# Fitness and Lean Mass Increases during Combined Training Independent of Loading Order 

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#### Abstract

SCHUMANN, M., M. KÜÜSMAA, R. U. NEWTON, A. SIRPARANTA, H. SYVÄOJA, A. HÄKKINEN and K. HÄKKINEN. Fitness and Lean Mass Increases during Combined Training Independent of Loading Order. Med. Sci. Sports Exerc., Vol. 46, No. 9, pp. 1758-1768, 2014. 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 ( $\mathrm{E}+\mathrm{S}$ vs $\mathrm{S}+\mathrm{E}$ ) is rare. This study investigated the effects of moderate frequency $\mathrm{E}+\mathrm{S}$ versus $\mathrm{S}+\mathrm{E}$ training on physical fitness, body composition, and blood lipids. Methods: Physically active and healthy young men performed $\mathrm{E}+\mathrm{S}(n=16)$ or $\mathrm{S}+\mathrm{E}(n=18)$ training $2-3$ times a week for 24 wk. Endurance (by incremental bike test) and strength (by dynamic leg press) performance as well as body composition (by dualenergy x-ray absorptiometry), muscle cross-sectional area of vastus lateralis (by ultrasound), and blood lipid levels were determined before and after the intervention. Results: Time to exhaustion, aerobic power ( W ), and one-repetition maximum strength significantly increased in the two groups at week $24(\mathrm{E}+\mathrm{S}, 12 \%-15 \%, P=0.003-0.001 ; \mathrm{S}+\mathrm{E}, 16 \%-17 \%, P<0.001)$, but no between-group difference was observed. Similarly, the two groups significantly increased total lean mass ( $\mathrm{E}+\mathrm{S}, 3 \%$; $\mathrm{S}+\mathrm{E}, 3 \%$; both $P=0.001$ ) and muscle cross-sectional area $(\mathrm{E}+\mathrm{S}, 14 \%, P=0.001 ; \mathrm{S}+\mathrm{E}, 16 \%, P<0.001)$ at week 24 to a similar extent. No significant changes in body fat or blood lipid levels were observed in either of the two groups at week 24. Conclusions: These results showed that moderatefrequency (2-3 times per week) combined $\mathrm{E}+\mathrm{S}$ or $\mathrm{S}+\mathrm{E}$ training led to significant improvements in physical fitness and lean body mass but did not induce significant changes in body fat or blood lipid levels. Furthermore, because 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


The benefits of combined endurance and strength training for the maintenance and development of physical fitness and body composition have been extensively investigated, and their importance especially for sedentary and

[^0]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 (1), which may lead to decreases in total and abdominal body fat (14) and total cholesterol levels as well as a more positive distribution of LDL-cholesterol (LDL-C) and HDLcholesterol (HDL-C) (38). Whereas endurance training may
directly decrease total body fat and weight, strength training can positively affect body composition via increases in muscle cross-sectional area (CSA) and lean body mass $(14,36)$.

The suggested amount of weekly physical activity commonly ranges from 150 to $300 \mathrm{~min} \cdot \mathrm{wk}^{-1}(14,18)$ of combined aerobic and resistance training. However, research findings have repeatedly emphasized impaired biological adaptations (interference) when high volumes of endurance and strength training are combined over a longer period (40). It seems 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 using a low volume and frequency of training sessions (i.e., 2-3 times per week), on the other hand, may not have inhibitory effects on neuromuscular and morphological adaptations $(24,28)$. A recent study by Fisher et al. (15) has shown that a combined training frequency of aerobic traning once a week and resistance training once a week 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 lipid levels vs increases in muscle size, etc.), intensity and volume of training performed, as well as dietary prescriptions (i.e., diet restriction vs freely chosen diet) and the population studied $(14,23,38)$.

The biological adaptations of concurrent training performed on separate days were previously examined in numerous studies. However, scientific examinations 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 because of the missing recovery when loadings are performed consecutively $(8-10,12,20)$. Because this training method can be considered 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 commit to regular physical activity while allowing sufficient time for other responsibilities.

The purpose of this study was twofold, namely, 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 lipid levels and 2) whether loading orderspecific adaptations (order effect) in these variables can be observed. Consequently, although 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 $(\mathrm{E}+\mathrm{S})$ and strength followed by endurance $(\mathrm{S}+\mathrm{E})$ training performed over 24 wk . We hypothesized that our training volume will induce significant improvements in physical fitness, body composition, and blood lipid levels 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 were 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 three times per week and lack of systematic engagement in any endurance or strength training before inclusion into the study. Subjects were informed about possible risks of all study procedures before giving a written informed consent. A completed health questionnaire and resting ECG were reviewed by a cardiologist before 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. Of the 42 originally recruited subjects, eight did not complete the study or were not included in the data analysis because of a training adherence of less than $90 \%$. The demographic characteristics of all included subjects were as follows: mean $\pm$ SD age, $30 \pm 5 \mathrm{yr}$; height, $179 \pm 6 \mathrm{~cm}$; weight, $78 \pm 11 \mathrm{~kg}$; body mass index (BMI), $24 \pm$ $3 \mathrm{~kg} \cdot \mathrm{~m}^{-2}$. An additional group of subjects undergoing the same prescreening process was recruited for reproducibility tests of the measurement procedures ( $n=21$; age, $30 \pm 6 \mathrm{yr}$; height, $180 \pm 7 \mathrm{~cm}$; weight, $82 \pm 9 \mathrm{~kg}$; BMI, $25 \pm 2 \mathrm{~kg} \cdot \mathrm{~m}^{-2}$ ). 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

After the prescreening, all experimental subjects were assigned to an $\mathrm{E}+\mathrm{S}(n=16)$ or $\mathrm{S}+\mathrm{E}(n=18)$ training group. The subjects performed either endurance immediately followed by strength training $(\mathrm{E}+\mathrm{S})$ or strength immediately followed by endurance training $(\mathrm{S}+\mathrm{E})$ for 24 wk . Because this study 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 ( $\mathrm{E}+\mathrm{S}$ vs $\mathrm{S}+\mathrm{E}$ ) was conducted before 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 were adjusted to the specifics of the subject. Testing of physical fitness, body composition, and blood lipids was then performed on three separate days before the start of the training (week 0). To allow for sufficient recovery, endurance, strength, body composition, and blood tests were separated by at least 48 h of rest. All measurements were repeated after 12 and 24 wk and were performed at the same time of the day within $\pm 1 \mathrm{~h}$ of the timing of baseline measurements. The additional subjects recruited for measurement reproducibility testing were familiarized with the
measurement procedures in the same manner as the intervention groups and were tested both before and after a $12-w k$ 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). After a warm-up (one set of five repetitions at $70 \%$ of estimated 1RM, one set of two repetitions at $80 \%-85 \%$ of estimated 1RM, and one set of one repetition at $90 \%-95 \%$ of estimated 1RM), a maximum of five trials was allowed to obtain a true 1 RM. The device was set up so that the knee angle in the initial flexed position was approximately $60^{\circ}$ (mean $\pm$ $\mathrm{SD}, 58^{\circ} \pm 2^{\circ}$ ), and a successful trial was accepted when the knees were fully extended (approximately $180^{\circ}$ ). The greatest load that the subject could lift to full knee extension at an accuracy of 1.25 kg was accepted as 1 RM . In addition, a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland) was used to determine maximal isometric bilateral leg press force $\left(\mathrm{MVC}_{\text {max }}\right)$. Subjects were seated with a hip and knee angle of $110^{\circ}$ and $107^{\circ}$, respectively, and were instructed to produce maximal force as rapidly as possible on verbal command and to maintain the force plateaued for $3-4 \mathrm{~s}$. At least three trials separated by a rest period of 1 min were conducted, and up to two additional trials were performed if the maximum force during the last trial was greater by $5 \%$ compared with that during the previous attempt. The trial with the highest maximal force measured in newtons was used for statistical analysis. The force signal was lowpass filtered ( 20 Hz ) and analyzed (Signal software version 4.04; Cambridge Electronic Design Ltd., Cambridge, United Kingdom). Rapid force production $\left(\mathrm{MVC}_{500}\right)$ was calculated from the force curve and defined as the average force produced during the first 500 ms of the maximal contraction.

Endurance performance. A graded protocol on a cycle ergometer (Ergometrics 800; Ergoline, Bitz, Germany) was used to determine $\dot{\mathrm{V}}_{2 \text { max }}$. The initial load for all subjects was 50 W and was increased by 25 W every 2 min . 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 frequency (rpm) for more than 15 s . HR was monitored throughout the test (Polar S410; Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 s at each load. 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 $16 \% \mathrm{O}_{2}$ and $4 \% \mathrm{CO}_{2}$. $\dot{\mathrm{V}} \mathrm{O}_{2 \text { max }}$ was accepted when $\dot{\mathrm{V}} \mathrm{O}_{2}$ plateaued despite a further
increase in power and when the RER exceeded 1.05 . $\dot{\mathrm{V}} \mathrm{O}_{2 \text { max }}$ used for statistical analysis was calculated as the highest $\mathrm{V}_{\mathrm{O}}^{2}$ value averaged over 60 s . In addition, maximal aerobic power ( W ) was calculated using the following equation (25): aerobic power $=W_{\text {com }}+25(t / 120)$, where $W_{\text {com }}$ is the load of the last completed stage and $t$ is the time of the last incomplete stage (in seconds) 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 microliters of blood was collected via the 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 (5).

Body composition and venous blood sampling. Anatomical muscle CSA of vastus lateralis (VL) was measured by the extended field of view mode (2) using a B-mode axial plane ultrasound (model SSD-a10; Aloka Co. Ltd., Tokyo, Japan) with a $10-\mathrm{MHz}$ 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 ImageJ software (version 1.44p; National Institutes of Health, Bethesda, MD). 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 dualenergy x-ray absorptiometry (Lunar Prodigy Advance; GE Medical Systems, Madison, WI). To control the experimental conditions, each scan was conducted in the morning after 12 h of fasting. Legs were secured by nonelastic straps at the knee and ankles, and the arms were aligned along the trunk with the palms facing the thighs. All metal objects were removed from the subject before 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 the 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 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 range of interest was then copied to the dualenergy x-ray absorptiometry scans obtained at weeks 12 and

24 , respectively, to assure that analyses were conducted from the same areas at all measurement times.

Venous blood samples were drawn after 12 h of fasting to obtain concentrations of total cholesterol, LDL-C, 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 3500 rpm (Megafure 1.0 R Heraeus; DJB Lab Care, Germany) and immediately analyzed by spectrophotometry (Konelab 20XTi; Thermo Fisher Scientific, Vantaa, Finland). LDL-C concentration was estimated using the Friedewald (17) equation: LDL-C $=$ total cholesterol - HDL-C - (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 min of rest.

During the first 12 wk , the subjects performed according to their corresponding training group two times $1 \mathrm{E}+1 \mathrm{~S}$ or two times $1 \mathrm{~S}+1 \mathrm{E}$ per week. The frequency was then increased during the second 12 wk so that two combined training sessions were performed in every first and fourth week and three combined training sessions in every second and third week (i.e., two times $1 \mathrm{E}+1 \mathrm{~S}$ or two times $1 \mathrm{~S}+1 \mathrm{E}$ or three times $1 \mathrm{E}+1 \mathrm{~S}$ or three times $1 \mathrm{~S}+1 \mathrm{E}$, 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, lowering the loads during the strength loading, and 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 HR (Polar S410; Polar Electro Oy, Kempele, Finland) associated with subject's individual aerobic and anaerobic threshold determined during measurements at weeks 0 and 12, respectively. Subjects were instructed to maintain a constant
pedaling frequency of 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 highintensity interval sessions (below and above the anaerobic threshold) were incorporated into the training program. The duration of endurance cycling progressively increased throughout the 12 wk of training from 30 to 50 min . During the second $12-\mathrm{wk}$ period, the major endurance program structure was maintained whereas both training volume and intensity were further increased. The aerobic threshold represented intensities ( $\% \mathrm{HR}_{\text {max }}$ ) of $65 \% \pm 5 \%$ and $67 \% \pm 6 \%$ in $\mathrm{E}+\mathrm{S}$ and $68 \% \pm 8 \%$ and $67 \% \pm 6 \%$ in $\mathrm{S}+\mathrm{E}$ at weeks 0 and 12 , respectively. The anaerobic threshold represented intensities of $85 \% \pm 5 \%$ and $86 \% \pm 5 \%$ in $\mathrm{E}+\mathrm{S}$ and $82 \% \pm 8 \%$ and $86 \% \pm 5 \%$ in $\mathrm{S}+\mathrm{E}$ at weeks 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 -wk periods. Exercises for the lower body were bilateral dynamic leg press and 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, lat pulldown, and exercises commonly used to improve trunk stability (crunches, torso rotation, and lower back extension). During the first 2 wk , training was performed as a circuit using 2-4 sets of 15-20 repetitions at an intensity of $40 \%-60 \%$ of 1 RM. Thereafter, protocols aiming for muscle hypertrophy ( $2-5$ sets of $8-10$ repetitions at $80 \%-85 \%$ of $1 \mathrm{RM}, 1.5-$ to $2-\mathrm{min}$ interset rest) and maximal strength (2-5 sets of $3-5$ repetitions at $85 \%-95 \%$ of 1RM, 3- to 4-min interset rest) and, during the last 2 wk , protocols targeting explosive strength (two sets of 8-10 repetitions at $40 \%$ of 1 RM with maximal velocity, 3- to 4 -min interset rest) were performed. During the second $12-\mathrm{wk}$ period, the major strength program structure was maintained whereas both training volume and frequency were slightly increased 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 $30-50 \mathrm{~min}$, resulting in a total duration of approximately $60-100 \mathrm{~min}$ for each combined training session (i.e., $\mathrm{E}+\mathrm{S}$ and $\mathrm{S}+\mathrm{E}$, respectively).

Dietary intake. To control nutritional intake, food diaries were collected for 3 d including one weekend day at weeks 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 a nutrient analysis software (Nutri-Flow; Flow-team Oy, Oulu, 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 before the start of each test and were asked to maintain similar nutritional intake before the measurements at weeks 0,12 , and 24 . During each training

|  | Week 0 |  | Week 24 |  |
| :---: | :---: | :---: | :---: | :---: |
|  | E+S | S+E | E+S | S+E |
| Physical fitness |  |  |  |  |
| Dynamic leg press 1RM (kg) | $157 \pm 30$ | $143 \pm 23$ | $175 \pm 27^{*}$ | $166 \pm 20^{*}$ |
| Isometric leg press MVC max $^{(N)}$ | $2648 \pm 689$ | $2338 \pm 540$ | $2881 \pm 725^{* *}$ | $2603 \pm 563$ ** |
| Isometric leg press MVC500 ( N ) | $1828 \pm 9845$ | $1616 \pm 315$ | $1970 \pm 526$ | $1808 \pm 304^{* *}$ |
| Time to exhaustion (min) | $19 \pm 3$ | $18 \pm 3$ | $22 \pm 3^{*}$ | $21 \pm 3^{*}$ |
| Aerobic power (W) | $268 \pm 40$ | $245 \pm 35$ | $300 \pm 38$ * | $284 \pm 37^{*}$ |
| $\dot{\mathrm{V}} \mathrm{O}_{2 \text { max }}\left(\mathrm{mL} \cdot \mathrm{kg}^{-1} \cdot \mathrm{~min}^{-1}\right)$ | $42 \pm 7$ | $43 \pm 7$ | $45 \pm 5^{* *}$ | $46 \pm 7^{* * *}$ |
| Body composition |  |  |  |  |
| Body weight (kg) | $80 \pm 12$ | $75 \pm 9$ | $80 \pm 10$ | $76 \pm 9^{* *}$ |
| BMI (kg.m ${ }^{-2}$ ) | $25 \pm 3$ | $23.5 \pm 2$ | $25 \pm 3$ | $23.9 \pm 2^{* *}$ |
| Body fat (\%) | $23 \pm 8$ | $21 \pm 5$ | $21 \pm 7$ | $20 \pm 5$ |
| Total fat mass (g) | $19,095 \pm 9217$ | 15,733 $\pm 5456$ | 17,104 $\pm 7415$ | 15,266 $\pm 5382$ |
| Abdominal fat mass (g) | $2484 \pm 1414$ | $1998 \pm 909$ | $2166 \pm 1092$ | $1932 \pm 943$ |
| Total lean mass (g) | $57,963 \pm 5116$ | $56,406 \pm 4942$ | $59,739 \pm 5673$ * | 58,062 $\pm 4726^{*}$ |
| Upper body lean mass (g) | $28,878 \pm 2798$ | $28,453 \pm 2705$ | 29,616 $\pm 3035^{* * *}$ | 28,999 $\pm 2291$ ** |
| Leg lean mass (g) | $29,085 \pm 2768$ | $27,953 \pm 2491$ | 30,123 $\pm 2978 *$ | 29,063 $\pm 2701$ * |
| Average CSA VL ( $\mathrm{cm}^{2}$ ) | $21 \pm 3$ | $21 \pm 3$ | $24 \pm 4^{*}$ | $24 \pm 3^{*}$ |

${ }^{*} P<0.001$, ${ }^{* *} P<0.05$, and ${ }^{* * *} P<0.01$ compared with corresponding value at week 0 .
session, a standardized low dose of glucose (according to body weight, 2-4 tablets, each containing 2.1 g of glucose) was provided at the midpoint of each combined session (after E or S, respectively) whereas 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). All results are presented as absolute values (Tables 1 and 2), and relative changes from week 0 , as means $\pm$ 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 $d$. Partial correlations with adjustment for groups were performed to determine relations between dependent variables across all experimental subjects. Measures of reliability are presented as intraclass correlation coefficients (ICC) of absolute agreement for single measures. Significance for all tests
was defined as $P=0.05$, whereas values $<0.06$ were accepted as a significant trend.

## RESULTS

The training adherence was $99 \% \pm 2 \%$ in both the $\mathrm{E}+\mathrm{S}$ and $\mathrm{S}+\mathrm{E}$ training groups. 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 ICC 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 weeks 0,12 , and 24 were $9.3 \pm 1.8 \mathrm{MJ}, 10.2 \pm 2.6 \mathrm{MJ}$, and $9.5 \pm 2.6 \mathrm{MJ}$ in $\mathrm{E}+\mathrm{S}$ and $9.4 \pm 2.0 \mathrm{MJ}, 9.3 \pm 1.7 \mathrm{MJ}$, and $7.9 \pm 1.7 \mathrm{MJ}$ in $\mathrm{S}+\mathrm{E}$. The average nutritional intake as percentage of total energy for CHO , fat, and protein were $42 \%-45 \%, 31 \%-36 \%$, and $17 \%-19 \%$ in $\mathrm{E}+\mathrm{S}$ and $42 \%-44 \%, 33 \%-36 \%$, and $18 \%$ in

TABLE 2. Absolute values of blood lipid levels in the two groups at weeks 0,12 , and 24 .

| Group | Week 0 | Week 12 | Week 24 | $\begin{gathered} \text { Weeks 0-12 } \\ (\Delta \mathrm{mM}) \end{gathered}$ | Weeks 13-24 <br> ( $\Delta \mathrm{mM}$ ) | Within-Group ES, Weeks 0-24 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Total Cholesterol (mM) |  |  |  |  |  |  |
| E+S | $4.8 \pm 0.9$ | $4.6 \pm 0.8$ | $4.6 \pm 1.0$ | $-0.3 \pm 0.5$ | $-0.2 \pm 0.5$ | 0.210 |
| S+E | $4.6 \pm 0.8$ | $4.6 \pm 0.6$ | $4.6 \pm 0.6$ | $0.0 \pm 0.6$ | $0.0 \pm 0.3$ | 0.000 |
| LDL-C (mM) |  |  |  |  |  |  |
| E+S | $2.8 \pm 0.9$ | $2.5 \pm 0.7$ | $2.7 \pm 0.9$ | $-0.3 \pm 0.4$ | $-0.1 \pm 0.3$ | 0.111 |
| S+E | $2.7 \pm 0.6$ | $2.8 \pm 0.7^{*}$ | $2.7 \pm 0.5$ | $0.1 \pm 0.4$ | $0.0 \pm 0.4$ | 0.000 |
| HDL-C (mM) |  |  |  |  |  |  |
| E+S | $1.6 \pm 0.4$ | $1.6 \pm 0.5$ | $1.5 \pm 0.4$ | $0.0 \pm 0.2$ | $-0.1 \pm 0.2$ | 0.250 |
| S+E | $1.4 \pm 0.3$ | $1.3 \pm 0.3^{* *}$ | $1.4 \pm 0.3$ | $-0.1 \pm 0.1$ | $0.0 \pm 0.1$ | 0.000 |
| Triglycerides (mM) |  |  |  |  |  |  |
| E+S | $1.0 \pm 0.3$ | $1.0 \pm 0.3$ | $0.8 \pm 0.3$ | $0.0 \pm 0.2$ | $-0.1 \pm 0.3$ | 0.666 |
| S+E | $1.4 \pm 1.0$ | $1.2 \pm 0.8$ | $1.1 \pm 0.5$ | $-0.2 \pm 0.6$ | $-0.2 \pm 0.6$ | 0.379 |

${ }^{*} P<0.05$ between the two groups at corresponding time point.
${ }^{* *} P<0.05$ compared with corresponding value at week 0 .


FIGURE 1—Relative changes in 1RM strength after 12 and 24 wk of combined E+S or S+E training. ${ }^{*} P<0.05, * * * P<0.001$, within the bar compared with pretraining values, outside the bar as indicated.

S + E throughout the 24 wk of training. No significant within- or between-group differences were observed.

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

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

Body composition. Absolute values of body composition measures at weeks 0 and 24 are presented in Table 1. A significant increase in body weight and BMI was observed in $\mathrm{S}+\mathrm{E}$ only $(1.7 \% \pm 2.4 \%$ and $1.7 \% \pm 2.6 \%$ at weeks 12 and 24 , respectively, $P<0.05$ ). No significant changes in body fat percentage, 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 VL (Fig. 3). Both groups significantly improved average CSA of VL at weeks $12(\mathrm{E}+\mathrm{S}, 8 \% \pm 7 \%, P=0.002, \mathrm{ES}=0.490 ; \mathrm{S}+\mathrm{E}$, $9 \% \pm 7 \%, P<0.001, \mathrm{ES}=0.643)$ and $24(\mathrm{E}+\mathrm{S}, 14 \% \pm$ $7 \%, P=0.001, \mathrm{ES}=0.822 ; \mathrm{S}+\mathrm{E}, 16 \% \pm 8 \%, P<0.001$,


FIGURE 2-Relative changes in time to exhaustion and maximal aerobic power (W) after 12 and 24 wk of combined $E+S$ or $S+E$ training. * $P<0.05$, ${ }^{* *} P<0.01, * * * P<0.001$, within the bar compared with pretraining values, outside the bar as indicated.


FIGURE 3-Absolute values of muscle CSA at $\mathbf{3 0 \%}, \mathbf{5 0 \%}$, and $70 \%$ of VL (bars) and relative changes of average VL CSA (line) after 12 and 24 wk of combined $\mathrm{E}+\mathrm{S}$ or $\mathrm{S}+\mathrm{E}$ training. $* P<0.05,{ }^{* *} P<0.01, * * * P<0.001$ within the bar (next to the SD bars in the line diagram) compared with pretraining values; $\dagger P<0.05, \dagger \dagger P<0.01, \dagger \dagger \dagger P<0.001$ compared with week 12 .
$\mathrm{ES}=1.178)$, whereby the increase from week 12 to week 24 was significant (both groups, $P<0.001$ ).

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 weeks $12(\mathrm{E}+\mathrm{S}, 2 \% \pm 3 \%, P=0.042$, $\mathrm{ES}=0.203$; $\mathrm{S}+\mathrm{E}, 3 \% \pm$ $2 \%, P<0.001, \mathrm{ES}=0.310)$ and $24(\mathrm{E}+\mathrm{S}, 3 \% \pm 3 \%, P=$ $0.001, \mathrm{ES}=0.329 ; \mathrm{S}+\mathrm{E}, 3 \% \pm 2 \%, P=0.001, \mathrm{ES}=0.342$ ). Similarly, both groups increased upper body lean mass (Fig. 4b) at weeks 12 (significant in $\mathrm{S}+\mathrm{E}$ only, $2 \% \pm 3 \%$,
$P=0.022, \mathrm{ES}=0.212)$ and $24(\mathrm{E}+\mathrm{S}, 3 \% \pm 3 \% P=0.005$, $\mathrm{ES}=0.253 ; \mathrm{S}+\mathrm{E}, 2 \% \pm 3 \%, P=0.025, \mathrm{ES}=0.218)$ and leg lean mass (Fig. 4c) both at weeks 12 ( $\mathrm{E}+\mathrm{S}, 2 \% \pm 3 \%$, $P=0.024, \mathrm{ES}=0.210 ; \mathrm{S}+\mathrm{E}, 3 \% \pm 2 \%, P<0.001, \mathrm{ES}=$ 0.373 ) and $24(\mathrm{E}+\mathrm{S}, 4 \% \pm 3 \%, P<0.001, \mathrm{ES}=0.361 ; \mathrm{S}+\mathrm{E}$, $4 \% \pm 2 \%, P<0.001, \mathrm{ES}=0.427$ ). The increase in leg lean mass from week 12 to week 24 was significant in $\mathrm{E}+\mathrm{S}$ only ( $P<0.05$ ). No significant between-group differences for the measures of body composition were obtained.

Blood lipids. Only minor changes in total cholesterol, HDL-C, LDL-C, and triglyceride levels were observed after

24 wk of training（Table 2）．A significant between－group difference was observed for LDL－C levels at week 12 （ $P<0.05$ ）but was diminished after 24 wk of training．

Correlations of physical fitness and body com－ position across all experimental subjects．All abso－ lute values of physical fitness at baseline $\left(1 \mathrm{RM}, \mathrm{MVC}_{\max }\right.$ ， $\mathrm{MVC}_{500}$ ，aerobic power，time to exhaustion，and $\dot{\mathrm{V}} \mathrm{O}_{2 \max }$ ） were significantly correlated with the corresponding relative changes obtained at weeks 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 percentage at baseline and the relative change in body fat percentage（ $r=-0.450$ ， $P=0.006$ ），for the absolute values of total fat at baseline and the corresponding relative change（ $r=-0.364, P=0.037$ ）， and for total fat and abdominal fat mass at baseline and the relative change in body fat percentage（ $r=-0.458, P=0.006$ ； $r=0.431, P=0.006$ ，respectively）．In addition，absolute values of 1RM strength at baseline were significantly corre－ lated with relative changes in body fat percentage and relative changes of total and abdominal fat mass obtained at weeks 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 lipid levels 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 per week）endur－ ance and strength training combined into the same training session（ $\mathrm{E}+\mathrm{S}$ vs $\mathrm{S}+\mathrm{E}$ ）on physical fitness，body composi－ tion，and blood lipid levels in moderately active and healthy young men．This study showed that both training orders（ $\mathrm{E}+\mathrm{S}$ and $\mathrm{S}+\mathrm{E}$ ）led to significant increases in muscular and cardio－ respiratory fitness，muscle CSA，and lean body mass after 12 and 24 wk of training，but no reductions in total body or abdominal fat mass，body fat percentage，or blood lipid levels 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 with 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 second loading performed to be adversely affected by fatigue induced by the first load－ ing．In recent studies，these adverse effects were reflected by increased work economy when endurance loading was performed immediately after a strength loading（13）and re－ duced neuromuscular performance measured immediately after 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 en－ durance 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 $\mathrm{E}+\mathrm{S}$ and $\mathrm{S}+\mathrm{E}$ training groups significantly improved physical fitness，as reflected in 1RM strength $(12 \%-17 \%)$, MVC $_{\max }(10 \%-13 \%)$ ，time to exhaus－ tion $(15 \%-17 \%)$ ，aerobic power（ $13 \%-16 \%$ ），and $\mathrm{VO}_{2 \max }$ （7\％），to a similar extent and no between－group differences were observed．Our findings are in line with results of Collins and Snow（12）and Chtara et al．（9）who also re－ ported that either loading order was similarly effective in improving endurance and strength performance after pro－ longed combined $\mathrm{E}+\mathrm{S}$ or $\mathrm{S}+\mathrm{E}$ training．However，other studies have found limited increases in $\dot{\mathrm{VO}}_{2 \text { max }}$ after the $E+S$ order in women（20）or $S+E$ order in men（10）and impaired strength adaptations after $\mathrm{E}+\mathrm{S}$ training in older men（8）when compared with the reverse loading order． Despite these findings of studies combining endurance and strength training into the same training session and those that report diverse biological adaptations induced by endur－ ance 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 sin－ gle isometric contraction $(32,33)$ ，suggesting similar acute neural responses to both types of loadings．Furthermore，pre－ vious 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 train－ ing．Therefore，the present endurance cycling combined with the hypertrophic and maximal strength training pro－ tocols may have 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．（40） who revealed that endurance running may be more detri－ mental to strength adaptations when compared with endur－ ance 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 CSA and leg lean mass in all subjects across the two training groups． Both training groups significantly increased muscle CSA after the 24 wk of training，independent of the loading or－ der．Although animal studies have shown that endurance and strength training might induce distinct genetic and
molecular pathways critical for muscle hypertrophy $(4,22)$, other studies of human subjects have indicated the cumulative effect of both loadings to possibly compromise beneficial morphological adaptations $(11,22)$. Coffey et al. (11) 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. Although the magnitude of interference when compared with 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 wk of training, independent of loading order. Muscle strength and possibly muscle mass have been associated with reduced mortality even in young subjects (30). Because 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 $(14,36)$. Our findings are thus of great importance because 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 percentage and total or abdominal fat mass in either training group. Furthermore, no significant changes in total cholesterol, HDL-C and LDL-C, or triglyceride levels were observed. Previous studies have shown a strong association between body fat and blood lipid levels (6), indicating that a reduction in fat mass positively correlates to changes in blood lipid levels. Typically, aerobic exercise has been considered as being most effective to induce reductions in fat oxidation during and in the hours after an exercise loading $(14,38)$, whereas 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 in old women (15). Therefore, our results may indicate endurance and strength training combined into the same training session to be less favorable for reductions in body fat and blood lipid levels 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. (1) showed that running induces higher rates of fat oxidation when compared with those in cycling, and a meta-analysis by Wilson et al. (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 with endurance cycling, which may provide additional explanations for our findings compared with those of previous investigations.

In addition, the important difference of the present study design compared with 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 $(2-3 \times 1 \mathrm{E}+\mathrm{S}$ or $2-3 \times 1 \mathrm{~S}+$ E per week $=$ $2-3$ total sessions instead of $2-3 \times 1 \mathrm{~S}+2-3 \times 1$ E per week $=$ 4-6 total sessions). Although energy expenditure (as measured by postexercise oxygen consumption) during exercise increases in proportion to the work performed, it does not return to baseline immediately postexercise but may remain elevated for a prolonged time (7). Previous studies have shown a dose-response relation between the duration and magnitude of postexercise oxygen consumption and the duration and intensity of both endurance and strength loadings performed (7), but very few studies have directly compared the effects of splitting exercise sessions with the similar workload performed during only one session. From these studies, however, it seems that performing prolonged endurance cycling (3) may lead to a smaller overall increase in postexercise oxygen consumption when compared with the same workload performed in two separate exercise sessions. Because we decreased the overall training frequency in the present study by combining endurance and strength training into the same training session, the overall weekly energy expenditure may have been lower than that observed during conventional concurrent training programs (i.e., separate-day combined training). However, because postexercise oxygen consumption or energy expenditure was 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 lipid levels 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. Because only half of the total training time was performed as endurance cycling, the overall duration and intensity of aerobic training may not have been sufficient (as also observed by the relatively small increases in $\left.\dot{\mathrm{V}} \mathrm{O}_{2 \text { max }}\right)$ to result in significant reductions of body fat and changes in blood lipid levels.

Lastly, when interpreting the present results, one must bear in mind that the subjects of the present study were normalweight, moderately active, and healthy males with normal blood lipid levels, which in turn provided a relatively small window for adaptations (14). 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 wk of training, which may support the finding 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 percentage and the relative reductions in these variables observed after 24 wk 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 are 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 with 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.

The authors express their gratitude to the technical staff involved in the data collection and would like to acknowledge the subjects who made this data collection possible.

The funding for this study has been provided by the Finnish Ministry of Education and Culture.

The authors do not have conflicts of interests.
The results of the present study do not constitute endorsement by the American College of Sports Medicine.
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    Submitted for publication November 2013.
    Accepted for publication February 2014.
    0195-9131/14/4609-1758/0
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    DOI: 10.1249/MSS. 0000000000000303

