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Fitness and lean mass increases during combined training independent of loading order

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ABSTRACT

Purpose: Although the benefits of combined endurance (E) and strength (S) training for the development of physical fitness and health are well known, scientific examination of the effect of loading order when E and S are combined into the same training session (E+S vs. S+E) is rare. This study investigated the effects of moderate frequency E+S versus S+E training on physical fitness, body composition and blood lipids. **Methods:** Physically active and healthy young men performed E+S (n=16) or S+E (n=18) training, 2-3 x·wk⁻¹ for 24 weeks. Endurance (by incremental bike test) and strength (by dynamic leg press) performance as well as body composition (by DXA), muscle cross-sectional area of vastus lateralis (by ultrasound) and blood lipids were determined before and after the intervention. **Results:** Time to exhaustion, aerobic power (W) and 1RM strength significantly increased in the two groups at week 24 (E+S 12-15%, p=0.003-0.001; S+E 16-17%, p<0.001) but no between-group difference was observed. Similarly, the two groups significantly increased total lean mass (E+S 3%, S+E 3%, both p=0.001) and muscle cross-sectional area (E+S 14%, p=0.001; S+E 16%, p<0.001) at week 24 to a similar extent. No significant changes in body fat or blood lipids were observed in either of the two groups at week 24. **Conclusion:** These results showed that moderate frequency (2-3 x·wk⁻¹) combined E+S or S+E training led to significant improvements in physical fitness and lean body mass but did not induce significant changes in body fat or blood lipids. Furthermore, as no between-group differences were observed, these results indicate that loading order does not seem to affect training adaptations of healthy moderately active young men.

Key words: order effect, aerobic training, resistance training, concurrent endurance and strength training, muscle cross-sectional area, body composition, hypertrophy, health

INTRODUCTION

The benefits of combined endurance (E) and strength (S) training for the maintenance and development of physical fitness and body composition have been extensively investigated and their importance especially for sedentary and moderately active populations is well known (15, 19, 21, 23, 34). Over recent years, a growing body of scientific knowledge strongly suggests that regular performance of aerobic and resistance training is a major factor in the prevention and treatment of cardiovascular disease including risk factors such as obesity, diabetes and blood lipid levels (23).

The long-term physiological adaptations of endurance and strength training are dissimilar in nature. Prolonged endurance training may enhance oxidative energy metabolism and simultaneously increase whole-body rates of fat oxidation (2), which may lead to decreases in total and abdominal body fat (1) and total cholesterol, as well as a more positive distribution of low (LDL-C) and high (HDL-C) density lipoproteins (38). While endurance training may directly decrease total body fat and weight, strength training can positively affect body composition via increases in muscle cross-sectional area and lean body mass (1, 36).

The suggested amount of weekly physical activity commonly ranges from 150 to 300 min·wk⁻¹ (1, 18) of combined aerobic and resistance training. However, research findings have repeatedly emphasized impaired biological adaptations (interference) when a high volume of endurance and strength training are combined over a longer period of time (40). It appears that this interference may be more pronounced in strength or power development (21) but to some extent also in muscle growth (24, 40), reducing the positive effects of physical training on fitness, body composition and health. Combined training utilizing a low volume and frequency of training sessions (i.e. 2-3 x·wk⁻¹), on the other hand, may not have inhibitory effects on neuromuscular and morphological adaptations (24, 28). A recent study by Fisher et al. (2013) (15) has shown that a combined training frequency of 1 x wk⁻¹ aerobic and 1 x wk⁻¹ resistance training led to similar improvements in overall fitness as a twice or three times higher training frequency in older women. The exact amount of exercise needed to achieve favorable training adaptations seems to depend on the size and type of the expected outcome variables (i.e. modest vs. large reductions in body fat or body weight, reductions in blood lipids vs. increases in

muscle size etc.), intensity and volume of training performed, as well as on dietary prescriptions (i.e. diet restriction vs. freely chosen diet) and the population studied (1, 23, 38).

The biological adaptations of concurrent training performed on separate days were previously examined in numerous studies. However, scientific examination of the physiological adaptations of endurance and strength training combined into the same training session are still rare but provide some evidence of loading order-specific adaptations due to the missing recovery when loadings are performed consecutively (9-11, 13, 20). As this training method can be considered as extremely time effective and time constraints are among the major reasons for restraining from regular physical activity in young adults (31), the aforementioned training regimen may help young adults to commit to regular physical activity while allowing sufficient time for other responsibilities.

The purpose of this study was two-fold to determine; 1) whether a moderate volume of endurance and strength training combined into the same training session is sufficient to yield significant changes in physical fitness, body composition and blood lipids and 2) whether loading order-specific adaptations (order effect) in these variables can be observed. Consequently, while the magnitude of possible interference was beyond the scope of this study, the focus of this study was to investigate the order effect of endurance followed by strength (E+S) and strength followed by endurance (S+E) training performed over 24 weeks. We hypothesized that our training volume will induce significant improvements in physical fitness, body composition and blood lipids but the magnitude of these adaptations will be related to the loading order performed.

METHODS

Subjects

Forty-two healthy men were recruited to participate in this study. Subjects' initial health and activity status was assessed by a standardized phone interview. The subjects were moderately physically active as characterized by irregular performance of walking, cycling or occasionally team sports at light to moderate intensity and duration for not more than 3 times per week and did not engage systematically in any endurance or strength training prior to inclusion into the study. Subjects were informed about possible risks of all study procedures before giving written informed consent. A completed health questionnaire and resting ECG were reviewed by a

cardiologist prior to the first exercise testing and training. All subjects were free of acute and chronic illness, disease and injury and did not report use of any medications that would contraindicate the performance of intense physical activity. Out of the 42 originally recruited subjects, 8 did not complete the study or were not included in the data analysis due to a training adherence of less than 90%. Demographic characteristics of all included subjects were as follows (mean \pm SD): age 30 \pm 5 years, height 179 \pm 6 cm, weight 78 \pm 11 kg, BMI 24 \pm 3. An additional group of subjects undergoing the same pre-screening process was recruited for reproducibility tests of the measurement procedures (n=21, age 30 \pm 6 years, height 180 \pm 7 cm, weight 82 \pm 9 kg, BMI 25 \pm 2). The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the ethics committee at the University of Jyväskylä

Study design

Following the pre-screening, all experimental subjects were assigned to an Endurance+Strength (E+S, n=16) or Strength+Endurance (S+E, n=18) training group. The subjects performed either endurance immediately followed by strength training (E+S) or strength immediately followed by endurance training (S+E) for 24 weeks. As this study was aimed to investigate the order effect of combined training, a no-training control group was not used. For familiarization, one combined training session in the order of the corresponding training group (E+S vs. S+E) was conducted prior to the baseline measurements and training. Thereafter, subjects reported to the laboratory for a second familiarization session during which the strength measurements were practiced and the equipment adjusted to the specifics of the subject. Testing of physical fitness, body composition and blood lipids were then performed on three separate days prior to the start of the training (wk 0). To allow for sufficient recovery, endurance, strength as well as body composition and blood tests were separated by at least 48 h of rest. All measurements were repeated after 12 and 24 weeks and were performed at the same time of day within \pm 1h of the timing of baseline measurements. The additional subjects recruited for measurement reproducibility testing (n=21) were familiarized with the measurement procedures in the same manner as the intervention groups and were tested both before and after a 12-week period without undergoing prescribed training but maintaining their habitual activities of daily living.

Testing procedures

Strength performance

Subjects' one repetition maximum (1RM) of leg extensors was determined using a dynamic horizontal leg press device (David 210, David Health Solutions, Helsinki, Finland). Following a warm up (1 set of 5 repetitions at 70% of estimated 1RM, 1 set of 2 repetitions at 80-85% of estimated 1RM, 1 set of 1 repetition at 90-95% of estimated 1RM) a maximum of 5 trials was allowed to obtain a true 1RM. The device was set up so that the knee angle in the initial flexed position was approximately 60 degrees (mean \pm SD, 58 \pm 2 degrees) and a successful trial was accepted when the knees were fully extended (~180 degrees). The greatest load that the subject could lift to full knee extension at an accuracy of 1.25 kg was accepted as 1RM. In addition, a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Finland) was used to determine maximal isometric bilateral leg press force (MVC_{max}). Subjects were seated with a hip and knee angle of 110 and 107 degrees, respectively and were instructed to produce maximal force as rapidly as possible on verbal command and to maintain the force plateaued for 3-4 seconds. At least three trials separated by a rest period of 1 minute were conducted and up to two additional trials were performed if the maximum force during the last trial was greater by 5% compared to the previous attempt. The trial with the highest maximal force measured in Newtons was used for statistical analysis. The force signal was low-pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). Rapid force production (MVC₅₀₀) was calculated from the force curve and defined as the average force produced during the first 500ms of the maximal contraction.

Endurance performance

A graded protocol on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) was used to determine $\dot{V}O_{2max}$. The initial load for all subjects was 50 Watts and increased by 25 Watts every 2 minutes. Subjects were asked to maintain a pedaling frequency of 70 rpm throughout the test. The test was stopped when the subjects failed to keep up the required rpm for more than 15 s. Heart rate was monitored throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 seconds at each stage. Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). On each testing day, air flow calibration was performed using a manual flow calibrator. Before each test, automatic air flow calibration was performed and the gas analyzer was calibrated using a certified gas

mixture of 16% O₂ and 4% CO₂. $\dot{V}O_{2max}$ was accepted when $\dot{V}O_2$ plateaued despite a further increase in power and when the respiratory exchange ratio exceeded 1.05. $\dot{V}O_{2max}$ used for statistical analysis was calculated as the highest $\dot{V}O_2$ value averaged over 60 s. In addition, maximal aerobic power (W) was calculated using the equation (25): Aerobic power = $W_{com} + (t/120) * 25$, where W_{com} is the load of the last completed stage and t is the time of the last incomplete stage (in s) and time to exhaustion was defined as the total duration of the test. Blood lactate concentrations were determined by capillary blood samples taken from the fingertip during the final seconds of each load. Twenty μ l of blood were collected by small capillaries, inserted into reaction capsules containing a hemolyzing and anticoagulant agent and lactate concentrations were analyzed using a Biosen analyzer (C_line Clinic, EKF, Magdeburg, Germany). Subjects' individual aerobic and anaerobic thresholds were determined using deflection points obtained by plotting the curves of blood lactate concentrations, ventilation, oxygen consumption and carbon dioxide production (6).

Body composition and venous blood sampling

Anatomical muscle cross-sectional area (CSA) of vastus lateralis (VL) was measured by the extended field of view mode (3), using a B-mode axial-plane ultrasound (model SSD-a10, Aloka Co Ltd, Japan) with a 10-MHz linear-array probe. A customized convex-shaped probe support was used to assure a perpendicular measurement and to constantly distribute pressure on the tissue. The transducer was moved manually from lateral to medial along a marked line on the skin. Three panoramic CSA images were taken at 30%, 50% and 70% of the femur length (lateral aspect of the distal diaphysis to the greater trochanter), respectively and CSA was analyzed manually using Image-J software (version 1.44p, National Institute of Health, USA). The mean of the two closest values (at 30%, 50% and 70%, respectively) was used for statistical analyses. To assess total CSA of VL, values of the three measurement points were averaged.

Whole body tissue composition was assessed by DXA (Lunar Prodigy Advance, GE Medical Systems, Madison, USA). To control the experimental conditions, each scan was conducted in the morning after 12 hours of fasting. Legs were secured by non-elastic straps at knee and ankles and arms were aligned along the trunk with palms facing the thighs. All metal objects were removed from the subject prior to the scan. Automatic analyses (Encore, version 14.10.022) provided total and upper body lean (including muscle) and fat mass.

Automatic generated regions of the legs were manually adjusted by the same investigator to include hamstrings and gluteal muscles. Thus, legs were separated from the trunk by a horizontal line right above the iliac crest providing lean and fat mass for legs and upper body separately. Abdominal fat mass was calculated by manually defining a range of interest (ROI) confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest (37). This customized ROI was then copied to the DXA scans obtained at week 12 and 24, respectively in order to assure analyses were conducted from the same areas at all measurement times.

Venous blood samples were drawn after 12h of fasting in order to obtain concentrations of total cholesterol, low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and triglycerides. Subjects were asked to rest for at least 8 h during the preceding night and were required to restrain from strenuous physical activity for at least 48 h. Blood samples were taken from the antecubital vein into serum tubes (Venosafe, Terumo Medical Co., Leuven, Hanau, Belgium) using standard laboratory procedures. Serum samples were stored for 10 min after which they were centrifuged at 3 500 rpm (Megafure 1.0 R, Heraeus, Germany) and immediately analyzed by spectrophotometry (Konelab 20XTi, Thermo Fisher Scientific, Vantaa, Finland). LDL-C was estimated using the Friedwald (1972) (17) equation: $LDL-C = \text{total cholesterol} - HDL-C - (\text{triglycerides}/2.2)$.

Combined endurance and strength training

Subjects were asked to maintain individual habitual physical activity (e.g. light walking, cycling and occasional team sports) throughout the study period. All prescribed training in the study was consistently supervised by qualified instructors. The training was designed to reflect a program aimed for physically active populations according to recommendations outlined by the American College of Sports Medicine (39) but modified to reduce overall training volume and frequency. The main objective was to improve physical fitness and health through a periodized program including both moderate and vigorous intensity aerobic exercises combined with hypertrophic and maximal strength exercise protocols. The endurance training was conducted on a cycle ergometer and the strength training program included exercises for all major muscle groups with a major focus on the lower extremities. Subjects were asked to proceed from one loading (i.e. E or S, respectively) to the subsequent loading (i.e. S or E, respectively) after a maximum of 10 minutes of rest.

During the first 12 weeks, the subjects performed according to their corresponding training group 2x (1E+1S) or 2x (1S+1E) per week. The frequency was then increased during the second 12 weeks so that 2 combined training sessions were performed in every 1st and 4th week and 3 combined training sessions in every 2nd and 3rd week (i.e. 2x [1E+1S] or 2x [1S+1E] or 3x[1E+1S] or 3x [1S+1E], respectively). To reflect tapering before testing, both week 12 and week 24 were conducted by maintaining the training frequency but reducing training volume and intensity by reducing the number of sets and lowering the loads during the strength loading as well as a reducing both the total duration and time spent at high intensity (i.e. above the anaerobic threshold) during endurance cycling.

The intensity of the endurance training was controlled by heart rate (HR) (Polar S410, Polar Electro Oy, Kempele, Finland) associated with subject's individual aerobic and anaerobic threshold determined during measurements at week 0 and 12, respectively. Subjects were instructed to maintain a constant pedalling frequency at approximately 70 rpm during each training session, while the magnetic resistance of the ergometer was adjusted to achieve the required exercise intensity. During weeks 1-7 steady-state cycling of low to moderate intensity (below and above the aerobic threshold) was performed and during the remaining weeks, additional high-intensity interval sessions (below and above the anaerobic threshold) were incorporated into the training program. The duration of endurance cycling progressively increased throughout the 12 weeks of training from 30 to 50 minutes. During the second 12-week period, the major endurance program structure was maintained, while both training volume and intensity were further increased. The aerobic threshold represented an intensity (% of HR_{max}) of 65 ± 5 % and 67 ± 6 % in E+S and 68 ± 8 and 67 ± 6 % in S+E at week 0 and 12, respectively. The anaerobic threshold represented an intensity of 85 ± 5 % and 86 ± 5 % in E+S and 82 ± 8 % and 86 ± 5 % in S+E at week 0 and 12, respectively.

The loads used during the strength training were determined by the number of repetitions and execution velocity and progressively increased throughout the two 12-week periods. Exercises for the lower body were bilateral dynamic leg press as well as bilateral (weeks 1-7 and 13-18) and unilateral (weeks 8-12 and 19-24) dynamic knee extension and flexion. Additional exercises for the upper body included dynamic seated vertical press and lat-pull down as well as exercises commonly used to improve trunk stability (crunches, torso rotation and lower back extension). During the first two weeks training was performed as a circuit using 2-4 sets of 15-

20 repetitions at an intensity of 40-60% of 1RM. Thereafter, protocols aiming for muscle hypertrophy (2-5 sets of 8-10 repetitions at 80-85% of 1RM, 1.5-2 min inter-set rest) and maximal strength (2-5 sets of 3-5 repetitions at 85-95% of 1RM, 3-4min inter-set rest) as well as during the last 2 weeks protocols targeting explosive strength (2 sets of 8-10 repetitions at 40% of 1RM with maximal velocity, 3-4 min inter-set rest) were performed. During the second 12-week period the major strength program structure was maintained, while both training volume and frequency were slightly increased in order to maximize fitness and health outcomes and to avoid a training plateau. The overall duration of the strength protocol within each combined training session was 30-50 min, resulting in a total duration of ~60-100 min for each combined training session (i.e. E+S and S+E, respectively).

Dietary intake

To control nutritional intake, food diaries were collected for three days including one weekend day at week 0, 12 and 24. Subjects received both verbal and written nutritional recommendations and were instructed on how to report nutritional intake in the diaries. The food diaries were analyzed by nutrient analysis software (Nutriflow, Flow-team Oy, Finland). Subjects were asked to maintain constant dietary intake throughout the study period. In preparation for all testing, subjects were instructed to consume a light meal 2-3 h prior to the start of each test and were asked to maintain similar nutritional intake prior to the measurements at week 0, 12 and 24. During each training session, a standardized low dose of glucose (according to bodyweight 2-4 tablets, each containing 2.1g of glucose) was provided at the mid-point of each combined session (after E or S, respectively), while water was allowed ad libitum.

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences (version 20.0, IBM Inc., Chicago, IL, USA). All results are presented as absolute values (Tables 1 and 2) and relative changes from week 0 as means with standard deviations (\pm SD). Normality of distribution was determined by the Shapiro-Wilk test at a significance level of $p < 0.05$. To achieve normality, all body composition and blood variables were log transformed. Within and between-group differences were assessed by a mixed ANOVA design with repeated measures. Effect sizes (ES) are given as Cohen's d. Partial correlations with adjustment for groups were performed to determine relationships between dependent variables across all experimental subjects. Measures

of reliability are presented as intra-class correlations (ICC) of absolute agreement for single measures. Significance for all tests was defined as $p=0.05$, while values <0.06 were accepted as a significant trend.

RESULTS

The training adherence was $99\pm 2\%$ in both the E+S and S+E training group. All subjects completed at least 90% of the overall training volume.

Measurement reproducibility

The analysis of reliability revealed an ICC > 0.7 for all test measures, indicating high reproducibility. The intra-class correlations of endurance and strength performance, body composition and blood lipid measures were 0.737 - 0.955, 0.786 - 0.975 and 0.763 - 0.866, respectively.

Nutrition

Total energy intake at week 0, 12 and 24 was 9.3 ± 1.8 MJ, 10.2 ± 2.6 MJ, 9.5 ± 2.6 MJ in E+S and 9.4 ± 2.0 MJ, 9.3 ± 1.7 MJ, 7.9 ± 1.7 MJ in S+E. The average nutritional intake as percentage of total energy for carbohydrates, fat and protein was 42-45%, 31-36% and 17-19% in E+S and 42-44%, 33-36% and 18% in S+E throughout the 24 weeks of training. No significant within or between-group differences were observed.

Physical Fitness

Absolute values of physical fitness at week 0 and 24 are presented in Table 1. Significant main effects for time were observed in 1RM ($F=73$, $p<0.001$), MVC_{max} ($F=14$, $p<0.001$) and MVC_{500} ($F=15$, $p<0.001$). Both groups significantly improved 1RM strength (Fig. 1) at week 12 (E+S $9\pm 8\%$, $p<0.001$, $ES=0.456$; S+E $12\pm 8\%$, $p<0.001$, $ES=0.772$) and 24 (E+S $12\pm 9\%$, $p=0.001$, $ES=0.620$; S+E $17\pm 12\%$, $p<0.001$, $ES=1.032$). The increase from week 12 to 24 was significant in both groups ($p<0.05$). Similarly, MVC_{max} significantly increased in both groups at week 12 (E+S $10\pm 10\%$, $p=0.010$, $ES=0.345$; S+E $9\pm 12\%$, $p=0.019$, $ES=0.337$) and 24 (E+S $10\pm 12\%$, $p=0.025$, $ES=0.302$; S+E $13\pm 18\%$, $p=0.024$, $ES=0.482$) while MVC_{500} increased significantly in S+E only at week 12 ($13\pm 15\%$, $p=0.002$, $ES=0.623$) and 24 ($14\pm 18\%$, $p=0.005$; $ES=0.620$).

+++ Fig 1 somewhere near here +++

Significant main effects for time were observed in time to exhaustion ($F=83$, $p<0.001$), maximal aerobic power ($F=71$, $p<0.001$) and $\dot{V}O_{2\max}$ ($F=12$, $p<0.001$). Both groups significantly improved time to exhaustion (Fig. 2a) at week 12 (E+S $9\pm 9\%$, $p=0.003$, $ES=0.387$; S+E $10\pm 6\%$, $p<0.001$, $ES=0.551$) and 24 (E+S $15\pm 9\%$, $p<0.001$, $ES=0.859$; S+E $17\pm 7\%$, $p<0.001$, $ES=1.027$) as well as maximal aerobic power (Fig. 2b) at week 12 (E+S $8\pm 9\%$, $p=0.011$, $ES=0.820$; S+E $9\pm 7\%$, $p<0.001$, $ES=0.630$) and 24 (E+S $13\pm 9\%$, $p<0.001$, $ES=0.830$; S+E $16\pm 7\%$, $p<0.001$, $ES=1.074$). The increases in aerobic power in both groups from week 12 to 24 were significant ($p<0.01-0.001$). The observed increases in $\dot{V}O_{2\max}$ were significant at both week 12 (E+S $4.8\pm 7\%$, $p=0.051$, $ES=0.266$; S+E $7.3\pm 8\%$, $p=0.003$, $ES=0.339$) and 24 (E+S $6.1\pm 8\%$, $p=0.041$, $ES=0.366$; S+E $6.4\pm 12\%$, $p=0.006$, $ES=0.396$). No significant between-group differences were obtained for the measures of physical fitness.

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

Body composition

Absolute values of body composition measures at week 0 and 24 are presented in Table 1. A significant increase in bodyweight and BMI was observed in S+E only ($1.7\pm 2.4\%$ and $1.7\pm 2.6\%$ at week 12 and 24 respectively, $p<0.05$). No significant changes in body fat %, total fat mass or abdominal fat mass were observed in the two groups at either week 12 or week 24. A significant main effect for time was observed for muscle CSA at 30% ($F=18$, $p<0.001$), 50% ($F=50$, $p=0.001$) and 70% ($F=60$, $p<0.001$) of vastus lateralis (Fig. 3). Both groups significantly improved average CSA of VL at week 12 (E+S $8\pm 7\%$, $p=0.002$, $ES=0.490$; S+E $9\pm 7\%$, $p<0.001$, $ES=0.643$) and 24 (E+S $14\pm 7\%$, $p=0.001$, $ES=0.822$; S+E $16\pm 8\%$, $p<0.001$, $ES=1.178$) whereby the increase from week 12 to 24 was significant (both groups $p<0.001$).

+++ Table 1 somewhere near here +++

+++ Fig 3 somewhere near here +++

A significant main effect for time was observed for total lean mass ($F=8$, $p=0.001$), upper body lean mass ($F=13$, $p<0.001$) and leg lean mass ($F=49$, $p=0.001$). Both groups significantly increased total lean mass (Fig. 4a) at week 12 (E+S $2\pm 3\%$, $p=0.042$, $ES=0.203$; S+E $3\pm 2\%$, $p<0.001$, $ES=0.310$) and week 24 (E+S $3\pm 3\%$, $p=0.001$, $ES=0.329$; S+E $3\pm 2\%$, $p=0.001$, $ES=0.342$). Similarly both groups increased upper body lean mass

(Fig. 4b) at week 12 (significant in S+E only, $2\pm 3\%$, $p=0.022$, $ES=0.212$) and 24 (E+S $3\pm 3\%$ $p=0.005$, $ES=0.253$; S+E $2\pm 3\%$, $p=0.025$, $ES=0.218$) and leg lean mass (Fig 4c) both at week 12 (E+S $2\pm 3\%$, $p=0.024$, $ES=0.210$; S+E $3\pm 2\%$, $p<0.001$, $ES=0.373$) and 24 (E+S $4\pm 3\%$, $p<0.001$, $ES=0.361$; S+E $4\pm 2\%$ $p<0.001$, $ES=0.427$). The increase in leg lean mass from week 12 to 24 was significant in E+S only ($p<0.05$). No significant between-group differences for the measures of body composition were obtained.

+++ Fig 4a – 4c somewhere near here +++

Blood lipids

Only minor changes in total cholesterol, HDL-C, LDL-C and triglycerides were observed after 24 weeks of training (Table 2). A significant between-group difference was observed for LDL-C at week 12 ($p<0.05$) but was diminished after 24 weeks of training.

+++ Table 2 somewhere near here +++

Correlations of physical fitness and body composition across all experimental subjects

All absolute values of physical fitness at baseline (1RM, MVC_{max} , MVC_{500} , aerobic power, time to exhaustion and $\dot{V}O_{2max}$) were significantly correlated with the corresponding relative changes obtained at week 12 and 24 ($r=-0.376$ to -0.725 , $p=0.031$ to <0.001). Similarly, significant correlations at week 24 were also found for body fat % at baseline and the relative change in body fat % ($r=-0.450$, $p=0.006$) as well as for the absolute values of total fat at baseline and the corresponding relative change ($r=-0.364$, $p=0.037$) and total fat and abdominal fat mass at baseline and the relative change in body fat % ($r=-0.458$, $p=0.006$ $r=-0.431$, $p=0.006$, respectively). In addition, absolute values of 1RM strength at baseline were significantly correlated with relative changes in body fat % as well as relative changes of total and abdominal fat mass obtained at week 12 and 24 ($r=-0.365$ to -0.456 , $p=0.025-0.006$). Similarly, changes in 1RM strength performance and changes in leg lean mass and VL CSA were significantly correlated ($r=0.476-0.629$, $p=0.037-0.007$) at week 24.

DISCUSSION

Physical fitness, body composition and blood lipids are strongly associated with health and mortality even in relatively young and healthy subjects (23, 30). The purpose of the present study was to assess the effects of

exercise order of moderate frequency ($2-3 \times \text{wk}^{-1}$) endurance and strength training combined into the same training session (E+S vs. S+E) on physical fitness, body composition and blood lipids in moderately active and healthy young men. This study showed that both training orders (E+S and S+E) led to significant increases in muscular and cardiorespiratory fitness, muscle cross-sectional area and lean body mass after 12 and 24 weeks of training, but no reductions in total body or abdominal fat mass, body fat % or blood lipids were observed in either of the two training groups. In addition, the magnitude of training-induced adaptations did not differ between the two groups.

Compared to concurrent training performed on separate days, endurance and strength training combined into the same training session does not allow any recovery between the two modes, leading to the loading performed second to be adversely affected by fatigue induced by the first loading. In recent studies, these adverse effects were reflected by increased work economy when endurance loading was performed immediately after a strength loading (14) and reduced neuromuscular performance measured immediately following intensive running or cycling (27), possibly influencing physiological training adaptations. As previous studies of combined endurance and strength training have shown possible compromised adaptations in strength and power but not endurance performance (21), it is likely that the acute effects of endurance loading on strength performance are more critical for the long-term development of physical fitness than the acute effects of strength loading on work economy during endurance performance.

Interestingly, the present E+S and S+E training groups significantly improved physical fitness as reflected in 1RM strength (12-17%), MVC_{max} (10-13%), time to exhaustion (15-17%), aerobic power (13-16%) and $\dot{\text{V}}\text{O}_{2\text{max}}$ (7%) to a similar extent and no between-group differences were observed. Our findings are in line with results of Collins and Snow (1993) (13) and Chtara et al. (2008) (10) who also reported that either loading order was similarly effective in improving endurance and strength performance following prolonged combined E+S or S+E training. However, other studies have found limited increases in $\text{VO}_{2\text{max}}$ following the E+S order in women (20) or S+E order in men (11) as well as impaired strength adaptations following E+S training in older men (9) when compared to the reverse loading order. Despite these findings of studies combining endurance and strength training into the same training session and those which report diverse biological adaptations

induced by endurance and strength training alone (22), the present results indicate that our subjects adapted to both training stimuli simultaneously and to the similar magnitude.

When combining endurance and strength into the same training session, it seems that the type of endurance training performed needs to be carefully considered. Endurance cycling is biomechanically similar to many of the strength exercises performed in the present study (16) and may essentially lead to a similar magnitude of fatigue as indicated, for example, by inhibited neuromuscular performance observed during a single isometric contraction (32, 33), suggesting similar acute neural responses to both types of loadings. Furthermore, previous studies have shown that endurance cycling training may also lead to small but significant increases in muscle CSA (28) and strength (24) in physically active subjects with no experience in regular endurance or strength training. Therefore, it is possible that the present endurance cycling combined with the hypertrophic and maximal strength training protocols led to synergistic rather than adverse effects on strength and endurance performance. This hypothesis may also be supported by the review by Wilson et al. (2012) (40) who revealed that endurance running may be more detrimental to strength adaptations when compared to endurance cycling, possibly related to a larger magnitude of muscle damage induced by the eccentric components of prolonged running (29).

The present increases in 1RM strength were significantly correlated with increases in anatomical muscle-cross-sectional area and leg lean mass in all subjects across the two training groups. Both training groups significantly increased muscle CSA after the 24 weeks of training independent of the loading order. Although animal studies have shown that endurance and strength training might induce distinct genetic and molecular pathways critical for muscle hypertrophy (5, 22), other studies of human subjects have indicated the cumulative effect of both loadings to possibly compromise beneficial morphological adaptations (12, 22). Coffey et al. (2009) (12) found in an acute study that neither of the two loading orders (E+S vs. S+E) showed superior signaling responses over the other but concluded that endurance and strength training performed in close proximity did not induce optimal activation of pathways to promote significant anabolic processes. While the magnitude of interference when compared to strength training alone was beyond the scope of this study, these previous findings possibly explain why no between-group differences in muscle growth were observed.

Similar to anatomical muscle CSA, leg, upper body and total lean mass were increased in the present two groups during the 24 weeks of training independent of loading order. Muscle strength and possibly muscle mass have been associated with reduced mortality even in young subjects (30). Since lean body mass has been shown to be a major determinant of basal metabolic rate by representing 60-75% of an individual's daily energy expenditure (35), increases in muscle and lean mass may have potential health benefits by inducing enhanced fat oxidation (1, 36). Our present findings are thus, of great importance as they show that a moderate volume of combined endurance and strength training may be beneficial in significantly increasing muscle strength and lean mass whereby the loading order does not seem to influence the magnitude of these adaptations.

However, the positive adaptations in physical fitness and lean body mass were not accompanied by significant reductions in body fat % and total or abdominal fat mass in either training group. Furthermore, no significant changes in total cholesterol as well as high- (HDL-C) and low (LDL-C) density lipoproteins or triglycerides were observed. Previous studies have shown a strong association between body fat and blood lipids (7), indicating that a reduction in fat mass positively correlates to changes in blood lipids. Typically aerobic exercise has been considered as being most effective to induce reductions in fat oxidation during and in the hours following an exercise loading (1, 38), while the direct effects of strength training on reductions in body fat and blood lipids are minimal (19, 26). Studies combining endurance and strength training on separate days often show reductions in both variables with varying training frequency and volume in young (19) and old men (34) as well as old women (15). Therefore, our present results may indicate endurance and strength training combined into the same training session to be less favorable for reductions in body fat and blood lipids than combined training performed on separate days. In contrast to our study, however, it needs to be noted that most of the previous studies were performed with endurance running. Achten et al. (2003) (2) showed that running induces higher rates of fat oxidation when compared to cycling and a meta-analysis by Wilson et al. (2012) (40) found combined training programs in which the aerobic training is carried out by running to be possibly more beneficial in reducing body fat when compared to endurance cycling which may provide additional explanations for our findings compared to previous investigations.

In addition, the important difference of the present study design compared to combined studies in which endurance and strength training was performed on separate days is that by performing both types of loadings

subsequently in the same training session, the total training frequency is essentially reduced (2-3x 1E+S or 2-3x 1S+E per week = 2-3 total sessions instead of 2-3x 1S + 2-3x per week = 4-6 total sessions). While energy expenditure (as measured by post-exercise oxygen consumption) during exercise increases in proportion to the work performed, it does not return to baseline immediately post-exercise but may remain elevated for a prolonged time (8). Previous studies have shown a dose-response relationship between the duration and magnitude of post-exercise oxygen consumption and the duration and intensity of both endurance and strength loadings performed (8) but very few studies have directly compared the effects of splitting exercise sessions compared to the similar workload performed during only one session. From these studies it, however, appears that performing prolonged endurance cycling (4) may lead to a smaller overall increase in post-exercise oxygen consumption when compared to the same workload performed in two separate exercise sessions. As we decreased the overall training frequency in the present study by combining endurance and strength training into the same training session, it is possible that the overall weekly energy expenditure was lower than that observed during conventional concurrent training programs (i.e. separate day combined training). However, as post-exercise oxygen consumption or energy expenditure were not measured in this study, these speculations remain to be investigated.

Further possible explanations for our findings of no significant reductions in body fat and blood lipids may be related to the present endurance training program. In line with our purpose to provide a moderate volume training program, we limited the duration of each training session to a maximum of 100 min leading to a total of maximal 200 min during weeks 0-12 and 200-300 min during weeks 13-24. As only half of the total training time was performed as endurance cycling, the overall duration and intensity of aerobic training may have not been sufficient (as also observed by the relatively small increases in $\dot{V}O_{2max}$) to result in significant reductions of body fat and changes in blood lipids.

Last, when interpreting the present results one must bear in mind that the subjects of the present study were normal weight, moderately active and healthy males with normal blood lipid levels which in turn provided a relatively small window for adaptations (1). Moreover, the nutritional intake was controlled but not restricted and the analysis of food diaries revealed that the subjects in both training groups maintained their caloric intake constant throughout the 24 weeks of training which may support that no significant changes in fat mass and

blood lipids were observed. However, the observed correlations between the present absolute values of fat mass and body fat % and the relative reductions in these variables observed after 24 weeks of training in all subjects independent of the training group indicate that our training program was especially effective for subjects with an initially high percentage of body fat, suggesting that the present training program may be desirable for overweight or obese populations.

In conclusion, this study demonstrated that both endurance training immediately followed by strength training and the reversed loading order are beneficial in enhancing physical fitness and body composition in healthy moderately active subjects even when the training frequency and volume is moderate. Although no significant reductions in body fat and blood lipids were observed, the significant increases in lean body mass may provide prolonged health benefits with the present training design. However, further studies should compare endurance and strength training combined into the same training session to that performed on separate days by possibly modifying the type and volume of endurance training performed, providing dietary restrictions or including additional populations such as overweight, obese or elderly subjects.

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CONFLICT OF INTERESTS

The authors do not have conflicts of interests and state that the results of the present study do not constitute endorsement by ACSM.

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FIGURE AND TABLE LEGENDS

Table 1. Absolute values of physical fitness and body composition in the two groups at week 0 and 24. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to corresponding value at week 0.

Table 2. Absolute values of blood lipids in the two groups at week 0, 12 and 24. * $p < 0.05$ compared to corresponding value at week 0; ^y $p < 0.05$ between the two groups at corresponding time point.

Fig 1. Relative changes in 1RM strength after 12 and 24 weeks of combined E+S or S+E training. * $p < 0.05$, *** $p < 0.001$; within the bar compared to pre-training values, outside the bar as indicated.

Fig 2. Relative changes in time to exhaustion and maximal aerobic power (W) after 12 and 24 weeks of combined E+S or S+E training. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; within the bar compared to pre-training values, outside the bar as indicated.

Fig 3. Absolute values of muscle cross-sectional area at 30%, 50% and 70% of VL (bars) and relative changes of average VL (line) after 12 and 24 weeks of combined E+S or S+E training. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ within the bar (next to the SD bars in the line diagram) compared to pre-training values, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ compared to week 12.

Fig 4. Relative changes in total, leg and upper body lean mass after 12 and 24 weeks of combined E+S or S+E training. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; within the bar compared to pre-training values, outside the bar as indicated.