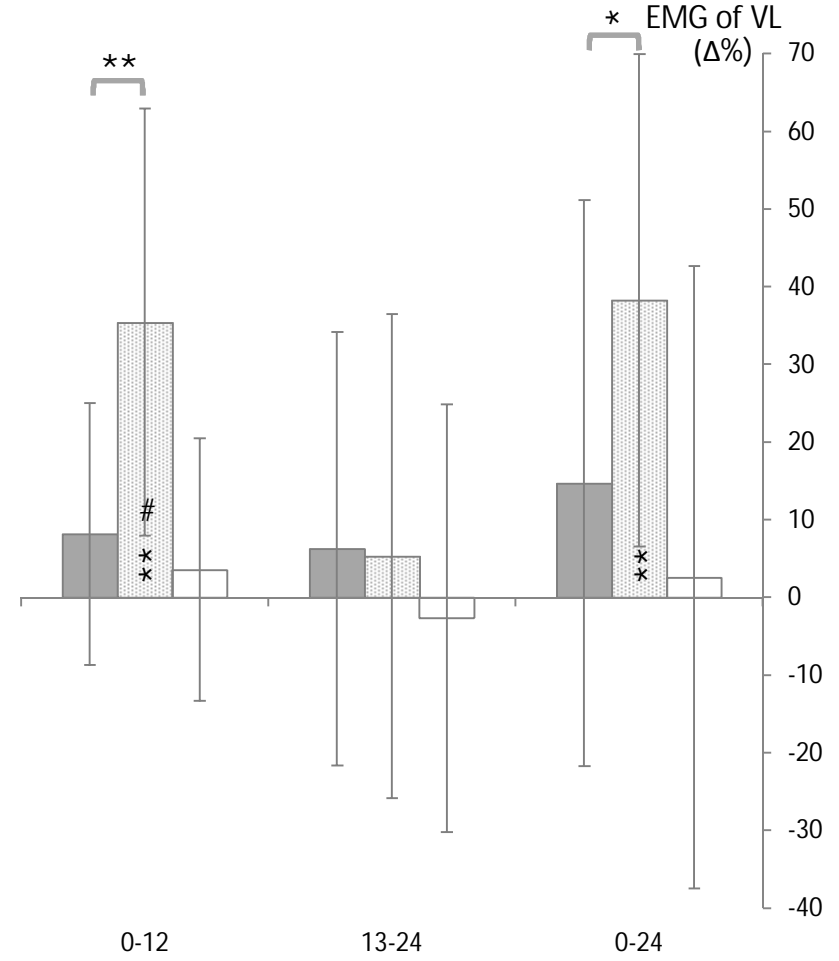
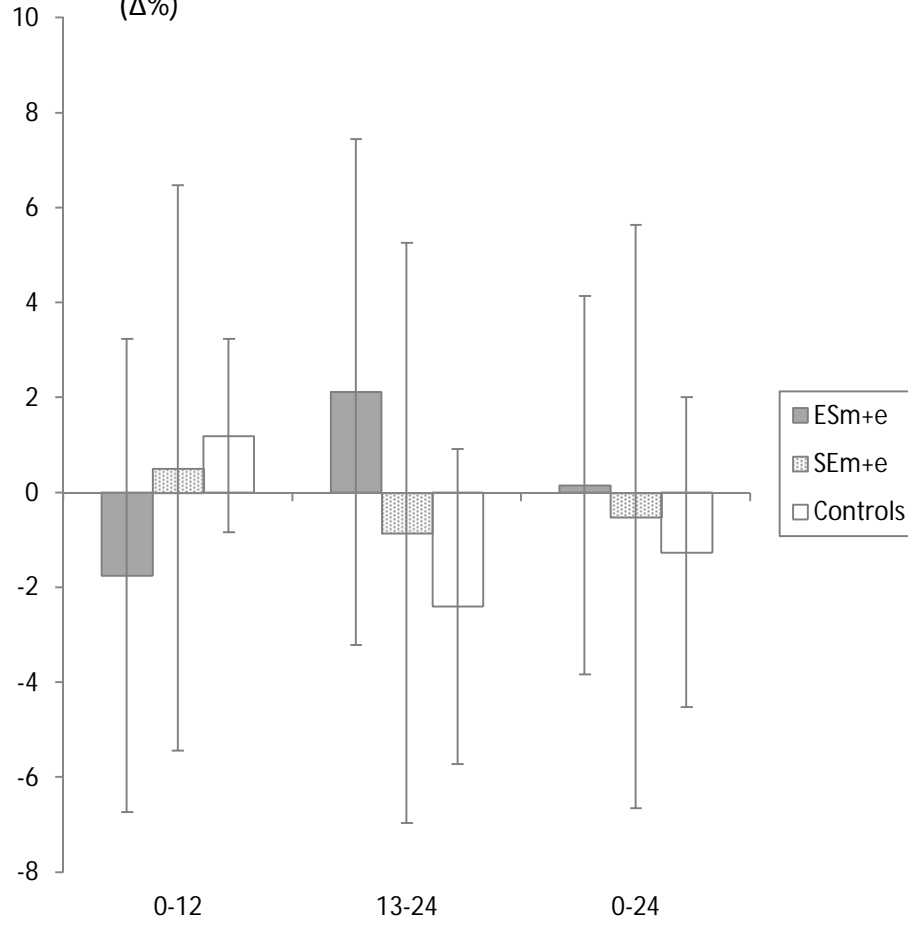


Fig. 2

a) Morning measurements

Voluntary activation %
($\Delta\%$)



b) Evening measurements

Voluntary activation
($\Delta\%$)

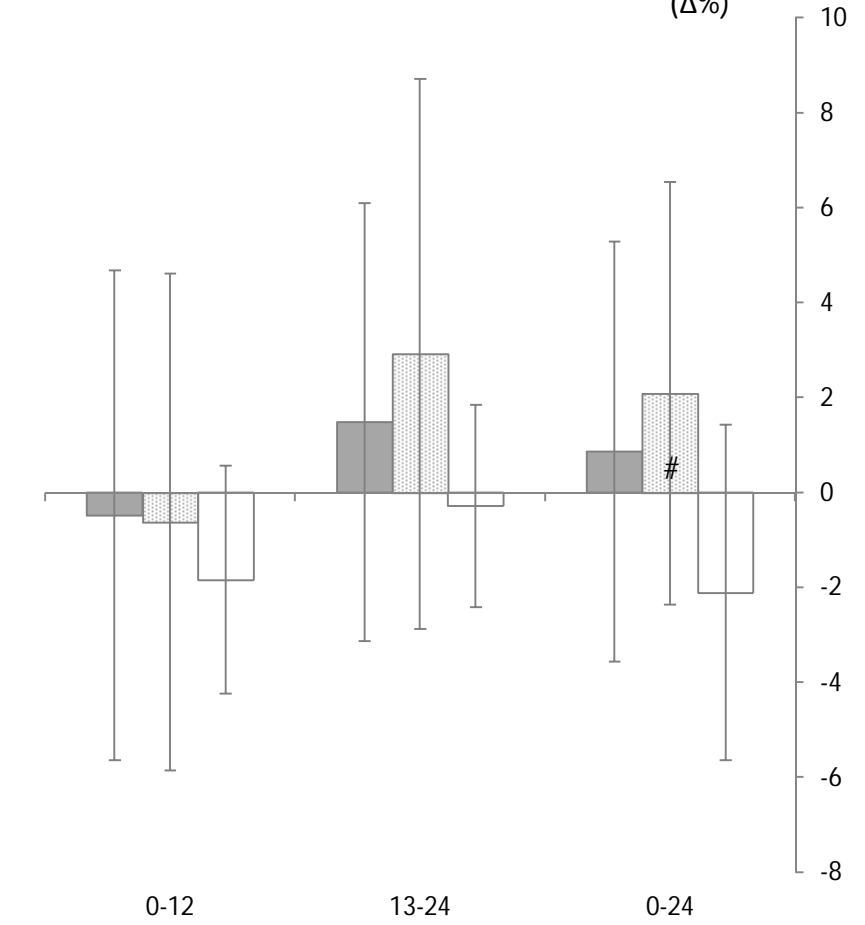


Fig. 3

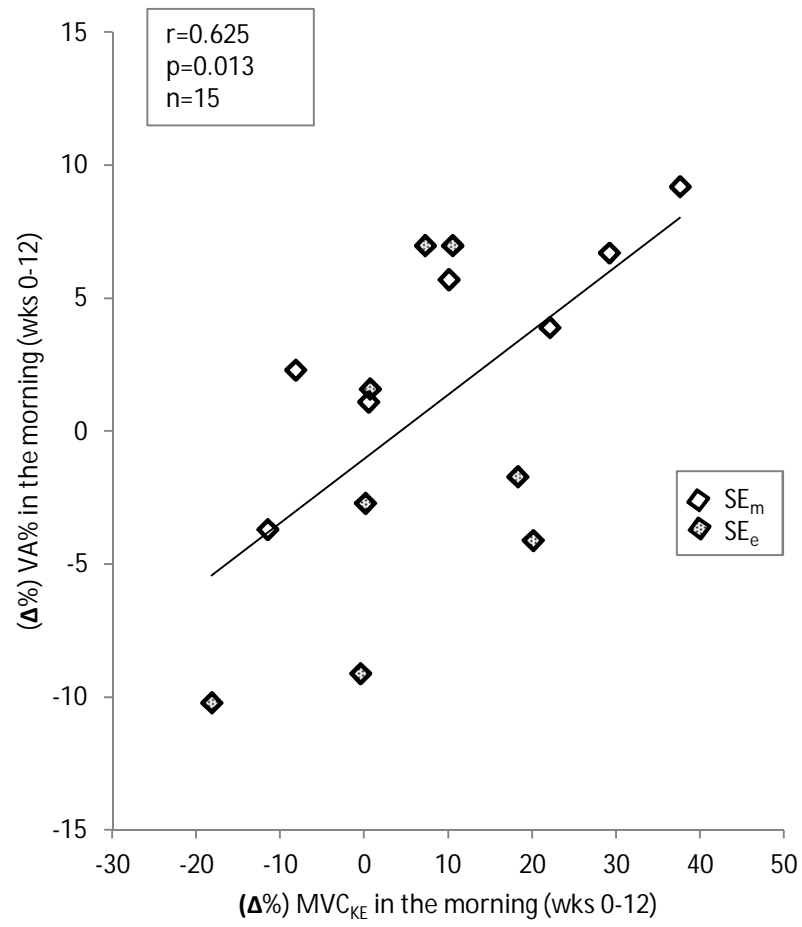


Fig. 4

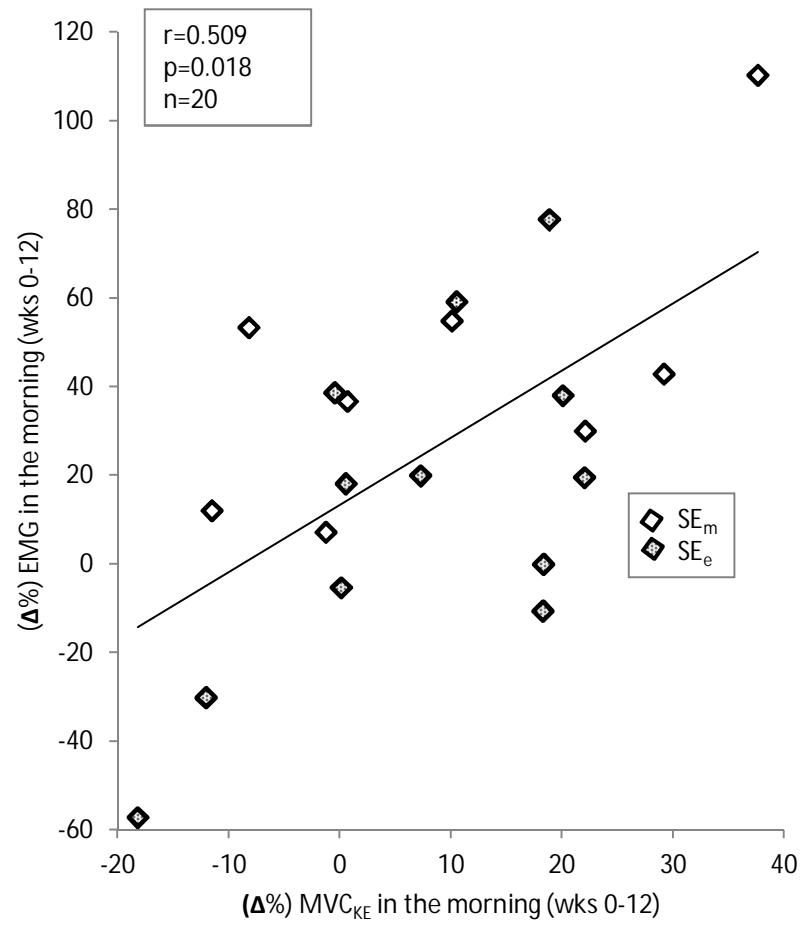
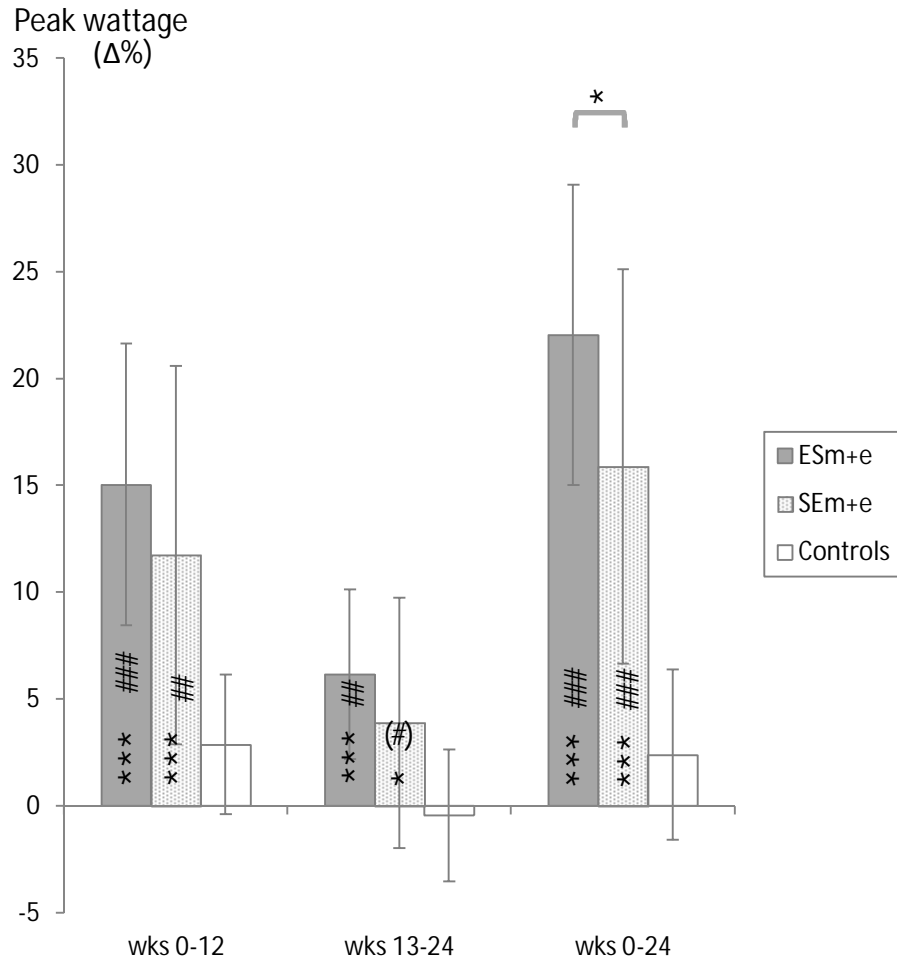


Fig. 5

a) Morning measurements



b) Evening measurements

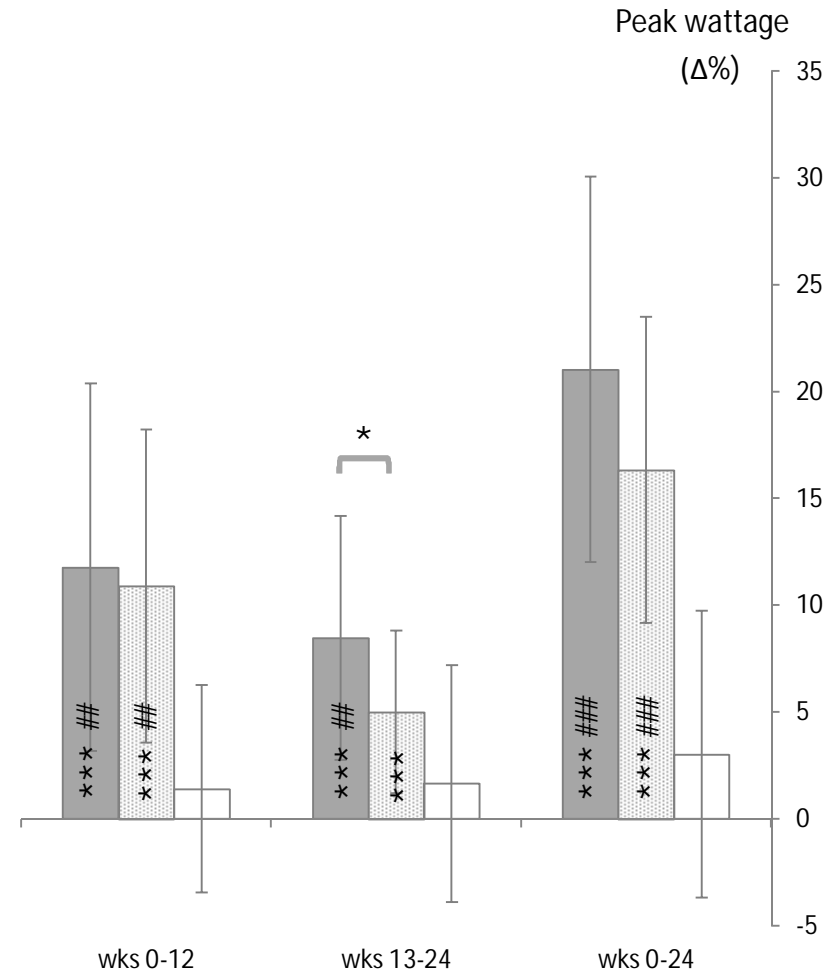


Fig. 6

Table 1. Absolute values \pm SD of isometric knee extension force (MVC_{KE}), rmsEMG of vastus lateralis, voluntary activation % (VA%) and peak wattage (W_{peak}) at pre-, mid- and post-measurements in the morning and in the evening.

	Pre		Mid		Post	
	Morning	Evening	Morning	Evening	Morning	Evening
MVC_{KE} (N) \pm SD						
SE _{m+e}	574 \pm 119	567 \pm 128	615 \pm 113	632 \pm 120*,§	628.9 \pm 129*	646 \pm 121*,§
ES _{m+e}	625 \pm 95	646 \pm 96	643 \pm 110	652 \pm 116	668.6 \pm 107	670 \pm 105
Controls	584 \pm 90	615 \pm 86	619 \pm 82*	599 \pm 95	566.45 \pm 7	571 \pm 86
rmsEMG \pm SD						
SE _{m+e}	0.26 \pm 0.08	0.24 \pm 0.05	0.32 \pm 0.16	0.33 \pm 0.11*,§	0.34 \pm 0.14*	0.34 \pm 0.14*,§
ES _{m+e}	0.24 \pm 0.08	0.28 \pm 0.08	0.28 \pm 0.09	0.29 \pm 0.07	0.30 \pm 0.08*	0.32 \pm 0.13
Controls	0.36 \pm 0.18	0.40 \pm 0.21	0.44 \pm 0.23	0.39 \pm 0.15	0.36 \pm 0.12#	0.36 \pm 0.08
VA% \pm SD						
SE _{m+e}	91.8 \pm 4.1	91.0 \pm 3.4	92.2 \pm 4.6	90.4 \pm 4.9	91.2 \pm 4.5	92.8 \pm 3.1
ES _{m+e}	92.4 \pm 4.2	91.3 \pm 5.4	90.7 \pm 5.4	90.7 \pm 5.5	92.5 \pm 4.0	92.0 \pm 5.3
Controls	92.2 \pm 5.2	95.2 \pm 2.3	95.0 \pm 2.3	93.5 \pm 2.9	92.7 \pm 3.2	93.2 \pm 3.8
W_{peak} (W) \pm SD						
SE _{m+e}	249 \pm 43	251 \pm 43	275 \pm 33*	276 \pm 34*	286 \pm 38#,*	290 \pm 34#,*
ES _{m+e}	245 \pm 32	249 \pm 37	281 \pm 36*	277 \pm 36*,§	298 \pm 36#,*,§	300 \pm 35#,*
Controls	267 \pm 35	270 \pm 40	274 \pm 32*	273 \pm 34*	272 \pm 34#,*	277 \pm 31#,*

SE_{m+e} = morning and evening training groups who performed strength before endurance training; ES_{m+e} = morning and evening groups who performed endurance before strength; * significant change from Pre; # significant change from Mid; § significant difference between changes in SE_{m+e} and ES_{m+e} at time point. Detailed levels of significance are presented in the results section.