Four weeks of speed endurance training reduces energy expenditure during exercise and maintains muscle oxidative capacity despite a reduction in training volume

F. Marcello Iaia,[1,2] Ylva Hellsten,[1] Jens Jung Nielsen,[1] Maria Fernström,[3,4] Kent Sahlin,[3,4] and Jens Bangsbo[1]

[1]*Copenhagen Muscle Research Centre, Department of Exercise and Sport Sciences, Section of Human Physiology, University of Copenhagen, Copenhagen, Denmark;* [2]*Faculty of Exercise Sciences, State University of Milan, Milan, Italy;* [3]*Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; and* [4]*Department of Sports and Health Science, Stockholm University College of Physical Education and Sports, Stockholm, Sweden*

Iaia FM, Hellsten Y, Nielsen JJ, Fernström M, Sahlin K, Bangsbo J. Four weeks of speed endurance training reduces energy expenditure during exercise and maintains muscle oxidative capacity despite a reduction in training volume. *J Appl Physiol* 106: 73–80, 2009. First published October 9, 2008; doi:10.1152/japplphysiol.90676.2008.—We studied the effect of an alteration from regular endurance to speed endurance training on muscle oxidative capacity, capillarization, as well as energy expenditure during submaximal exercise and its relationship to mitochondrial uncoupling protein 3 (UCP3) in humans. Seventeen endurance-trained runners were assigned to either a speed endurance training (SET; $n = 9$) or a control (Con; $n = 8$) group. For a 4-wk intervention (IT) period, SET replaced the ordinary training ($\sim$45 km/wk) with frequent high-intensity sessions each consisting of 8–12 30-s sprint runs separated by 3 min of rest (5.7 $\pm$ 0.1 km/wk) with additional 9.9 $\pm$ 0.3 km/wk at low running speed, whereas Con continued the endurance training. After the IT period, oxygen uptake was 6.6, 7.6, 5.7, and 6.4% lower ($P < 0.05$) at running speeds of 11, 13, 14.5, and 16 km/h, respectively, in SET, whereas remained the same in Con. No changes in blood lactate during submaximal running were observed. After the IT period, the protein expression of skeletal muscle UCP3 tended to be higher in SET (34 $\pm$ 6 vs. 47 $\pm$ 7 arbitrary units; $P = 0.06$). Activity of muscle citrate synthase and 3-hydroxyacyl-CoA dehydrogenase, as well as maximal oxygen uptake and 10-km performance time, remained unaltered in both groups. In SET, the capillary-to-fiber ratio was the same before and after the IT period. The present study showed that speed endurance training reduces energy expenditure during submaximal exercise, which is not mediated by lowered mitochondrial UCP3 expression. Furthermore, speed endurance training can maintain muscle oxidative capacity, capillarization, and endurance performance in already trained individuals despite significant reduction in the amount of training.

uncoupling protein 3; citrate synthase; 3-hydroxyacyl-CoA dehydrogenase; oxygen uptake; fiber type distribution

RUNNING ECONOMY, DEFINED AS the energy used at a given submaximal running speed, has been reported to be an important component of endurance performance (9, 39, 51). In already trained subjects, running economy has been shown to increase after a period of interval (2, 19, 23, 57), plyometric (42, 52, 59, 62), and strength (27, 37) training. Such adaptations are likely ascribed to changes in a number of physiological and biomechanical parameters (51, 64), including reduced ventilatory demands (19), greater muscular power generation (41), as well as improved musculotendinous stiffness (59) and shorter ground contact times (40). Although the energy cost of running has been widely investigated the effect of high-intensity intermittent training, i.e., speed endurance training, on energy expenditure during exercise is still unclear and some of the underlying mechanisms remain poorly understood.

One of the potential candidates of training-induced improvements in running economy is an increased mitochondrial efficiency (i.e., increased ATP/O_2) due to reduced uncoupled respiration. The mitochondrial uncoupling protein 3 (UCP3) has, among other things, been suggested to be involved in thermogenesis by mediating mitochondrial proton leak, and thus energy is dissipated as heat instead of being converted to ATP (4, 22). An improved running economy may therefore be related to reduced levels of UCP3. However, the role of UCP3 for mitochondrial efficiency has recently been questioned (53), and additional information from in vivo human experiments is required. Cross-sectional studies have shown that, compared with untrained people, endurance-trained subjects exhibit lower muscle UCP3 expression both at mRNA (48, 55) and protein levels (38, 49, 50), which has been associated with a greater mechanical efficiency. Similarly, longitudinal studies dealing with previously untrained subjects have reported lowered UCP3 protein abundance after a period of endurance training (17, 49, 54). Also, speed endurance training has been shown to downregulate UCP3 expression in skeletal muscle (49). However, because the subjects in the latter study were not performing regular training, the physiological response obtained may have been an effect of the increase in the amount of physical activity rather than an effect of speed endurance training specifically. Thus it is unclear how a period of speed endurance training in already trained subjects would affect UCP3 expression and whether this is related to change in running economy or energy expenditure during exercise.

Over the past decades, training at supramaximal exercise intensities has received much attention (30, 47). High-intensity intermittent training in habitually active subjects has been found to improve endurance capacity as well as several molecular and cellular markers of muscle oxidative potential (6, 7, 20), including capillarization (26). Some studies have also

examined the effect of adding high-intensity intermittent to the regular endurance training on performance of endurance-athletes. Laursen et al. (32) and Stepto et al. (60) observed a lower time (2–4%) to complete a simulated-laboratory 40-km time trial when well-trained cyclists carried out a 3- to 4-wk training program including sessions of intense intermittent cycling exercise (12 × 30 s at 175% of the intensity corresponding to peak aerobic power output). However, in these studies the participants maintained a great portion of their weekly aerobic training volume, and only performance aspects were investigated. Thus the aim of the present study was to test the hypothesis that an alteration from regular endurance to speed endurance training would result in reduced energy expenditure and in lowered UCP3 protein expression in skeletal muscle. Furthermore, it was examined whether, in already trained runners, a reduced training volume but increased training intensity would maintain, or even improve, muscle oxidative enzyme activity, capillarization, and endurance performance.

METHODS

Subjects

Seventeen moderately endurance-trained male runners [age, 33.9 ± 1.5 (mean ± SE) yr; height, 180.1 ± 1.9 cm; body mass, 72.9 ± 2.4 kg; and maximum oxygen uptake ($\dot{V}_{O_{2max}}$), 55.5 ± 1.4 ml·kg^{-1}·min^{-1}] with a minimum of 4 yr of running experience participated in the study. The participants were fully informed of any possible risks and discomforts associated with the experimental procedures before giving their written informed consent to participate. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg communities according to code of Ethics of the World Medical Association (Declaration of Helsinki).

Experimental Design

A parallel, two-group, longitudinal (pretraining, posttraining), blinded design was used. Subjects were matched and randomly assigned to either an experimental speed endurance training group (SET; $n = 9$) or a control group (Con; $n = 8$). The study was carried out in the last phase of the competitive season (November to December) and consisted of *1*) pretraining testing, *2*) a 4-wk intervention training (IT) period, and *3*) posttraining testing.

Training Intervention

Before the study, all subjects were regularly performing moderate-intensity exercise three to five times per week for a total weekly distance of ~45 km. No intense intermittent training was carried out during the 3 mo preceding this intervention. During the IT period, SET replaced their endurance training (205.8 ± 19.3 min/wk) with sessions of high-intensity intermittent exercise, i.e., speed endurance training, each consisting of 8–12 running bouts of 30-s duration interspersed by 3 min of rest. The training intensity was 22.4 ± 0.4 km/h, corresponding to 93 ± 0.5% of the speed achieved in a 30-s "all-out" sprint run, and it was modified accordingly over the course of the IT period. The subjects trained on alternate days, 3.4 ± 0.1 times a week. In each training session, subjects performed additional 15.4 ± 0.2 min of warm-up and recovery activities at a speed of 11.3 ± 0.3 km/h (9.9 ± 0.3 km/wk). The training sessions were carried out on a 400-m running track and were carefully supervised. During the IT period, SET performed no other exercise training aside from the one prescribed. Con continued its sessions (52.3 ± 2.4 min) of aerobic moderate-intensity training (13.0 ± 0.4 km/h) 4.0 ± 0.4 times a week, for a total distance run per day and week of 11.3 ± 0.5 and 45.2 ± 5.1 km, respectively. Con completed daily training diaries which were analyzed at the end of each week. Heart rate was recorded

(Polar S610 heart rate monitor, Polar Electro Oy, Kempele, Finland) in both groups during all training sessions. To reduce the interference of uncontrolled variables, all subjects were instructed to maintain their habitual lifestyle and normal dietary intake before and during the study.

Pre- and Posttraining Testing

Before and after the 4-wk IT period, the participants underwent a resting muscle biopsy procedure ~48 h after the last training session. On the same days, they performed a treadmill test. One week later they completed a 10,000-m running time trial (10 km). Between the two tests, subjects performed additional training sessions. The participants were familiarized to treadmill running, and they had experience with 10-km racing on the track.

Details of Experimental Procedures

On the day of testing, the participants reported to the laboratory or to the running track 3 h after consuming a light meal. Subjects did not consume beverages containing caffeine in the experimental days and refrained from physical activity and alcohol consumption 48 h before testing. To minimize diet-induced changes in physiological variables, subjects were also requested to record their individual food intake in the 48 h preceding the pretraining tests and to replicate their dietary pattern before the posttraining testing. During the test trials, subjects had a Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland) fitted around the chest for continuous heart rate recordings. Heart rate was collected at 5-s intervals.

Muscle biopsy. In preparation for the muscle biopsy a small incision (0.5–1 cm) through the skin and fascia over the medial part of vastus lateralis muscle was made under local anesthesia (1 ml; 20 mg/l lidocaine without epinephrine) and was afterward secured with sterile reinforced skin closure (Steri-Strip, 3M Health Care, St. Paul, MN). The subject's left or right leg was randomly selected. The same leg was used for pretraining and posttraining IT period biopsies. A muscle sample (~100 mg) was obtained using the needle biopsy technique with suction (1). The muscle tissue was immediately frozen in liquid nitrogen and stored at −80°C.

Treadmill test. Before the test, subjects had catheter (18 gauge, 32 mm) inserted in the medial antecubital vein, covered by a wrist bandage. The test was performed on a motorized treadmill under standard laboratory conditions (20°C; 40% relative humidity). The treadmill calibration was checked before each trial. The first part of the test consisted of four 6-min running stages at 11, 13, 14.5, and 16 km/h, respectively, separated by 2-min rest periods. Then, 10 min after the cessation of the submaximal part of the test, subjects carried out a $\dot{V}_{O_{2max}}$ test consisting of 1 km/h increments every minute until volitional exhaustion. Pulmonary $\dot{V}_{O_2}$ was measured throughout the protocol by a breath-by-breath gas analyzing system (MedGraphics CPX/D, St. Paul, MN). The analyzer was calibrated before each test with two gases of known oxygen and carbon dioxide concentrations as well as by the use of a 3-liter syringe for the tube flowmeter calibration (44). $\dot{V}_{O_2}$ was determined as the highest value achieved over a 20-s period. A plateau in $\dot{V}_{O_2}$, despite an increased speed, and a respiratory exchange ratio (RER) >1.15 were used as criteria for $\dot{V}_{O_{2max}}$ achievement. Blood samples were collected at rest and at the end of each exercise bout using 2-ml heparinized syringes.

The 10-km running time trial. The 10-km running time trial was carried out on a 400-m running track after 15 min of standardized warm-up. To avoid the effect of racing tactics and strategies, the test was conducted on individual basis with participants starting in random order at 1-min intervals. During the trial, subjects received information regarding the number of laps to go. Performance times were subsequently verified using video recordings.

Blood Analysis

Immediately after sampling, a part of the blood (100 μl) was hemolyzed using a 1:1 dilution with a buffer solution (Yellow Spring Instruments, Yellow Springs, OH) to which 20 g/l Triton X-100 was added (18), for analysis of lactate concentration ([La$^-$]) (YSI lactate analyzer, model 23, Yellow Spring Instruments).

Muscle Analysis

Mitochondrial UCP3. Portions of freeze-dried muscle were cleaned from blood, fat, and connective tissue, and they were homogenized in cold lysis buffer (2 mM HEPES 1 mM EDTA, 5 mM EGTA, 10 mM MgCl$_2$, 50 mM β glycerol phosphate, 1 mM NaVO$_4$, 2 mM dithiothreitol, 1% Triton X-100, and protease inhibitors, pH 7.4). Protein concentration was determined in muscle homogenate using Pierce protein assay (kit no. 23223). Muscle homogenate was solubilized in Laemmli sample buffer and denatured by boiling. One hundred micrograms of protein was added per lane on 12% polyacrylamide gels and separated by sodium dodecyl sulfate-polyacrylamide gel for 60 min at 135 V. The separated polypeptides were transferred to a polyvinylidene difluoride membrane at 10 V for 60 min, and blocked in Tris-buffered saline with 5% nonfat milk. Membranes were incubated overnight with polyclonal antibody against UCP3 (no. 3046, Chemicon), diluted 1:1,000, washed and incubated with secondary antibody goat anti-rabbit (IgG-horseradish peroxidase; no. sc-2030, Santa Cruz). The membrane was again washed and incubated with chemiluminescence detection reagent enhanced chemiluminescence (no. RPN 2106, Amersham). Finally, an X-ray film was exposed to the membrane for 10 min. The optical density of the bands was quantified by using Quantity One 1-D Analysing software (Bio-Rad). Samples were analyzed, and the density of the bands were related to a standard sample loaded on the same gel.

Mitochondrial enzymes. For the determination of enzymatic activity, a portion of the muscle biopsy sample was freeze-dried, and all connective tissue, visible fat, and blood were carefully dissected away under a stereomicroscope in a room with a temperature of 18°C and a relative humidity below 30%. About 2 mg dry wt of muscle tissue was homogenized (1:400) in a 0.3 M phosphate buffer adjusted to pH 7.7 containing 0.5 mg/ml of bovine serum albumin. Citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) maximal activities were determined by using fluorometric methods with NAD-NADH coupled reactions (34).

Muscle fiber type distribution. Immediately after extraction from the leg, a portion of the muscle tissue was mounted in an embedded medium (OCT Compound Tissue-Tek, Sakura Finetek, Zoeterwoude, The Netherlands) and frozen in isopentane that was cooled to the freezing point in liquid nitrogen. The samples were stored at −80°C until analyzed for fiber type distribution and capillarization. Serial 10-μm-thick sections were cut at −20°C and incubated for myofibrillar ATPase reactions at pH 9.4, after preincubation at pH 4.3, 4.6, and 10.3 (5). Based on the myofibrillar ATP staining, slow-twitch (ST) and fast-twitchs a, x, and c (FTa, FTx, and FTc) fiber types were defined under light microscopy. The number of fibers was determined using the software program Tema version 1.04 (CheckVision).

Muscle capillarization. Staining of capillaries was performed on 8-μm-thick transverse sections of frozen skeletal muscle samples. The sections were fixed in 2% formaldehyde for 2 min at room temperature and −20°C acetone for 30 s. The sections were rinsed with PBS containing 1% BSA and thereafter blocked with 1% PBS containing BSA. The sections were then incubated for 1 h at room temperature with primary monoclonal antibody, mouse α-CD31 (50 μg/ml; Clone JC70A, DAKO, Glostrup, Denmark) for detection of endothelial cells. The sections were rinsed and thereafter incubated with a biotin-coupled rabbit α-mouse antibody (E0354, DAKO). Antibody binding was visualized with ABC complex with alkaline phosphatase (ABC/Complex/AP, DAKO). Negative controls were achieved with staining without the primary antibody. Immunoreactive cells were examined in a Zeiss Axioplan Microscope. The number of capillaries was determined using the software program Tema version 1.04 (CheckVision).

Calculations

Energy expenditure was expressed in kilojoules per minute and calculated by multiplying the V̇o$_2$ (l/min) determined during the last minute of each running speed, for the caloric equivalent corrected for the corresponding RER. Running economy (ml·kg^{-1}·km^{-1}) was defined as the pulmonary V̇o$_2$ measured during the last minute of each running stage, divided by running speed and body mass.

Statistics

Before using a statistical parametric technique, the assumption of normality was verified by the Kolmogorov-Smirnov test. Student's unpaired *t*-tests were used to compare subjects' characteristics between the two training groups before the IT period. Changes in the treadmill test, 10-km time trial performance as well as muscle oxidative enzymes were examined using a two-factor repeated-measures ANOVA. In case of significant main effects or interactions, a Newman-Keuls post hoc test was used to identify the points of difference.

In SET, possible differences in muscle variables between before and after the IT period were evaluated by using a Student's paired *t*-test. Correlation coefficients were determined and tested for significance using the Pearson's regression test. A significance level of $P < 0.05$ was set for all the analysis. Data are presented as means ± SE.

RESULTS

Body Mass and V̇o$_{2max}$

The body mass was the same before and after the IT period in both SET (73.1 ± 2.7 vs. 73.2 ± 2.7 kg) and Con (72.6 ± 2.9 vs. 73.2 ± 2.8 kg). V̇o$_{2max}$ was not altered with the IT period either in SET (54.8 ± 1.7 and 53.5 ± 1.8 ml·kg^{-1}·min^{-1}) or in Con (56.1 ± 2.1 and 56.4 ± 2.0 ml·kg^{-1}·min^{-1}).

Mitochondrial Oxidative Enzymes

In SET, maximal activity of CS was 30.9 ± 1.5 and 29.4 ± 1.5 μmol·g dry wt^{-1}·min^{-1}, before and after the IT period, respectively, and was also unchanged in Con (38.4 ± 2.3 vs. 38.2 ± 3.6 μmol·g dry wt $^{-1}$·min^{-1}). Similarly, HAD maximal activity was the same after as before the IT period both in SET (33.0 ± 2.4 vs. 33.5 ± 2.1 μmol·g dry wt^{-1}·min^{-1}) and in Con (30.7 ± 2.4 vs. 31.4 ± 0.9 μmol·g dry wt^{-1}·min^{-1}).

Muscle UCP3 Protein Expression, Fiber Type Distribution, and Capillaries (Only SET)

In SET, the expression of skeletal muscle UCP3 tended to be higher (~41%; $P = 0.06$) after the IT period (Fig. 1). The relative number of FTx fibers, in SET, was higher ($P < 0.05$) after compared with before the IT period (14.0 ± 3.1 vs. 9.7 ± 1.9%, respectively), but no significant changes were observed either in ST or in FTa and FTc fibers (57.2 ± 4.1 vs. 61.2 ± 3.2, 24.8 ± 2.1 vs. 26.5 ± 1.6 and 4.0 ± 1.4 vs. 2.6 ± 0.7%, respectively). In SET, capillary supply, expressed as capillary-to-fiber ratio, was similar (3.24 ± 0.25 vs. 3.47 ± 0.18) before and after the IT period, respectively.

V̇o$_2$, Energy Expenditure, and Running Economy During Submaximal Running

In SET, V̇o$_2$ at 11, 13, 14.5, and 16 km/h was lower ($P < 0.05$) after than before the IT period (Fig. 2). In Con, V̇o$_2$
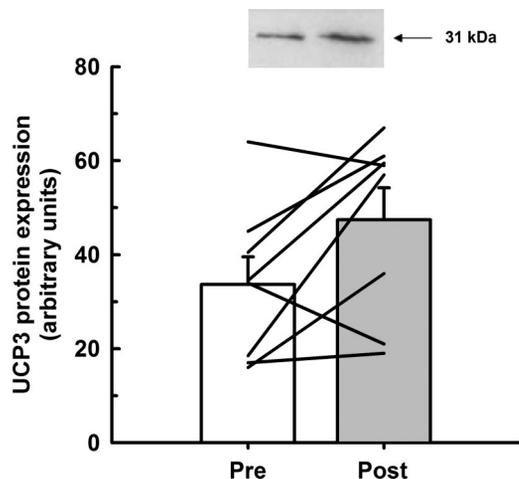
Fig. 1. Effect of 4 wk of speed endurance training (SET group) on protein expression of vastus lateralis muscle mitochondrial uncoupling protein 3 (UCP3). *Insets* at *top* show representative pretraining (Pre) and posttraining (Post) Western blot bands. A standard muscle sample extract was used as an internal reference and corresponds to 100 arbitrary units.

remained unaltered at all running speeds (Table 1). Correspondingly, in SET, energy expenditure was 6.0 ± 2.0, 7.2 ± 2.1, 5.3 ± 1.8, and $6.3 \pm 2.0\%$ lower after the IT period at 11, 13, 14.5, and 16 km/h, respectively, whereas no changes were observed in Con at either speeds (Table 1). Also, running economy was lower ($P < 0.05$) in SET after the IT period and unchanged in Con (Table 1).

Ventilation and RER During Submaximal Running

In SET, before the IT period mean ventilation ($\dot{V}_E$) at 13 and 14.5 km/h was 80.7 ± 4.7 and 101.5 ± 4.4 l/min, respectively, and it was lower ($P < 0.05$) after the IT period (75.5 ± 3.3 and 93.6 ± 3.3 l/min; Table 1), whereas no changes were observed at the other speeds. In Con, $\dot{V}_E$ was unaltered at all running velocities (Table 1).

After the IT period, in SET, the RER at 11 km/h was higher ($P < 0.05$) than before the IT period (0.93 ± 0.01 vs. 0.90 ± 0.01, respectively), whereas it was the same at the other running speeds. No differences in RER before and after the IT period were found for Con (Table 1).

Heart Rate During Submaximal Running

In SET, heart rate after the IT period was 152 ± 4, 167 ± 4 and 178 ± 4 beats/min, at 13, 14.5, and 16 km/h, respectively, which was lower ($P < 0.05$) than before the IT period (158 ± 4, 171 ± 3 and 182 ± 3 beats/min, respectively). In Con, heart rate was not changed (Table 1).

Blood [La⁻] During Submaximal Running

In SET, before the IT period, venous blood [La⁻] reached 1.7 ± 0.2, 2.3 ± 0.3, 4.2 ± 0.6 and 6.4 ± 0.8 mM at 11, 13, 14.5, and 16 km/h, respectively, with the levels being the same after the IT period (Fig. 3). In Con, no significant changes in blood [La⁻] were observed during the treadmill test.

10-km Time Trial Performance

Performance time in the 10-km running trial was unchanged in both SET ($40:52 \pm 1:09$ vs. $40:59 \pm 1:30$ min:s) and Con

($40:53 \pm 2:41$ vs. $40:36 \pm 2:27$ min:s). In SET, peak and average heart rate during the 10-km time trial before the IT period were 184 ± 4 and 177 ± 4 beats/min, respectively, which were similar to after the IT period (181 ± 4 and 175 ± 4 beats/min, respectively). In Con, peak heart rate was 179 ± 3 beats/min before the IT period and 177 ± 3 beats/min after the IT period, with the average heart rate values being 170 ± 3 and 168 ± 2 beats/min, before and after the IT period, respectively. Before and after the IT period, the performance time in the 10-km trial was inversely related to $\dot{V}_{O_{2max}}$ ($r^2 = -0.71$ and $r^2 = -0.70$, $n = 15$, respectively). No correlations were found between 10-km performance time and either running economy or percentage of $\dot{V}_{O_{2max}}$.

DISCUSSION

The major findings of the present study were that 4 wk of speed endurance training in already endurance-trained individuals led to lowered energy expenditure during submaximal running. These changes were not associated with a reduced muscle UCP3 protein expression. In addition, despite a two-thirds reduction in the total amount of training, levels of muscle oxidative enzymes, capillarization, $\dot{V}_{O_{2max}}$, and performance in a 10-km time trial were maintained. These findings demonstrate that intense exercise training can improve running economy while maintaining adaptations gained from previous endurance training.

The present findings show that in already endurance-trained subjects speed endurance training can lower $\dot{V}_{O_2}$ during submaximal running despite a marked reduction in the total volume of training. The changes in running economy observed are of the same magnitude (5–8%) as those reported after strength and plyometric training programs (42, 52, 59, 62). At a running speed of 11 km/h the RER value was higher after the speed endurance training, indicating that carbohydrate oxidation was elevated after the IT period, which in part can explain the lower $\dot{V}_{O_2}$. However, no differences in RER were observed at the other speeds and also the estimated energy expenditure was lower at all speeds. There were no changes in blood [La⁻] during submaximal running after the 4 wk of speed endurance
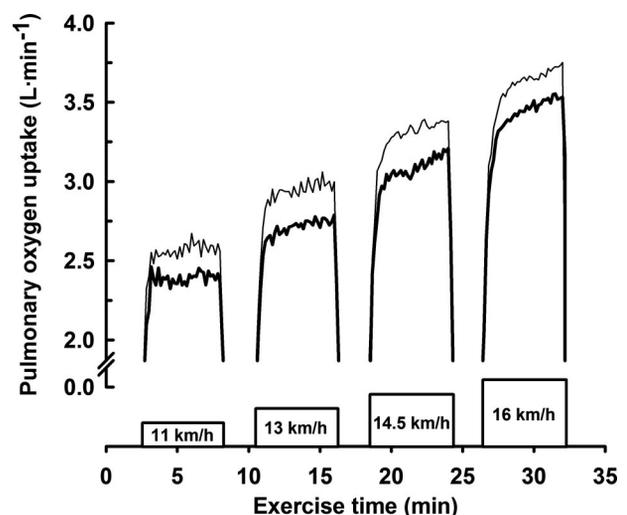

Fig. 2. Pulmonary oxygen uptake during 4 treadmill stages of 6-min each before (thin line) and after (thick line) 4 wk of speed endurance training (SET group).

Table 1. *Physiological variables measured during four treadmill stages of 6 min each before (pretraining) and after (posttraining) the 4-wk intervention period for speed endurance training and control groups*

	Running Speed, km/h							
	11		13		14.5		16	
	Pretraining	Posttraining	Pretraining	Posttraining	Pretraining	Posttraining	Pretraining	Posttraining
SET group								
$\dot{V}_{O_2}$, ml·kg^{-1}·min^{-1}	35.3±1.4	32.9±1.2*	41.1±1.7	37.9±1.4*	46.3±1.6	43.6±1.4*	49.4±1.6	46.3±1.8*
RE, ml·kg^{-1}·km^{-1}	196±8	183±6*	192±5	177±5*	190±5	179±4*	188±3	176±4*
EE, kJ/min	53.4±2.4	50.0±2.0*	62.6±2.8	57.9±2.2*	71.0±2.9	67.0±2.3*	76.4±3.3	71.8±3.6*
$\dot{V}_E$, l/min	63.6±3.4	61.4±2.6	80.7±4.7	75.5±3.3*	101.5±4.4	93.6±3.3*	118.0±4.8	114.5±4.5
RER, ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	0.90±0.01	0.93±0.01*†	0.95±0.02	0.96±0.01	1.00±0.02†	1.00±0.02†	1.03±0.02	1.05±0.02
Heart rate, beats/min	142±3	138±4*	158±4†	152±4*	171±3†	167±4*†	182±3	178±4
Con group								
$\dot{V}_{O_2}$, ml·kg^{-1}·min^{-1}	32.5±1.6	32.0±1.4	38.5±1.5	38.1±1.1	44.2±2.1	43.7±1.6	48.9±2.4	48.8±2.1
RE, ml·kg^{-1}·km^{-1}	184±4	182±6	180±3	179±5	182±5	181±5	184±5	184±5
EE, kJ/min	52.4±3.8	51.8±2.5	61.1±2.8	60.9±2.4	70.5±3.3	70.2±3.2	78.7±3.7	78.8±2.8
$\dot{V}_E$, l/min	60.8±3.4	61.8±3.5	75.3±3.5	75.8±3.9	92.9±4.9	91.2±6.4	115.5±5.7	112.7±6.4
RER, ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	0.88±0.01	0.89±0.01	0.91±0.01	0.92±0.01	0.94±0.01	0.95±0.01	0.99±0.01	1.00±0.01
Heart rate, beats/min	130±6	131±3	145±4	143±2	157±2	156±3	170±4	167±3

Values are means ± SE. IT, intervention; SET, speed endurance training; Con, control; $\dot{V}_{O_2}$, oxygen uptake, $\dot{V}_{CO_2}$, carbon dioxide production; RE, running economy; EE, energy expenditure; $\dot{V}_E$, ventilation; RER, respiratory exchange ratio. *Significant difference from pretraining IT period value, $P < 0.05$. †Significant difference from Con group, $P < 0.05$.

training, suggesting that the anaerobic energy contribution was not altered and that the total energy expenditure was lowered after the training period. At some of the submaximal running speeds, a reduced $\dot{V}_E$ and heart rate were observed after the IT period. The lower ventilator and cardiac work could be estimated to account for maximally 8 (12) and 0.5% (28), respectively, of the difference in $\dot{V}_{O_2}$ observed between before and after the IT period. Therefore, these factors are unlikely to be major contributors to the reduced energy expenditure.

We hypothesized that alterations in $\dot{V}_{O_2}$ could have been linked to a change in muscle UCP3 levels, because it has been shown that mechanical energy efficiency is negatively related to both UCP3 mRNA (55) and protein expression (38, 50). However, the speed endurance training period that resulted in diminished energy expenditure during running did not lead to lowered level of UCP3 protein. On the contrary, UCP3 tended to increase, which rather seems to support the theory that the main function of UCP3 is not in regulation of mitochondrial

efficiency (53). Although the improved running economy after SET cannot be explained by reduced UCP3-mediated proton leak, we cannot rule out the possibility that mitochondrial efficiency is improved by other mechanisms. Irrespective of the functional role of UCP3, mitochondrial efficiency is dependent on the degree of proton leak through the mitochondrial membrane. The nature and control of proton leak and thus mitochondrial efficiency are largely unknown.

It may be speculated why the SET group tended to increase the UCP3 expression. UCP3 has been proposed to have an antioxidant function (15). Therefore, the increase in UCP3 may have been a way to protect the muscle against mitochondrially derived reactive oxygen species generated by the higher training intensity. An augmented UCP3 expression could also have served to facilitate fatty acid oxidation in skeletal muscle (10), and to protect mitochondria against nonmetabolizable fatty acids-induced oxidative damages (53). Alternatively, the upregulation of UCP3 content in skeletal muscle may have been associated with the change in fiber type composition. UCP3 is highly expressed in FTb and FTx compared with ST fibers (25, 49, 50), and after the IT period the SET group showed an elevated percentage of FTx fibers. Thus it is plausible that the tendency to a greater UCP3 expression observed after the speed endurance training could be attributed to the higher number of FTx fibers.

The higher relative distribution of FTx fibers after the speed endurance training period may seem surprising because most studies involving high-intensity training observed a shift from ST and FTx fibers toward FTa fibers (47). However, the reduction in training volume may have been so drastic (∼44 vs. ∼15 km/wk) that the muscles had similar adaptations as observed during a de-training period (11). On the other hand, in a study by Liljedahl et al. (33), the relative proportion of FTb fibers tended to increase after a 4-wk cycling sprint training program of physically active subjects. It is difficult to evaluate whether the greater distribution of FTx influenced the energy turnover during submaximal running. A higher recruitment of
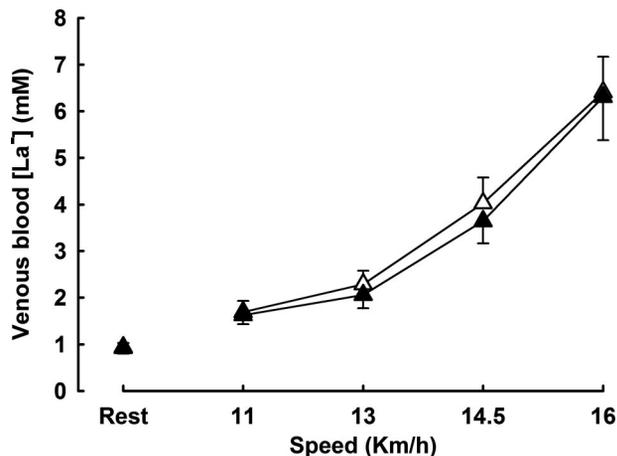

Fig. 3. Venous blood lactate concentration ([La$^-$]) at the end of 4 treadmill stages of 6-min each, before (open symbols) and after (filled symbols) 4 wk of speed endurance training (SET group).

FT fibers has been repeatedly suggested to be related to higher energy cost (3, 14, 21, 29), although we observed that the energy expenditure during running was lowered despite the speed endurance training leading to an increased percentage of FTx fibers. However, it is doubtful whether the FTx fibers were recruited at the velocities used in the treadmill test.

In the present study, the activity of mitochondrial oxidative enzymes remained unchanged after the 4-wk period indicating that, in moderately trained endurance runners, speed endurance training provided sufficient stimuli to maintain the oxidative potential. Although endurance training has been used traditionally to improve the oxidative capacity, a number of studies with untrained subjects demonstrated that it is possible to increase levels of oxidative enzymes and endurance performance with a limited amount of anaerobic work constituted by brief, very intense bouts. For example, Burgomaster et al. (7) reported a 38% increase in the maximal activity of CS and a twofold increase in cycle endurance capacity after six sessions of sprint interval training. These findings confirm what had been demonstrated in previous studies where marked improvements in muscle oxidative potential (~38 and ~60% for CS and HAD, respectively) resulted after 2 wk of daily sprint training (43, 46), and the improvements were similar to those reported by Chesley et al. (8) and Spina et al. (58) in which the CS activity was elevated by 20–30% after 7 days of endurance cycle exercise (2 h/day at 60–70% of peak $\dot{V}o_2$). A few studies have examined the effect of high-intensity training on muscle oxidative enzymes in already trained individuals (16, 56, 63) and similar to the present study, no changes in the activity of CS were observed when well-trained athletes replaced part of their habitual endurance training with intense interval sessions (80–90% of $\dot{V}o_{2max}$) (16, 63). However, to our knowledge, this is the first study to demonstrate unaltered levels of CS and HAD activity in already endurance-trained subjects after a period of speed endurance training associated with a marked reduction in training volume. Also $\dot{V}o_{2max}$ was unaltered in the SET group, which is in agreement with the findings by Madsen et al. (35), who observed that, over a 4-wk period, one 35-min high-intensity bout per week was enough to retain $\dot{V}o_{2max}$ levels. In SET, the average and peak heart rate during the 35- to 40-min training sessions, were around 73 and 92% of maximal heart rate, respectively. Apparently the stimuli during short but very intense training are sufficient to avoid a reduction in muscle oxidative enzyme activity and $\dot{V}o_{2max}$.

The present investigation showed that in SET also the number of capillaries per fiber was maintained, although the amount of training was significantly reduced. It appears that the stimuli for capillary growth in these subjects were more related to the exercise intensity rather than to the duration of training. A previous study has shown that high-intensity intermittent training at 150% of $\dot{V}o_{2max}$ was efficient in enhancing muscle capillarization (26). The specific training-induced stimuli underlying changes in angiogenesis have been suggested to be related to the increase in blood flow and thereby shear stress (24, 36) and to the mechanical stretch associated with muscle contractions (24, 45). Thus stimuli for capillary maintenance and growth are clearly present during intense exercise, which induces great changes in muscle blood flow as well as muscle stretch.

In the present study, 10-km performance was maintained even though the total training distance was severely (~65%) reduced. In agreement, it was recently shown that the endurance performance was unchanged when young rats underwent 4 wk of low volume, high-intensity interval treadmill running after a 10-wk continuous endurance training period (31). In two other studies, male endurance-trained cyclists replaced for a 3- to 4-wk period part of their training volume with two weekly sessions of high-intensity intermittent cycle exercise (12 × 30 s at 175% of an intensity corresponding to peak aerobic power output) and improved performance in a simulated-laboratory 40-km time trial by 2–4% (32, 60). We failed to see any improvements in the endurance performance, but differently from Laursen et al. (32) and Stepto et al. (60), in the present investigation the participants underwent a dramatic reduction in training volume, which may have been one of the reasons for the unchanged performance.

Distance running performance is a function of $\dot{V}o_{2max}$, running economy, and relative work intensity (13). In SET, $\dot{V}o_{2max}$ was unaltered and performance in the 10-km time trial was the same after the IT-period, even though the estimated running economy at racing speed increased by 6.1% after the IT period. Furthermore, in the present study, like in the one by Williams and Cavanagh (64), no correlation was found between 10-km performance and running economy. This finding is different from other studies showing performance improvements after changes in running economy (42, 59, 61). Nevertheless, since in SET the estimated percentage of $\dot{V}o_{2max}$ sustained in the 10-km time trial was reduced after the IT period (80.7 ± 1.6 vs. 84.1 ± 1.5%), it is possible that any potential advantage gained by an improved running economy may had been blunted by a diminished fractional utilization of $\dot{V}o_{2max}$ throughout the trial and be the explanation for the unchanged 10-km performance.

In summary, the present study showed that in already trained subjects an alteration from regular endurance to speed endurance training reduced energy expenditure during submaximal running. This change was not solely caused by elevated carbohydrate oxidation and by reduced ventilatory and heart work. The expression of muscle UCP3 tended to be elevated after the speed endurance training period, and it cannot explain the better running economy. Furthermore, levels of mitochondrial oxidative enzymes, muscle capillarization, $\dot{V}o_{2max}$ and endurance performance remained unaltered despite the dramatic reduction in training volume. Thus it appears that in already trained subjects, speed endurance training per se provides sufficient stimuli for maintaining the adaptations in the cardio-respiratory and muscular systems induced by previous endurance training. Further studies are required to investigate whether the improved running economy after SET can be explained by biomechanical or by bioenergetic factors (e.g., increased mitochondrial efficiency).

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