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Title: Fitness, body composition and blood lipids following three concurrent strength and endurance training modes

Running head: Health and combined training

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ABSTRACT

This study investigated changes in physical fitness, body composition and blood lipid profile following 24 weeks of three volume-equated concurrent strength (S) and endurance (E) training protocols. Physically active, healthy male and female participants (18-40 yrs) performed S and E sessions on different days (DD, men n=21, women n=18) or in the same session with E preceding S (ES, men n=16, women n=15) or vice versa (SE men n=18, women n=14). The training volume was matched in all groups. Maximal leg press strength (1RM) and endurance performance (VO_{2max} during cycling), body composition (DXA) and blood lipids were measured. 1RM and VO_{2max} increased in all groups in men (12-17% $p<0.001$ and 7-18% $p<0.05$-0.001, respectively) and women (13-21% $p<0.01$-0.001 and 10-25% $p<0.01$-0.001, respectively). VO_{2max} increased more in DD vs. ES and SE both in men ($p=0.003$-0.008) and women ($p=0.008$-0.009). Total body lean mass increased in all groups (3-5%, $p<0.01$-0.001). Only DD led to decreased total body fat (men -14±15% $p<0.001$, women -13±14% $p=0.009$) and abdominal-region fat (men -18±14% $p=0.003$, women -17±15% $p=0.003$). Changes in blood lipids were correlated with changes in abdominal-region fat in the entire group ($r=0.283$, $p=0.005$) and in DD ($r=0.550$ $p=0.001$). In conclusion, all modes resulted in increased physical fitness and lean mass, while only DD led to decreases in fat mass. Same-session SE and ES combined training is effective in improving physical fitness, while volume-equated, but more frequent DD-training may be more suitable for optimizing body composition and, possibly useful in early prevention of cardiovascular and metabolic diseases.

Keywords: combined training; physical performance; health; resistance training; aerobic training; metabolic health

INTRODUCTION

The importance of consistent adherence to physical activity for improved physical fitness and health has been well established in various populations (Ghahramanloo et al. 2009, Häkkinen et al. 2003, Sillanpää et al. 2009). Regular physical exercise is associated with reduced levels of cardiovascular risk factors (Naghii et al. 2011), favorable changes in body composition (Ho et al. 2012, Sigal et al. 2007) as well as blood lipid profile (Tambalis et al. 2009). In terms of the choice of exercise type, endurance (E) and strength (S) training result in specific improvements in physical fitness as well as positive changes in health-related outcomes. While E-training has been shown to increase cardiorespiratory fitness (Murias et al. 2010) and consequently reducing premature all-cause and cardiovascular disease mortality (Farrell et al. 1998, Kodama et al. 2009), S-training leads to increased muscle size and strength (Häkkinen et al. 2001), thus sustaining functional capacity (Landi et al. 2014).

While it has been proposed that either E or S training alone can improve cardiovascular health and physical fitness (Spence et al. 2013), including both exercise modes into the same training regimen (combined training) may be even more effective than adhering to only either training mode alone (Sillanpää et al. 2008). Greater benefits in terms of cardiovascular health (Ghahramanloo et al. 2009, Ho et al. 2012, Sigal et al. 2007), reduced total body and abdominal fat as well as improvements in overall physical fitness profile have been observed with combined training in comparison to either S or E training alone in both obese (Ho et al. 2012, Dutheil et al. 2013) and elderly (Lee et al. 2014, Sillanpää et al. 2008) populations.

However, combined training regimens with an overall high volume and frequency can compromise strength gains especially in previously untrained individuals (Hickson 1980), emphasizing the importance of utilizing a moderate volume and frequency of training program to prevent adverse effects (Häkkinen et al. 2003). Moderate volume and frequency combined training seems to be effective in increasing muscle strength and size as well as maximal aerobic capacity in previously untrained adults, regardless of whether S and E are performed on different days (DD) or in the same training session with different orders (i.e. E immediately followed by S, ES, or vice versa, SE) (Eklund et al. 2015). However, data on health-related outcomes following these combined exercise training modes is still lacking.

Although a previous study by our group showed that 24 weeks of ES and SE-training produced similar adaptations in physical fitness and increases in lean mass in previously untrained men, no decreases in body fat mass were observed in either training group (Schumann et al. 2014). However, as split exercise sessions may result in increased post-exercise oxygen consumption in comparison to a long one (Almuzaini et al 1998) and could consequently contribute to increased overall energy expenditure, it is left unclear how splitting S and E onto different days affects body composition in the long term. As reductions in adipose tissue have been associated with an improved blood lipid profile (Dutheil et al. 2013), it is of interest to investigate the effects of different modes of volume-equated combined training programs on changes in body composition and blood lipid content.

The aim of the present study was to investigate possible differences in body fat and lean mass, blood lipid levels and physical fitness profile following 24 weeks of volume-equated different-day and same-session combined strength and endurance training in previously untrained healthy men and women. More specifically, this was achieved through comparing adaptations following strength and endurance training performed on different days (DD) or in the same session with two different orders (ES and SE). The present study expands on our previous work (Eklund et al. 2015, Eklund et al. 2016, Schumann et al. 2014) in order to provide a more comprehensive understanding of how adaptations to same-day combined strength and endurance training compares to strength and endurance training performed on separate days.

MATERIALS AND METHODS

Study design

Subjects. Following institutional ethical approval and in accordance with the Declaration of Helsinki, written informed consent was obtained from recreationally active male (n=70) and female (n=70) volunteers. The recruited subjects were required not to have participated in systematic strength or endurance training for at least 1 year prior to the study. All subjects were free from chronic illnesses and injuries, below a BMI of 30 $m^2 \cdot kg^{-1}$ and non-smokers. Female subjects were not pregnant or lactating. As a part of the pre-screening process, all subjects completed a health questionnaire and underwent a resting ECG, which were approved by a cardiologist. Out of the 140 recruited subjects, 15 men and 24 women did not complete the study or were not included in the analysis due to a training adherence below 90%. Subject demographics of the included subjects are presented in Table 1.

Experimental approach. To examine the effects of combined strength and endurance training performed on different days (DD) or in the same session (with two different orders: ES and SE) on physical fitness profile, body composition and blood lipids, the subjects were assigned to one of three training groups for the 24-week training intervention. The subjects were measured before (Week 0), at the mid-phase (Week 12) and after (Week 24) the training intervention. The measured variables included maximal strength and endurance performance, body composition and blood lipids as well as collection of food diaries.

All subjects were initially familiarized with the training and measurement protocols and equipment. The strength and endurance measurements as well as measurement of body composition and blood lipids were separated from each other by a minimum of 2 days. The measurements were conducted for each subject at the same time of day (±1h) to minimize circadian fluctuation. Subjects were instructed to abstain from caffeine 12 h and alcohol 24 h prior to all measurements. For training and measurements of physical fitness the subjects arrived to the laboratory in a rested and hydrated state and at least 2 h postprandial. 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 body composition, blood lipids, maximal strength and endurance performance, each participant was randomly assigned to one of the three training modes for the entire 24-week duration of the study: 1) strength and endurance training performed on different days (DD, men n=21, women n=18), 2) strength and endurance performed in the same training session with endurance preceding strength (E+S, men n=16, women n=15) or 3) vice versa, i.e. strength and endurance performed in the same training session with strength preceding endurance (S+E, men n=18, women n=14).

Measurements of physical fitness

Maximal concentric strength. Bilateral leg press one-repetition maximum (1 RM) was measured using a David 210 weight stack horizontal leg press device (David Health Solutions Ltd., Helsinki, Finland). The participants were seated in the device with a starting knee angle of 60° (58° ± 2°). As a preparation for the 1 RM trials, participants 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 rest between sets. When verbally instructed, participants performed a dynamic action to a full leg extension (knee angle 180°). The load was increased upon a successful completion. After a maximum of 5 maximal trials, the trial with the highest successfully completed load was accepted as the 1 RM.

Isometric force. Maximal bilateral isometric leg press force (MVC) was measured at a knee angle of 107° (Häkkinen et al. 1998) on a horizontal leg press device designed and manufactured by the Department of Biology of Physical Activity (University of Jyväskylä,

Jyväskylä, Finland). Subjects were instructed to perform a bilateral leg press action to reach the maximum force as rapidly as possible and maintaining it for 2-3 s. A minimum of 3 and a maximum of 5 maximal trials were allowed. A fourth and fifth trial was allowed, if the difference from the third trial to the previous 2 exceeded 5 %. Force signals were recorded with Signal 2.16 software (Cambridge Electronic Design, Cambridge, UK) sampled at 2 000 Hz, processed with a low-pass filter (20 Hz) and analyzed using a customized, automated script (Signal 2.16 software, Cambridge Electronic Design, Cambridge, UK). The trial with the highest maximal force was used for further analysis.

Maximal oxygen uptake. 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 participant was 50 W, with 25 W increments applied every 2 min until volitional exhaustion. Participants were asked to keep the pedaling frequency at 70 revolutions per minute (rpm) throughout the test. The current rpm was visible for the participants throughout the test. When the participants failed to keep up the required rpm for longer than 15 s, the test was terminated. Oxygen uptake was determined continuously, breath-by-breath, with a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Maximal oxygen consumption (VO_{2max}) was averaged over each 60 s period during the test. The VO_2-value from the last complete minute during the test was defined as VO_{2max}.

Blood lipids. Blood samples were drawn from the antecubital vein at 7:00-9:00 following a 12h overnight fast to obtain concentrations of total cholesterol ($Chol_{tot}$), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides. Participants were instructed to abstain from strenuous physical activity 48 before the blood samples were taken. Blood samples were drawn by a trained technician from the antecubital vein into serum tubes

(Venosafe, Terumo Medical Co., Leuven, Hanau, Belgium) adhering to standard laboratory procedures. Serum samples were stored for 10 min before being centrifuged at 3 500 rpm (Megafure 1.0 R, Heraeus, Germany) followed by immediate spectrophotometry analyzes (Konelab 20XTi, Thermo Fisher Scientific, Vantaa, Finland). The Friedewald equation (Friedewald et al. 1972) was used for estimating concentrations of LDL:

LDL = total cholesterol - HDL-C - (triglycerides/2.2)

Body composition. Body composition was assessed by Dual-Energy X-ray Absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems, Madison, USA). The DXA-scans were always performed in the morning with the participant in a fasted (12h) state. Leg position was fixed with Velcro-straps at the knees and ankles. Arms were aligned along the trunk with the palms facing the thighs. Automated soft tissue analyses were conducted for lean and fat mass (Encore-software, version 14.10.022). To analyze lower body fat ($\mathrm{Fat_{lower}}$) and lean mass ($\mathrm{Lean_{lower}}$), a region of interest (ROI) was created where the legs were separated from the trunk by a horizontal line directly above the iliac crest. Total body and arm fat ($\mathrm{Fat_{tot}}$, $\mathrm{Fat_{arms}}$) and lean mass ($\mathrm{Lean_{tot}}$, $\mathrm{Lean_{arms}}$) as well as android fat mass ($\mathrm{Fat_{andr}}$, centrally located fat mass) (Hind et al. 2011) were obtained for each of the regions through the manufacturer's pre-defined ROI's.

Food diaries.

Nutritional intake was controlled through food diaries, which were filled in by the participants for three consecutive days at weeks 0, 12 and 24. Energy intake was analyzed based on the food diaries with a nutrient analysis software (Nutriflow, Flow-team Oy, Finland). The participants received written and verbal nutritional recommendations according to the national guidelines and were asked to maintain constant dietary intake throughout the intervention.

Training

The training program has been described in detail previously (Eklund et al. 2015). In short, the training was designed to reflect recommendations for physically active individuals as well as targeted at improving both maximal strength and endurance performance. During the initial 12 weeks, the same-session subjects completed two weekly sessions of [1E+1S] or [1S+1E] (respective to the assigned training order), and five sessions per two weeks (5x [1E+1S] or [1S+1E]) during weeks 13-24. The time between training modes was 5-10 min and recovery time between training sessions 48-72 h. The DD-group adhered to the same training program but performed S and E on alternating days, i.e. completing 4 weekly training sessions during the first 12 weeks and 10 sessions per two weeks during the latter 12 weeks. Training sessions were supervised by research staff.

Strength training mainly targeted the knee extensors and flexors as well as hip extensors, with the exercises consisting of horizontal leg press, seated hamstring curls and seated knee extensions. During the initial weeks, the exercises were performed in a circuit (2-4 sets of 15-20 repetitions with up to 60% of 1RM) and then continued through hypertrophy-inducing training (2-5 x 8-12 at 80-85% of 1RM, 1-2 min rest) towards maximal strength training (2-5 x 3-5 at 85-95% of 1RM, 3-4 min rest). A similar periodization scheme was used for the upper body. Dumbbells and cable pulley machines were used for upper body exercises, and both machines and body weight were utilized for exercises of the trunk. The periodization was repeated during weeks 13-24 with increased training intensity and volume. The duration of each strength session was 50-60 min.

Endurance training sessions were performed on a cycle ergometer. The training intensities were controlled through heart rate zones, which corresponded to the threshold values of aerobic and anaerobic thresholds. The training consisted of 30-50 min continuous cycling near the aerobic threshold (weeks 1-7 and 13-16), including interval training at and above the anaerobic threshold (weeks 8 and 17 onwards). The interval sessions were initiated and finished with 10-15 minute bouts below the aerobic threshold, with 5-minute altering bouts on the anaerobic threshold and below the aerobic threshold in between.

Statistical analysis. Data is presented as means ± standard deviations. All statistical analyses were carried out with IBM SPSS Statistics v.22 software (IBM Corporation, Armonk, New York, USA). Normality was checked using the Shapiro-Wilk test as well as through observing the Q-Q-plots. Normally distributed data was analyzed for within-group (time) changes with a repeated measures analysis of variance (ANOVA) using absolute values. Differences between the training modes (time×training) were analyzed using a repeated measures ANCOVA with absolute values for main effects and a One-Way ANOVA with absolute changes for pairwise comparisons. The covariates used were the baseline values for the variable in question. Bonferroni post-hoc adjustments were used where appropriate. Non-normally distributed data was log-transformed to achieve normality and thereafter analyzed as described above. The reported effect sizes are Cohen’s *d* with an effect size of ≥0.20 being considered small, ≥0.50 medium, and ≥0.80 large. The reported correlations are bivariate Pearson correlation coefficients (r). The level for significance was p≤0.05. A trend was accepted at p≤0.06.

RESULTS

Training adherence

The training adherence in men was 99±2%, 99±2% and 100±1% in ES, SE and DD, respectively, and in women 98±4%, 99±2% and 99±2% in ES, SE and DD, respectively.

Measurement reproducibility

The measurement reproducibility was high (intra-class correlation 0.7-0.9) for all test measures, as has been reported earlier by our research group (Schumann et al. 2014).

Body composition

Lean mass. Total body lean mass increased significantly in all three training groups in both men (effect sizes DD 0.39, ES 0.32, SE 0.35) and women (effect sizes DD 0.55, ES 0.30, SE 0.38) (Figure 1). The regional changes in lean mass are presented in Table 2. The change in lower body lean mass was significant ($p<0.05$) in all groups except DD men. Trunk lean mass increased significantly in all groups ($p<0.05$) except in SE-women and ES-men. The change in lean mass of the arms was significant ($p<0.05$) in all groups except ES-women and SE-men. Time×group interactions were not observed in lean mass either in the separated regions or in the total body.

Fat mass. Fat mass decreased in all regions in the DD-groups, while significant changes in ES and SE were not found during the training intervention (Figure 2 and Table 2). In women, significant time×group interactions were observed in $\mathrm{Fat}_{\mathrm{tot}}$ ($p=0.035$), $\mathrm{Fat}_{\mathrm{lower}}$ ($p=0.048$) and $\mathrm{Fat}_{\mathrm{andr}}$ ($p<0.001$). The decrease in $\mathrm{Fat}_{\mathrm{tot}}$ in women was significantly greater in DD than in ES and SE during weeks 0-24 ($p=0.005$ and $p=0.028$, respectively; effect size DD 0.48, ES 0.03, SE 0.09) and weeks 13-24 ($p=0.016$ and $p=0.047$, respectively; effect size DD 0.23, ES 0.01, SE 0.04). In $\mathrm{fat}_{\mathrm{lower}}$ the decrease in women in DD was significantly greater than in ES during weeks 13-24 (p=0.039; effect size DD 0.25, ES 0.03, SE 0.06) and approaching significance during weeks 0-24 (p=0.052; effect size DD 0.43, ES 0.07, SE 0.11). The magnitude of decrease in women in Fat_{andr} was greater in DD in comparison to ES and SE during weeks 0-12 (p=0.001 and p=0.028, respectively; effect size DD 0.34, ES 0.04, SE 0.07), weeks 0-24 (p<0.001 and p=0.002, respectively; effect size DD 0.51, ES 0.06, SE 0.06) and weeks 13-24 (p=0.012 and p=0.025, respectively; effect size DD 0.17, ES 0.01, SE 0.0). In men, a significant time×group interaction was noted in Fat_{andr} (p=0.038) with the decreases in DD being of greater magnitudes than SE at weeks 0-12 (p=0.038; effect size DD 0.18, ES 0.13, SE 0.03), weeks 0-24 (p=0.003; effect size DD 0.45, ES 0.27, SE 0.03) and weeks 13-24 (p=0.010; effect size DD 0.27, ES 0.14, SE 0.06).

Nutrition. Total energy intake (MJ) at week 0, 12 and 24 in men were as follows 9.3±1.8, 10.2±2.6 and 9.5±2.6 for ES; 9.4±2.0, 9.3±1.7 and 7.9±1.7 for SE; 8.4±2.3, 9.0±1.4 and 9.2±1.6 in DD, respectively. Total energy intake was in women 8±1.2, 7.8±1.8 and 8.2±2.1 for ES; 7.6±1.2, 7.7±1.6 and 7.1±2.1 for SE; 7.0±1.9, 6.9±1.6 and 7.0±1.8 for DD, respectively. The food energy intake did not significantly change in any of the groups.

Blood lipids. Total cholesterol changed significantly only in the male ES group (weeks 0-12 p=0.019 and 0-24 p=0.012) (Table 3). The change in total cholesterol was significantly different from the same sex DD (p=0.028) and SE (0.048) groups at weeks 0-12. $Chol_{HDL}$ changed significantly only in DD women (weeks 0-12 p=0.001 and weeks 13-24 p<0.001). Between-group interactions in $Chol_{HDL}$ were observed in men between DD and SE (weeks 0-12 p=0-005 and weeks 13-24 p=0.047). Favorable changes in $Chol_{LDL}$ were found in the male ES group (weeks 0-12 p=0.037) and triglycerides (weeks 13-24 p=0.017). The changes in $Chol_{TOT}$ and Fat_{andr} had a low correlation during weeks 0-12 (r=0.280, p=0.006) and weeks 0-24 (r=0.283, p=0.005) among all participants as well as a moderate correlation in the DD-group including both sexes (weeks 0-12 r=0.601 p<0.001 and weeks 0-24 r=0.550 p=0.001).

Strength and endurance performance. Changes in 1RM and VO_{2max} are presented in Figure 3 and have also partly been published elsewhere (Eklund et al. 2015, Eklund et al. 2016, Schumann et al. 2014, Schumann et al. 2015). 1 RM significantly increased in all groups in men (all groups p<0.001) and women (DD, SE p<0.001, ES p=0.002). In women, the increase in 1RM during weeks 0-12 was larger in DD than in ES (p=0.013). Maximal isometric leg extension force (MVC) increased in all groups by week 24 (Women: DD 21±13% from 1341±265 N p<0.001, ES 22±18% from 1610±302 N p<0.001, SE 12±13% from 1700±668 N p=0.016; Men DD 11±12% from 2332±590 N p<0.001, ES 9±13% from 2653±683 N p=0.032, SE 13±18% from 2338±540 N p=0.024). No significant time×group interactions were found in 1RM or MVC. Increases in VO_{2max} were significant in all groups (DD p<0.001, ES p=0.037, SE p=0.013) and women (DD p<0.001, ES p=0.009, SE p=0.002). The increase in VO_{2max} during weeks 0-24 was larger in the DD group than in ES or SE both in women (p=0.009 and p=0.008, respectively; effect size DD 1.23, ES 0.85, SE 0.67) and men (p=0.003 and p=0.008, respectively; effect size DD 0.94, ES 0.38, SE 0.40).

DISCUSSION

The main objective of the present study was to evaluate the effects of different-day (DD) strength and endurance training and same-session combined strength and endurance training with different orders (ES and SE) on body composition, blood lipid parameters and strength and endurance performance in healthy men and women. The primary finding of the study was that while all three training modes led to significant increases in lean body mass as well as strength and endurance performances, decreased body fat mass was observed only in the DD training groups. Only minor fluctuations in blood lipids were observed over the 24-week training intervention, but these changes were associated with the changes in fat mass.

Body composition and blood lipids

The increases in total body lean mass were similar following all three training modes in both sexes during the 24-week training period, despite the regional changes not reaching significance in all groups. These findings are in line with our earlier investigation (Eklund et al. 2015), in which similar increases in vastus lateralis cross-sectional area following different-day and same-session training with different orders in men were reported. While it has been suggested that endurance exercise performed immediately before strength exercise may interfere with the hypertrophic stimuli induced by the strength loading (Apro et al. 2015), the present intervention did result in considerable and statistically significant increases (3-4%) in lean mass measured by DXA in both same-session groups. However, as this investigation did not include a strength training-only group, it is not possible to conclude whether the gains in lean mass would have been larger without the coexistent endurance training. It also needs to be noted that cycling as the present choice of endurance exercise may aid rather than interfere with muscle growth, while running could have adverse effects on muscle hypertrophy (Wilson et al. 2012). Nonetheless, our results indicate that cycling endurance exercise performed in the immediate presence of strength training (either before or after) did not affect lean body mass differently than allowing for a full day of recovery through splitting strength and endurance training onto different days.

Interestingly, body fat mass was found to decrease only following the DD-training, even though changes in lean mass being similar in all groups and despite nutritional intake being maintained in each group. These decreases were significantly larger than those of the ES training in men, and significantly larger than both same-session modes in women. Similarly to what was observed in terms of lean mass, the same-session training groups did not significantly differ from each other in either sex in terms of decreases in body fat mass. This supports previous results in comparisons of prolonged ES or SE training in men (Schumann et al. 2014), where the training modes did neither result in significant fat loss, nor differed from each other.

Although the training volume was matched in all groups in the present study, the evident difference between the three groups was that the DD-group consistently performed the training sessions on different days while the same-session groups always performed both modes in the same training sessions. Even though the post-exercise energy consumption has not been investigated in a setting of combined training per se, split endurance sessions have been suggested to produce larger post-exercise energy costs than one long-duration session (Almuzaini et al. 1998), possibly contributing to a larger overall energy expenditure over time. As the DD-training could be considered to be a “split session” in comparison to same-session combined training, the assumption of a larger post-exercise energy consumption could be considered a feasible assumption to why the DD-training resulted in a larger degree of fat loss in comparison to combined session training. However, as post-exercise energy consumption was not measured in the present study, this hypothesis remains speculative until further investigation.

The excessive accumulation of adipose tissue especially in the abdominal area has been identified to be a cardiovascular risk factor (Mottillo et al. 2010) as well as to induce a pro-inflammatory environment associated with e.g. metabolic syndrome (Ritchie and Connell 2007). The present results are, therefore, of great importance from a public health perspective as a significant decrease in fat mass was prominently observed in the abdominal region in the DD-groups. Furthermore, these results support earlier findings that decreases in abdominal fat may be associated with decreased blood lipid concentrations (Dutheil et al. 2013), as observed in the present study both in the total subject population as well as in the DD-groups alone through correlations between the changes in total cholesterol and abdominal fat. Thus, the present findings suggest that DD-training could be an effective strategy for decreasing fat in the abdominal region (as represented by decreases in $\mathrm{Fat}_{\mathrm{andr}}$), and thus possibly contribute to improving both cardiovascular and metabolic health (Mottillo et al. 2010, Ritchie and Connell 2007). However, considering the lipid fractions, decreased LDL cholesterol was observed in the ES group among men as well as a modest effect on HDL cholesterol following SE training in women, without correlations to body composition. Therefore, the effects of different combined strength and endurance training regimens is important to investigate further in order to determine possible training protocol specific effects on lipid fractions.

When interpreting the results of the present study the slight differences in baseline level of body fat also needs to be taken into account. Despite the groups displaying similar BMI's in both sexes, the ES and SE groups were slightly leaner at the start of the study. To overcome the difference in the baseline conditions, our statistical method was designed to take into account the baseline level in order to identify true adaptations. Thus, as the magnitude of change was more than two-fold in the DD-groups in comparison to the same-session groups and importantly, without any change in fat mass in the same-session combined groups, it is possible that DD-training is more potent in decreasing body fat mass. However, as comparisons between different-day and same-session training have mainly been conducted focusing on exercise performance rather than detailed comparisons of changes in body composition (Robineau et al. 2014, Sale et al. 1990), additional similar interventions are needed in order to gain a better perspective into the differences between these training modes following prolonged training periods.

Physical performance profile

All training modes resulted in significant gains in maximal concentric strength and isometric force of the lower extremities despite some initial differences in the time course of adaptations in women. Although DD-training in women resulted in improved isometric force as well as significantly larger gains in dynamic strength by week 12 in comparison to E+S and S+E, the adaptations after 24 weeks were similar in the three groups. While Sale et al. (Sale et al. 1990) reported larger strength gains following different-day than same-session training after 20 weeks in men, the results of the present study displayed a similar effect after 12 weeks in women. A recent investigation reported that following a 7-week training intervention in athletes a different-day S and E training mode appeared to be more beneficial for strength adaptations than immediate sequencing of S and E loadings (Robineau et al. 2014). However, with this limited number of studies examining differences between same- and different day S and E training, the inconsistencies in the outcomes between these studies is difficult to identify. The reasons could possibly be related to the specifics of the training programs (e.g. training frequency and/or intensities and training periodization scheme) as well as the subject populations. From the scope of the present study, the baseline difference in 1 RM between the DD and ES group may also explain the difference in the time course of adaptations. The DD-group starting at a slightly lower baseline level may have provided an opportunity for more rapid initial strength gains. Nonetheless, the present study showed similar long-term efficiency for improving maximal strength performance both in the same- and different-day training groups.

A difference to the findings from Sale et al. (Sale et al. 1990) was found in training-induced changes in VO_{2max}. In the present study the increase in VO_{2max} was significantly larger following DD-training than E+S or S+E -training in both men and women, while the earlier study reported no difference between groups. However, the results of the present study are in agreement with those of Robineau et al. (Robineau et al. 2014), who reported that different-day training appeared more likely to improve VO_{2max} than same-day training. In the present study, it may be likely that the lower initial level of VO_{2max} in the DD-groups partly contributed to this difference, considering the possibility for a larger window of adaptation when commencing training at a lower level of fitness. Despite this, the more than two-fold increases in VO_{2max} in the DD-group suggest that increases may be more likely to occur with the DD rather than ES or SE training, but further research is needed to establish the findings with its exact mechanisms.

CONCLUSIONS

In summary, the present study showed that all of the three modes of combined strength and endurance training were effective in increasing maximal strength and endurance performance as well as lean body mass in healthy individuals following 24 weeks of combined strength and endurance training. However, the increases in endurance performance were larger in magnitude when strength and endurance were performed on different days in comparison to that produced by same-session training. Furthermore, body fat mass was decreased only following combined strength and endurance training performed on different days. As the decreases in fat mass were associated with positive changes in blood lipids, combined strength and endurance training on different days may be an effective strategy for early prevention of cardiovascular and metabolic diseases. While the mechanism for this phenomenon was beyond the scope of the present study, separating strength and endurance training into more frequent sessions performed on different days seems to be a valid option for healthy adults who wish to simultaneously optimize body composition and improving physical fitness.

CONFLICT OF INTERESTS

The authors state that there is no conflict of interest.

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TABLES

Table 1. Subject characteristics at Week 0.

	Women			Men		
	DD (n=18)	**ES** (n=17)	**SE** (n=15)	**DD** (n=21)	**ES** (n=17)	**SE** (n=18)
Age (y)	29.9 ± 7.5	29.1 ± 5.6	28.9 ± 4.4	28.9 ± 6.1	29.8 ± 6.0	29.8 ± 4.4
Height (cm)	168.0 ± 5.0	168.0 ± 7.0	164.0 ± 5.0	180.0 ± 0.07	178.0 ± 6.0	179.0 ± 5.0
Weight (kg)	66.5 ± 8.2	66.7 ± 10.1	62.4 ± 8.0	80.5 ± 11.1	80.3 ± 12.0	75.2 ± 8.5
BMI ($m^2 \cdot kg^{-1}$)	23.7 ±2.8	23.7 ± 3.3	23.2 ± 3.4	24.8 ± 3.2	25.2 ± 3.3	23.5 ± 2.1
1 RM (kg)	88 ± 12	102 ± 22 *	99 ± 18	142 ± 24	157 ± 30	143 ± 23
VO_{2max} (ml/min/kg)	28 ± 5	31 ± 4	34 ± 5 *	36 ± 7	42 ± 7 *	43 ± 7 *
% BF	37.8 ± 5.0	34.8 ± 8.5	31.6 ± 7.2	26.5 ± 6.5	22.9 ± 8.2	20.6 ± 5.3 *

1 RM = maximal leg press strength, VO2max=maximal oxygen uptake, %BF = percentage of body fat

DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session training, strength followed by endurance

* indicates difference to same-gender DD

Table 2. Changes (%) in body composition

	Women						Men					
Weeks	**0-12**	**p**	**0-24**	**p**	**13-24**	**p**	**0-12**	**p**	**0-24**	**p**	**13-24**	**p**
Lean$_{tot}$												
DD	4±3*	0.001	5±3*	<0.001	1±3		3±2*	<0.001	4±3*	<0.001	1±2	
ES	1±2*$^{[\ddagger\ p=0.059]}$	0.024	3±3*	0.001	2±2*	0.011	2±3*	0.033	3±3*	0.001	1±2	
SE	2±2*	0.007	3±2*	0.001	1±3		3±2*	<0.001	3±2*	<0.001	0±2	
Lean$_{arms}$												
DD	3±4*	0.015	3±4*	0.040	0±4		3±3*	<0.001	5±4*	<0.001	1±3	
ES	2±4	0.133	2±4	0.297	0±5		1±3		3±4*	0.027	2±6	
SE	3±4*	0.035	3±3*	0.004	0±4		2±3		3±4		1±4	
Lean$_{lower}$												
DD	3±2*	<0.001	4±2*	<0.001	1±2		2±3*	0.049	2±4		0±2	
ES	2±2$^{[*]}$	0.056	3±3*	0.002	2±1*	0.002	2±3*	0.018	4±3*	0.001	1±2	
SE	2±3*	0.020	3±2*	<0.001	1±3		3±3*	<0.001	4±3*	<0.001	0±2	
Lean$_{trunk}$												
DD	5±7		6±8*	0.014	1±7		3±3*	0.005	3±5		0±5	
ES	1±4		4±6*	0.046	3±5		2±5		3±4*	0.041	1±4	
SE	3±4$^{[*]}$	0.053	3±6	0.338	0±6		4±4*	0.004	3±3*	0.015	-1±3	
Fat$_{tot}$												
DD	-7±8*	0.017	-13±14*	0.009	-7±9*	0.025	-6±9*	0.005	-14±15*	<0.001	-9±10*	0.001
ES	-1±7[‡]	0.059	0±8‡	0.005	1±5‡	0.016	-3±13		-6±18		-3±11	
SE	-3±6		-4±10‡	0.008	-1±7‡	0.047	-2±11		-2±13‡	0.043	0±11‡	0.036
Fat$_{arms}$												
DD	-5±9		-11±15*	0.020	-6±11		-3±9		-9±17*	0.026	-6±13	
ES	-1±11		0±10		2±13		0±13		-5±15		-5±11	
SE	1±9		-6±9		-6±10		1±12		-2±16		-2±15	
Fat$_{lower}$												
DD	-5±8		-10±13*	0.018	-7±8*	0.014	-5±8*	0.026	-12±14*	0.004	-7±9*	0.005
ES	-1±7		-1±8[‡]	0.052	-1±4‡	0.029	-2±13		-5±16		-3±11	
SE	-2±5		-4±9		-1±7		-3±11		-4±12		0±10	
Fat$_{android}$												
DD	-11±10*	0.003	-17±15*	0.003	-7±10*	0.030	-7±10*	0.027	-18±14*	<0.001	-13±10*	<0.001
ES	2±10‡	0.001	3±8‡	<0.001	2±8‡	0.012	-5±15		-9±21		-4±13	
SE	-3±12‡	0.028	-4±15‡	0.002	-1±9‡	0.025	1±12‡	0.038	0±15‡	0.003	-1±12‡	0.010

* significant within-group change, ‡ significant difference to same-gender DD (with p-value), # significant between ES and SE. DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session training, strength followed by endurance

Table 3. Blood lipid concentrations (absolute values).

	Women			Men		
	0	**12**	**24**	**0**	**12**	**24**
Chol$_{tot}$						
DD	4.8±0.7	5.1±0.8	4.7±0.8	4.6±0.8	4.7±0.8	4.6±0.9$^{\alpha}$
ES	4.9±0.9	5.1±1.0	4.9±1.0	4.8±0.9	4.4±0.7*‡#	4.5±0.8*
SE	4.6±0.7	4.7±0.8	4.7±0.9	4.6±0.8	4.6±0.8	4.6±0.6
Chol$_{HDL}$						
DD	1.9±0.3	2.1±0.3*	1.9±0.2$^{\alpha}$	1.4±0.3	1.5±0.4	1.4±0.3
ES	1.9±0.4	2.0±0.5	1.9±0.4	1.5±0.3	1.5±0.4	1.4±0.3
SE	1.9±0.4	1.9±0.5‡	2.0±0.4 §	1.4±0.3	1.3±0.3* ‡	1.4±0.3
Chol$_{LDL}$						
DD	2.4±0.6	2.5±0.7	2.3±0.8	2.6±0.9	2.7±0.8	2.5±0.8
ES	2.5±0.6	2.6±0.7	2.6±0.7	2.8±0.9	2.5±0.7*	2.7±0.8
SE	2.2±0.8	2.3±0.8	2.3±0.8	2.6±0.7	2.8±0.8	2.7±0.5
HDL/LDL						
DD	0.8±0.4	1.0±0.6	0.9±0.4	0.6±0.2	0.6±0.3	0.6±0.2
ES	0.8±0.3	0.9±0.4	0.7±0.3	0.6±0.3	0.6±0.2	0.6±0.3
SE	1.0±0.5	0.9±0.4	0.9±0.3	0.6±0.5	0.5±0.3	0.5±0.2
Triglycerides						
DD	1.2±0.5	1.1±0.5	1.1±0.6	1.4±0.8	1.3±0.5	1.2±0.7
ES	1.1±0.4	1.1±0.4	1.0±0.4	1.0±0.3	1.0±0.3	0.8±0.3$^{\alpha}$
SE	1.1±0.5	1.1±0.5	1.0±0.3	1.3±1.0	1.2±0.8	1.2±0.6

* significant within-group change from week 0, $^{\alpha}$ significant within-group change from week 12

‡ significant difference to same-gender DD at time point, # significant difference between same-gender ES and

§ significantly different from the other groups 12-24

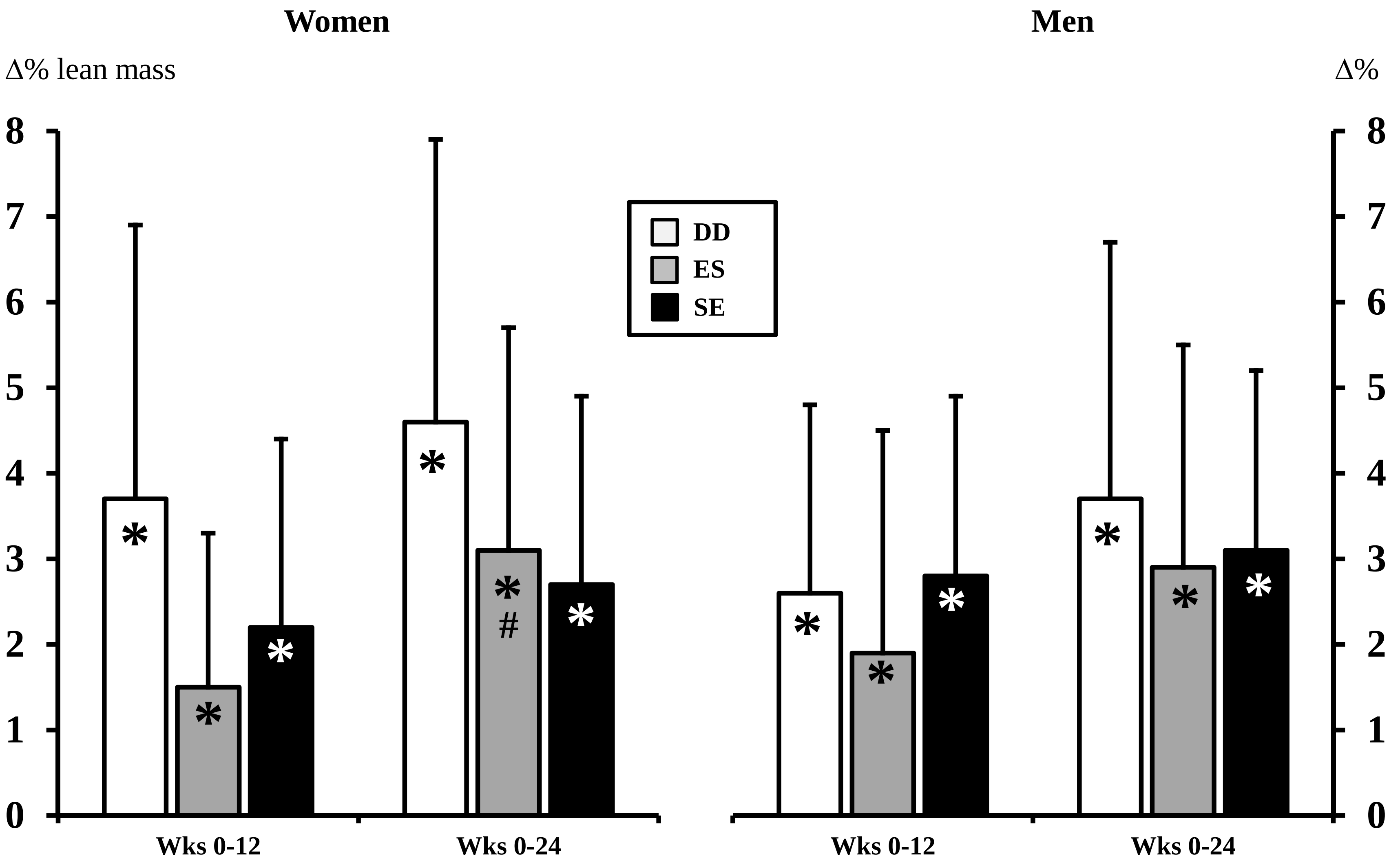
DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session

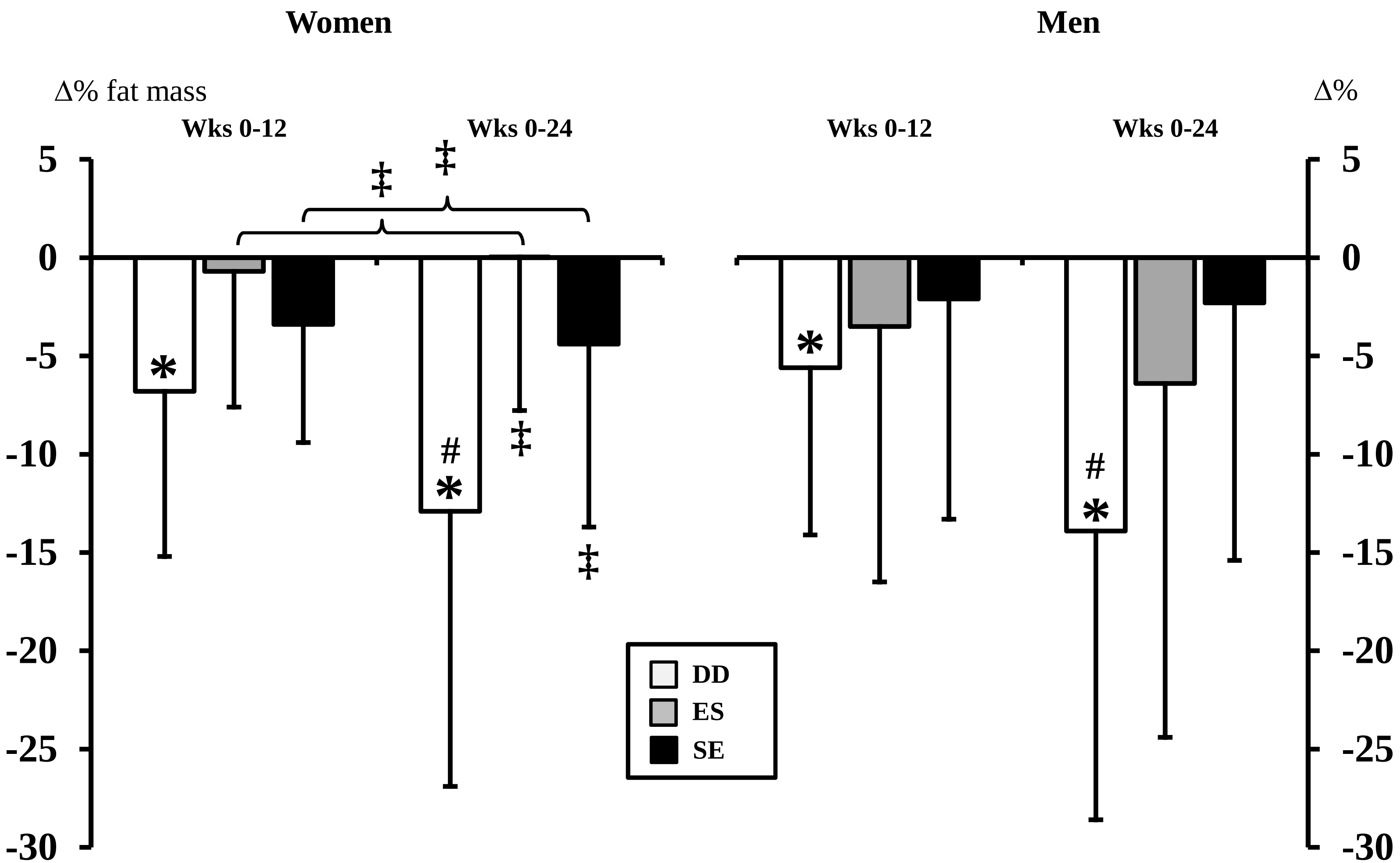
FIGURE CAPTIONS AND LEGENDS

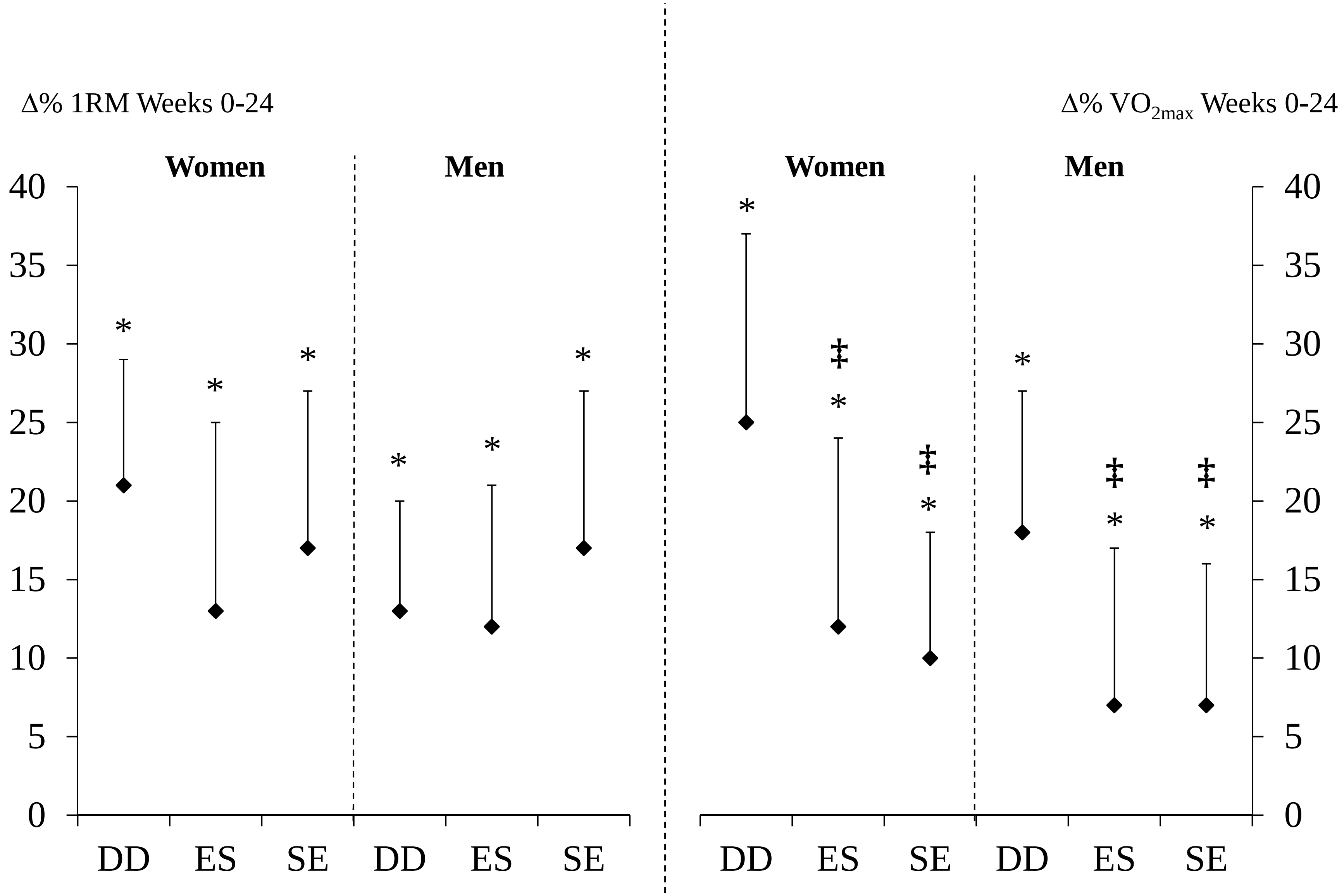
Figure 1. Mean (SD) changes in total body lean mass. * significant within-group change during weeks 0-12, # significant within group change during weeks 13-24. DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session training, strength followed by endurance.

Figure 2. Mean (SD) changes in total body fat mass. * significant within-group change during weeks 0-12, # significant within group change during weeks 13-24, ‡ significant difference to same-session DD. DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session training, strength followed by endurance.

Figure 3. Mean (SD) changes in 1 RM (left) and VO2max (right). * significant within-group change, ‡ significant difference to same-sex DD. DD = Different-day training, ES = Same-session training, endurance followed by strength, SE = Same-session training, strength followed by endurance


Women
Men
Δ% lean mass
Δ%
8
7
6
5
4
3
2
1
0
DD
ES
SE
*
#
Wks 0-12
Wks 0-24
Wks 0-12
Wks 0-24


Women
Men
Δ% fat mass
Δ%
Wks 0-12
Wks 0-24
Wks 0-12
Wks 0-24
5
0
-5
-10
-15
-20
-25
-30
‡
‡
*
#
*
‡
‡
*
#
*
DD
ES
SE


Δ% 1RM Weeks 0-24
Δ% VO_{2max} Weeks 0-24
Women
Men
Women
Men
40
35
30
25
20
15
10
5
0
*
‡
DD
ES
SE
DD
ES
SE
DD
ES
SE
DD
ES
SE