Concurrent speed endurance and resistance training improves performance, running economy, and muscle NHE1 in moderately trained runners

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The purpose of this study was to examine whether speed endurance training (SET, repeated 30-s sprints) and heavy resistance training (HRT, 80–90% of 1 repetition maximum) performed in succession are compatible and lead to performance improvements in moderately trained endurance runners. For an 8-wk intervention period (INT) 23 male runners [maximum oxygen uptake (V̇o2max) 59 ± 1 ml·min−1·kg−1; values are means ± SE] either maintained their training (CON, n = 11) or performed high-intensity concurrent training (HICT, n = 12) consisting of two weekly sessions of SET followed by HRT and two weekly sessions of aerobic training with an average reduction in running distance of 42%. After 4 wk of HICT, performance was improved (P < 0.05) in a 10-km run (42:30 ± 1:07 vs. 44:11 ± 1:08 min:s) with no further improvement during the last 4 wk. Performance in a 1,500-m run (5:10 ± 0.05 vs. 5:27 ± 0.08 min:s) and in the Yo-Yo IR2 test (706 ± 97 vs. 491 ± 65 m) improved (P < 0.001) only following 8 wk of INT. In HICT, running economy (189 ± 4 vs. 195 ± 4 ml·kg−1·km−1), muscle content of NHE1 (35%) and dynamic muscle strength was augmented (P < 0.01) after compared with before INT, whereas V̇o2max, muscle morphology, capillarization, content of muscle Na+/K+ pump sub-units, and MCT4 were unaltered. No changes were observed in CON. The present study demonstrates that SET and HRT, when performed in succession, lead to improvements in both short- and long-term running performance together improved running economy as well as increased dynamic muscle strength and capacity for muscular H+ transport in moderately trained endurance runners.

short (5–15 min) and longer (30–60 min) duration performance of endurance trained subjects can be improved by adding speed endurance training (SET; repeated 30-s sprints) to a reduced amount of aerobic training (7), or when including heavy resistance training (HRT; load of ∼80–90% of 1 repetition maximum) to aerobic training (3, 33–35, 60, 61). The mechanisms causing the improved endurance performance after a period of intense training are not fully elucidated. SET performed by runners accustomed to moderate-intensity endurance training increased the content of muscle transport proteins involved in hydrogen (11, 30, 38) and potassium handling (7, 38), which are speculated to be important for performance during short-duration intense exercise where accumulation of potassium in muscle interstitium (49) and muscle acidification (24) may impair muscle function. Moreover, both runners (7, 37) and soccer players (17, 30) improved running economy (RE), whereas maximal oxidative enzyme activity and capillary density remained unchanged following 2- to 9-wk periods with SET. HRT in endurance athletes leads to elevated maximal strength as well as a faster rate of force development (RFD) (3, 34, 35, 60), which may improve muscle perfusion during submaximal exercise, assuming that lower contraction time to produce the needed force reduces the contraction-induced occlusion of the blood vessels in the contracting muscles (2). In some (60, 61) but not all studies (3) movement economy while running or cycling is also improved following HRT, which may relate to augmented elastic energy released during exercise (25, 57) since HRT is reported to lead to a thicker and stiffer patella tendon (43, 55, 59).

It is common for athletes to perform different types of training on the same day. This may evoke cross-talk in muscle signaling such that one type of training may impair or stimulate the molecular response to another type of training. It has been proposed that endurance training may blunt signaling of pathways important for muscle hypertrophy when performed concurrently with resistance training (19, 51). This is supported by the finding that hypertrophy only occurred in fast-twitch fibers in a group doing resistance training in combination with endurance training, whereas muscle hypertrophy was present in both slow- and fast-twitch fibers in a group performing only resistance training (44). In contrast, resistance training (six sets with ~8–14 repetitions of leg press) carried out after endurance training (1 h at ~65% V̇o2max) has shown to enhance mRNA levels of PGC-1α important for mitochondrial biogenesis compared with only endurance training (64). Thus concurrent endurance and resistance training can impact the adaptations relative to single mode training. It is, however, unclear whether concurrent high-intensity training like SET and HRT affects each other when performed in succession.

High-volume moderate-intensity training may lower hematological variables (36, 58), impair immune system function (26), and increase muscle damage markers (14). SET and HRT, combined with aerobic training, may also lead to such changes and lack of performance improvements, since it has been shown that sufficient recovery from SET is needed for improving performance (53); however, this relationship has not yet been studied.

Thus the aims of the present study were to examine whether SET and HRT, when performed on the same day, are compatible leading to improvements in short- and long-term endurance performance and to explore the potential

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mechanisms for the changes in performance with the concurrent training. We hypothesized that the combined SET and HRT together with a reduction in the volume of aerobic moderate-intensity running of moderately trained endurance runners would cause an improved short- and long-term endurance performance, higher content of hydrogen and potassium transport proteins, increased dynamic muscle strength, as well as improved RE.

METHODS

Subjects

Twenty-three moderately trained endurance male runners took part in this study. They had an average age, height, body mass, and \( V_{\text{O2max}} \) of 31.1 ± 1.8 (means ± SE) yr, 180.0 ± 0.8 cm, 76.9 ± 1.8 kg, and 59.4 ± 1.1 ml-min\(^{-1}\)kg\(^{-1}\), respectively. Subjects had been running on a regular basis for 7.5 ± 1.5 yr and were running 29.7 ± 6.1 km/wk with a frequency of 3.3 ± 0.5 times/wk without including any SET or HRT. After receiving information about the study and the possible risks and discomforts associated with the experimental procedures, all subjects gave their written informed consent to participate. This study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of the capital region of Copenhagen (Region Hovedstaden).

Experimental Design

The study was conducted for a period of 15 wk encompassing a 4-wk lead-in period and an 8-wk training intervention period (INT) with three rounds of testing (Pre, Mid, and Post) each lasting ~1 wk (Fig. 1). The lead-in period was undertaken to evaluate the habitual running pattern of the subjects, to ensure that the subjects had a stable level of fitness and to familiarize the subjects with the testing procedures used to evaluate the response to INT. After the lead-in period, the subjects were randomly stratified to either a high-intensity concurrent training group (HICT, \( n = 12 \)) or a control group (CON; \( n = 11 \)), based on age, height, body mass, and \( V_{\text{O2max}} \). During INT two subjects withdrew from CON because of an injury and change of job. During the lead-in period total distance covered was 31 ± 6 vs. 28 ± 6 km/wk in HICT and CON, respectively, and distance covered by interval running was 3 ± 4 vs. 6 ± 9 km/wk, respectively, with no difference between HICT and CON. Before and after the 4-wk lead-in period body mass was quantified and \( V_{\text{O2max}} \) was assessed using an incremental test performed until exhaustion. Both variables were unchanged during the lead-in period in both HICT (77.2 ± 2.4 vs. 77.3 ± 2.5 kg and 4.69 ± 0.13 vs. 4.62 ± 0.14 l/min) and CON (77.2 ± 2.9 vs. 76.4 ± 2.9 kg and 4.54 ± 0.17 vs. 4.56 ± 0.16 l/min, after relative to before, respectively).

Training

The HICT group trained 4 times/wk. Supervised concurrent training (SET followed by HRT) was performed on Mondays and Fridays. SET consisted of repeated 30-s all-out running with 3 min of recovery in between and was progressed from 4 to 12 repetitions during INT (Table 1). Warm-up before SET consisted of ~2 km of running at a self-selected pace. HRT was carried out ~15 min after SET and was progressed from 3 sets of 8 repetitions at 15 repetition maximum (RM) to 4 sets of 4 repetitions at 4RM in weeks 5–8 (Table 1) using squat, deadlift, and leg press as exercises. Training was performed with an emphasis on explosiveness (i.e., as high concentric speed as possible). When the subjects were able to perform one repetition more than designated, resistance increased for the following set(s). The subjects had 3 min of passive rest between each set and exercise. Aerobic training was performed twice weekly. On Wednesdays, nonsupervised aerobic high-intensity intervals (AHI) were performed consisting of 4 × 4 min of running with a target heart rate (HR) > 85% HR\(_{\text{max}}\) separated by 2 min of passive recovery (Table 1). On Saturdays, subjects performed aerobic moderate intensity (AMI) continuous running with a target HR of 75–85% HR\(_{\text{max}}\) for 40–70 min (Table 1). During INT, 16 sessions of concurrent training and 8 sessions of both AHI and AMI were planned. The adherence to concurrent training, AHI and AMI was 76 ± 4% (12.2 ± 0.7 sessions), 79 ± 6% (5.5 ± 0.4 sessions), and 83 ± 7% (6.5 ± 0.7 sessions), respectively, and no catch-up sessions were allowed. A training log was kept and analyzed to record the intensity and duration of the nonsupervised training (AHI + AMI). Intervals during the AHI training were performed with an average of 86.1 ± 0.9% HR\(_{\text{max}}\), whereas the AMI training had an average duration of 51.9 ± 4.8 min performed with an average of 82.1 ± 1.3% HR\(_{\text{max}}\). The weekly running distance of the HICT group was 42% less (\( P < 0.05 \)) during INT compared with the lead-in period (18 ± 2 vs. 31 ± 6 km,

![Figure 1. Flow diagram of the experimental design with tests and measurements at baseline (BL), before (Pre), midway (Mid), and after (Post) an 8-wk intervention period with high-intensity concurrent training (HICT) or maintained training in a control group (CON). Two subjects in CON dropped out after Mid.](image)

Table 1. Weekly training schedule

<table>
<thead>
<tr>
<th>INT Weeks</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET: Mon and Fri (intensity)</td>
<td>4/6 (all-out)</td>
<td>7/7 (all-out)</td>
<td>8/8 (all-out)</td>
<td>6/8 (all-out)</td>
<td>9/8 (all-out)</td>
<td>10/8 (all-out)</td>
<td>11/9 (all-out)</td>
<td>12/9 (all-out)</td>
</tr>
<tr>
<td>HRT: Mon and Fri (sets × reps)</td>
<td>3 × 8 (15RM)</td>
<td>3 × 8 (12RM)</td>
<td>4 × 6 (8RM)</td>
<td>4 × 6 (8RM)</td>
<td>4 × 4 (4RM)</td>
<td>4 × 4 (4RM)</td>
<td>4 × 4 (4RM)</td>
<td>4 × 4 (4RM)</td>
</tr>
<tr>
<td>AHI: Wed (intensities)</td>
<td>4 × 4 + 2 min (~85% HR(_{\text{max}}))</td>
<td>4 × 4 + 2 min (~85% HR(_{\text{max}}))</td>
<td>4 × 4 + 2 min (~85% HR(_{\text{max}}))</td>
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<td>4 × 4 + 2 min (~85% HR(_{\text{max}}))</td>
</tr>
<tr>
<td>AMI: Sat (intensities)</td>
<td>~50 min (75–85% HR(_{\text{max}}))</td>
<td>~50 min (75–85% HR(_{\text{max}}))</td>
<td>~50 min (75–85% HR(_{\text{max}}))</td>
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<td>~50 min (75–85% HR(_{\text{max}}))</td>
</tr>
</tbody>
</table>

Weekly training schedule in the high-intensity concurrent training (HICT) group performing speed endurance training (SET, 30-s sprints separated by 3 min of rest) followed by heavy resistance training (HRT; intensity expressed as repetition maximum, RM) twice weekly, as well as a weekly session with aerobic high intensity (AHI) and a weekly session of aerobic moderate intensity (AMI) training for an 8-week period with training days listed in italic.
respectively). The calculations of running distance did not include distance covered during warm-up and cool-down. The subjects in CON were instructed to continue their running routines as during the lead-in period performing a total distance of 40 ± 8 km/wk, including 4 ± 5 km/wk of interval running during INT.

**Testing**

During the lead-in period 2 days were used to familiarize the subjects with resistance training and with the additional tests used to assess performance before (Pre), during (Mid), and after (Post) INT. On the first day a 1,500-m run was performed followed by resistance training with emphasis on correct posture and lifting technique. On the second day a Yo-Yo intermittent recovery level 2 test (Yo-Yo IR2) was performed followed by 1RM (squat and leg press) and 5RM (deadlift) strength testing familiarization. Due to the risk of lower-back injury associated with 1RM deadlifting, testing of 5RM was selected as the intensity for the deadlift exercise.

Before, midway, and after INT, the subjects completed a series of tests on separate days in the following order: 1) a treadmill test to quantify \( \dot{V}O_2 \) kinetics, running economy (RE), and \( V_{O2\max} \) Pre and Post INT; 2) 1RM squat and leg press and 5RM deadlift to determine maximal dynamic strength Pre, Mid, and Post INT; 3) a 10-km run Pre, Mid, and Post INT to determine long-term endurance; 4) a Yo-Yo IR2 Pre, Mid, and Post INT to determine intermittent intense endurance; 5) a 1,500-m run Pre, Mid and Post INT to determine short-term endurance; and 6) a muscle biopsy and a blood sample obtained at rest under standardized conditions after an overnight fast Pre, Mid, and Post INT.

All tests were carried out at least 48 h apart and were repeated at the same time of day to minimize the influence of different hormonal milieu and variations in body temperature due to the circadian rhythm (22). To ensure a reliable testing environment, all tests were executed without any oral support or cheering. Subjects refrained from strenuous physical activity 48 h prior to testing and abstained from alcohol and caffeine 24 h prior to testing and were instructed to replicate their diet 2 days prior to and during the series of tests. All testing was preceded by 15 min of a test-specific standardized warm-up.

\( V_{O2\max} \) kinetics, running economy, and \( \dot{V}O_2_{\max} \). The first incremental test before the lead-in period consisted of 2 min at 5 km/h, 3 min at 10 km/h, 2 min at 14 km/h, after which the speed increased by 1 km/h every minute until exhaustion. Before and after INT a modified protocol was used consisting of three 6-min intervals: a) moderate running at 12 km/h separated by 20 min of rest to quantify \( V_{O2} \) kinetics and RE. Walking for 2 min at 5 km/h preceded all intervals. At the end of the third interval speed was increased to 14 km/h and from here increases in speed of 1 km/h every minute were made until exhaustion to determine \( V_{O2\max} \) (highest value achieved over a 30-s period). Before the test, subjects were weighed and had a Polar S610 HR monitor (Polar Electro) fitted around their chest for continuous HR recordings. After initial calibration, pulmonary \( \dot{V}O_2 \) was measured by a breath-by-breath gas analyzing system (Oxycon Pro, Viasys Healthcare, Hoechberg, Germany).

To determine \( V_{O2} \) kinetics errant breaths, defined as any value lying more than 4 SDs away from the local mean caused by swallowing and coughing were initially removed. Then the \( \dot{V}O_2 \) responses for the three transitions were linearly interpolated to give 1-s values, and then averaged. The initial cardiodynamic component was ignored by eliminating the first 20 s of data after the onset of exercise. The data were fitted using a monoexponential model:

\[
\dot{V}O_2(t) = \dot{V}O_2(\text{baseline}) + A \left(1 - e^{-((t-\text{TD})/\tau)}\right)
\]

with \( \dot{V}O_2(t) \) being oxygen uptake to a given time. \( \dot{V}O_2 \) baseline was calculated as the average \( \dot{V}O_2 \) in the last minute walking at 5 km/h; and \( A, \text{TD}, \) and \( \tau \) were the amplitude, time delay, and time constant, respectively for the response modeled with \( \tau \) describing the speed of the \( \dot{V}O_2 \) response as the time to attain 63% of the amplitude.

RE was calculated with the following formula:

\[
\text{RE} \ (\text{mL} \cdot \text{kg}^{-1} \cdot \text{km}^{-1}) = \left[\frac{\dot{V}O_2(\text{ml/min}) \times 60 \times \text{min/h}}{\text{BM(kg)}}\right] / 12 \text{ km/h}
\]

where \( \dot{V}O_2 \) is the average value for the three intervals during the last 2 min of running at 12 km/h and BM is body mass.

**Strength testing.** The sequence of testing was 1RM squat, 1RM leg press, and 5RM deadlift with a resting period of 3 min separating trials within the specific exercise until 1 or 5RM was reached and 5 min of rest separating exercises. During squat testing subjects were instructed to tap a bench (40 cm of height) which was placed behind them to ensure the same range of motion during all tests and during leg press a mark of tape was used to secure that the same depth was obtained in all tests.

The 10-km and 1,500-m tests. The 10-km running test was performed on a carefully measured route in public parks and the 1,500-m running test was performed on a 400-m running track. Weather conditions were similar during testing.

Yo-Yo intermittent recovery test level 2. Yo-Yo IR2 was performed on an indoor surface. The test consists of 2 × 20 m shuttle runs at increasing speeds, interspersed by 10-s of active recovery (controlled by audio signals from a compact disc) (45). The test was terminated when an individual was no longer able to maintain the required speed, and each individual was allowed one warning in case their running speed was too low. The distance (in m) covered up to the end point represented the test result.

**Scores of overtraining.** To measure general and sport specific recovery and stress, a Danish version of Recovery Stress Questionnaire (RESTQ) based on an English version (42) was used. RESTQ consists of 56 elements, which on 19 scales measure recovery and stress based on questions with possible answers on a scale from 0 (never) to 6 (always). The 19 scales were divided into “superscores” of “General Stress” (7 scales), “General Recovery” (5 scales), “Sport-Specific Stress” (3 scales), and “Sport-Specific Recovery” (4 scales). RESTQ was handed out when the biopsies had been taken before (Pre), midway (Mid), and after (Post) INT.

**Muscle biopsies and blood sampling.** All invasive procedures were performed between 7 and 11 a.m. under standardized conditions after an overnight fast 48–72 h after testing of 1,500-m performance. A biopsy was collected at rest (Pre, Mid, Post) from the vastus lateralis muscle of the right leg under sterile conditions and with local anaesthesia (1 ml; 20 mg/l lidocaine without epinephrine) using the Bergström technique (10). A part of the muscle sample (~80 mg wet weight) was immediately frozen in liquid N₂ and stored at −80°C until further analysis (see Western blotting). The remaining muscle tissue was then mounted in an embedding medium (OCT Tissue-Tek, Sakura Finetek, Zoeterwoude, NL), frozen in precooled isopentane, and subsequently stored at −80°C until further analysis (see Immunofluorescence microscopy). Furthermore, a 10 ml blood sample was drawn from an antecubital vein in between the lidocaine injection and the muscle biopsy.

**Muscle Analysis**

**Western blotting.** The frozen muscle biopsies were weighed before and after freeze drying (Heto CD 52 Freeze dryer, Heto-Holten, Denmark) to determine the water content. All visible fat, blood, and connective tissue were then carefully dissected away under a stereo microscope in a room with a temperature of 18°C and a relative humidity below 30%. Next, ~5 mg of freeze-dried muscle tissue was homogenized on ice a fresh batch of ice-cold modified GSK3 buffer (MG-buffer) [80 μM l-muscle dry wt, 10% glycerol, 20 mM Na₂HPO₄, 1% Nonidet P-40, 2 mM PMSF, 150 mM NaCl, 50 mM HEPES (pH 7.5), 20 mM β-glycerophosphate, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 10 μg/ml aprotinin, 3 mM benzeni-
dine, 10 µg/ml leupeptin, 2 mM Na₂VO₃) for 2 hours of 30 s using a TissueLyser II (Retch, Germany). After being rotated end over end for 1 hour at 4°C, samples were centrifuged for 20 min at 17,500 g at 4°C and lysates were collected as the supernatant.

Protein determination was performed on lysates using Pierce BCA Protein Assay Kit no. 23225 (Pierce Biotechnology). Lysate samples were diluted 1:5 in double-distilled H₂O (ddH₂O); 3 × 10 µl of each diluted sample was added to a 96-well micro titer plate together with 3 × 10 µl of diluted MG-buffer, ddH₂O, and Pierce bovine serum albumin (BSA) standards with protein amounts ranging from 0.2 to 2.0 mg/ml. The spectrophotometric reaction was initiated by adding 200 µl of Pierce BCA reagent (Reagent A and Reagent B diluted 49:1) and the microtiter plates were incubated at 37°C for 30 min. The absorbance was read at 550 nm in a Multiskan FC microplate reader (Thermo Fisher Scientific) using SkanIt software 2.5.1 for Multiskan (Thermo Scientific). Finally, the protein concentrations were calculated from the standard curves after correcting for the absorbance of ddH₂O and MG-buffer.

The lysates were diluted to appropriate protein concentrations in an X6 sample buffer [0.5 M Tris base, 0.1-dithiothreitol (DTT), sodium dodecyl sulfate (SDS), glycerol, and bromophenol blue]. Criterion gels were used and all of the individual samples (Pre, Mid, Post) were loaded next to each other to avoid possible bias from side to side differences in transfer efficacy. A human standard was loaded on each side of the gel for the same reason. Further, a colored molecular marker (Precision Plus Protein Standards, no. 161-0374 or no. 161-0373, Bio-Rad, CA) was loaded at the ends of the gels to recognize the specific regions of interest and to act as a control of the molecular weight of the protein analyzed when the immunostaining was visualized.

The gels were exposed to electrophoresis in a Gel-apparatus (Mini Protein Tetra Cell, Bio-Rad, China) filled with running-buffer (120 g Tris base, 576 g glycin, 40 g SDS, 4 liters ddH₂O). The gels ran with 55 mA and maximum 150 V per gel (Power Pac HC, Bio-Rad). The gels were stained with 200 ml 96% ethanol, 1 liter ddH₂O. The proteins were visualized by incubation with a chemiluminescent HRP substrate (Immobilon Western, Millipore, MA) for 5 min immediately before the membrane image was digitalized (Bio-Rad ChemiDOC MP Imaging System).

Quantification was performed in Image Lab 4.0 software (Bio-Rad).

Muscle enzyme activity. For the determination of enzymatic activity, ~2.5 mg dry weight (dw) of muscle tissue was homogenized (1:400) in a 0.3 M phosphate buffer (17.117 g K₂HPO₄ Merck 5099, 10.207 g K₂HPO₄ Merck 4873 in 250 ml H₂O) and was titerated with KH₂PO₄ until pH 7.7 was reached. Next, 0.5 mg/ml of BSA was added for 2 rounds of 30 s using a TissueLyser II (Retch, Germany). Maximal activity of CS, HAD, and PKF was determined fluorometrically with NAD-NADH (NADP-NADPH) coupled reactions (46) on a Fluoroscan Ascent apparatus (Thermo Scientific) using Ascent Software version 2.6.

Immunofluorescence microscopy. For HICT, the embedded muscle samples were cut using a cryostat, and transverse sections 8 µm in thickness were placed onto glass slides. To verify the cross-sectional orientation of the individual muscle fiber, multiple samples were cut and examined under light microscopy until a cross-section of desirable size, orientation, and uniform polygonal appearance was visible. Only areas without artefacts or tendency to longitudinal cuts were analyzed. Staining targets were visualized pairwise. First, capillaries and myofiber type IIA were visualized using biotinylated Ulex europaeus agglutinin I lectin (VECTB-1065, VWR, Bie and Berntsen, Herlev, Denmark, 1:100) and a monoclonal antibody (SC-71, Hybridoma Bank, Iowa City, IA, 1:500), respectively. Second, myofiber borders were visualized using an antibody against laminin (DAKO Z0097, 1:1000) together with myosin heavy chain (Sigma, M8421, 1:1000) added for distinction of myofiber type I. Specific secondary antibodies [order listed: Streptavidin/FITC, (Dako F0422, Glostrup, Denmark), Alexa-555 donkey anti-mouse (Invitrogen, A-31570, Life Technologies Denmark, Naerum, Denmark, 1:100), Alexa-350 goat anti-rabbit (Invitrogen, P10994, 1:1000) and Alexa-488 donkey anti-mouse (Invitrogen, A21202, 1:1000)] were applied to each primary antibody. Specificity of the staining was assessed by single staining, and by staining without the primary antibody. Three individual muscle fiber types were identified as type I (green), type IIA (red), and type IIX (unstained/black) (13). Visualization was performed on a computer screen using a light microscope (Carl Zeiss, Germany), and all morphometric analysis were performed using a digital analysis program (ImageJ, NIH ImageJ). Two or more separate sections of a cross-section were used for analysis, and the cross-sectional area was assessed by manually drawing the perimeter around each selected section. The number of muscle fibers and capillaries within each section was counted, and capillary supply was subsequently expressed as capillaries per fiber (C:F-ratio) and capillary density (cap/mm²). Mean fiber area was assessed by manual drawing of the perimeter of each muscle fiber. All analysis was carried out manually by the same blinded investigator.

Blood Analysis

The resting venous blood sample was analyzed for ferritin, hemoglobin, leukocytes, reticulocytes, transferrin, immunoglobulin A (IgA), creatine kinase (CK), lactate dehydrogenase (LDH), cortisol, and testosterone by automated analyzers (Cobas Fara, Roche, Neuilly sur Seine, France).
Statistics

Student’s unpaired t-tests were used to compare subjects’ characteristics (age, height, body mass, training during lead-in, and V\textsubscript{O}2\text{max}) in HICT and CON before INT. A two-way ANOVA for repeated measurements was used to determine the effect of INT on body mass, V\textsubscript{O}2\text{max}, performance tests, V\textsubscript{O}2 kinetics, RE, RESTQ, maximal enzymatic activity, and blood markers with group (HICT vs. CON) and time (Pre vs. Mid. vs. Post INT) as factors. To determine the effect of INT on muscle protein expression, capillarization, and muscle morphology, a one-way ANOVA for repeated measurements was performed separately for HICT and CON (only HICT for the latter two). When an overall main effect or interaction was obtained, a Student-Newman-Keuls post hoc test was used as a multiple-comparison procedure to isolate which group or time point differed from the other. For all the analysis, the level of statistical significance was set to P < 0.05. All data on muscle protein expression were related to a mean of at least two human standards (2–3 human standards per gel) and a ratio (e.g., Pre/Post) was calculated. The individual Mid and Post signal intensities were related to individual Pre signal intensity before being log transformed. Associations between performance (10-km, 1,500-m, and Yo-Yo IR2) and physiological [V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}), RE, τ, 1RM squat, 1RM leg press, 5RM deadlift, maximal enzymatic activity of CS, PFK, and HAD, capillary density (cap/mm\textsuperscript{2}), C:F-ratio, fiber type distribution, fiber type area] variables were evaluated using Pearson’s correlation coefficient r analysis. Except for fiber type area and distribution, a one-tailed test design was applied with the a priori hypothesis that high values of, e.g., C:F-ratio and maximal enzymatic activity of CS correlated with high performance. Data are presented as means ± SE except for data on muscle protein which is presented as geometric means ± 95% confidence intervals.

RESULTS

Main effects for group (HICT and CON), time (Pre, Mid, and Post INT) and interactions are displayed in Table 2.

The 10-km, 1,500-m, and Yo-Yo IR2 Performance

Before INT no difference in 10-km (44:11 ± 1:08 vs. 41:52 ± 1:08 min:s), 1,500-m (5:27 ± 0:08 vs. 5:22 ± 0:07 min:s), and Yo-Yo IR2 (491 ± 65 vs. 429 ± 93) running performance was found between HICT and CON. 10-km performance was improved (P < 0.05) by 3.8% after 4 wk of HICT (42:30 ± 1:07 vs. 44:11 ± 1:08 min:s) with no further improvement in the following 4 wk (42:20 ± 1:03 min:s; Fig. 2A). In HICT, 1,500-m performance was unchanged after 4 wk, but improved (P < 0.001) by 5.5% after 8 wk compared with before INT (5:10 ± 0:05 vs. 5:27 ± 0:08 min:s) (Fig. 2B). Likewise, Yo-Yo IR2 performance was unchanged after 4 wk, and then improved (P < 0.001) by 44% after 8 wk of HICT compared with before INT (705 ± 97 vs. 491 ± 65) (Fig. 2C). Performance in CON was unaltered with INT (Fig. 2).

V\textsubscript{O}2 Kinetics, Running Economy, and V\textsubscript{O}2\text{max}

In HICT, the speed of the rise in V\textsubscript{O}2 (time constant, τ) in the transition from walking to running was not changed after compared with before INT (22 ± 21 ± 2 s). Steady-state V\textsubscript{O}2 at 12 km/h was lower (P < 0.01) after compared with before INT (2.98 ± 0.11 vs. 3.01 ± 0.11 l/min) corresponding to a 3.1% improvement (P < 0.01) in RE (189 ± 4 vs. 195 ± 4 ml·kg\textsuperscript{-1}·km\textsuperscript{-1}) (Fig. 3). V\textsubscript{O}2\text{max} was the same before and after INT in both HICT (60.7 ± 12. vs. 59.5 ± 0.8 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) and CON (58.9 ± 21. vs. 58.2 ± 2.3 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}). No difference in τ (19 ± 1 vs. 19 ± 2 s), steady-state V\textsubscript{O}2 at 12 km/h (2.80 ± 0.17 vs. 2.80 ± 0.13) or RE (178 ± 6 vs. 180 ± 4) was observed in CON after compared with before INT. Before INT, 10-km performance of pooled subject data (HICT+CON) correlated (P < 0.05) with V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) (n = 18, r\textsuperscript{2} = 0.20, τ = 18, r\textsuperscript{2} = 0.07) and RE (n = 18, r\textsuperscript{2} = 0.35). The 1,500-m performance correlated with V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) (n = 18, r\textsuperscript{2} = 0.50). And last, Yo-Yo IR2 performance correlated with V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) (n = 17, r\textsuperscript{2} = 0.22). After 8 wk of HICT, V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) correlated (P < 0.05) with 1,500-m (n = 12, r\textsuperscript{2} = 0.40) and Yo-Yo IR2 (n = 12, r\textsuperscript{2} = 0.80) performance. No other correlations were found between performance and τ, RE, or V\textsubscript{O}2\text{max} in either pooled subject data, HICT, or CON.

Maximal Dynamic Strength

HICT improved (P < 0.01) 1RM squat by 9% (123 ± 7 vs. 113 ± 6 kg) (Fig. 4A), 1RM leg press by 8% (249 ± 16 vs. 231 ± 14 kg) (Fig. 4B), and 5RM deadlift by 14% (114 ± 7 vs. 100 ± 8 kg) (Fig. 4C) during the first 4 wk of INT. After 8 wk of training, HICT had improved (P < 0.001) 1RM squat by 10.220.33.4 on April 19, 2017 http://jap.physiology.org/ Downloaded from

Table 2. Main effects for group (HICT vs. CON), time (Pre, Mid, Post) and interactions following an 8-week intervention period consisting of high-intensity concurrent training (HICT) or maintained training in a control group (CON)

<table>
<thead>
<tr>
<th>Group</th>
<th>HICT vs. CON</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre vs. post</td>
<td>Pre vs. mid</td>
</tr>
<tr>
<td>10 km (min:s)</td>
<td>0.609</td>
<td>0.398</td>
<td>0.475</td>
</tr>
<tr>
<td>1,500 m (min:s)</td>
<td>0.594</td>
<td>0.905</td>
<td>0.218</td>
</tr>
<tr>
<td>Yo-Yo IR2 (m)</td>
<td>0.165</td>
<td>0.018*</td>
<td>0.205</td>
</tr>
<tr>
<td>τ (s)</td>
<td>0.295</td>
<td>0.939</td>
<td>N/A</td>
</tr>
<tr>
<td>RE (ml·kg\textsuperscript{-1}·km\textsuperscript{-1})</td>
<td>0.121</td>
<td>0.001**</td>
<td>N/A</td>
</tr>
<tr>
<td>V\textsubscript{O}2\text{max} (ml·min\textsuperscript{-1}·kg\textsuperscript{-1})</td>
<td>0.450</td>
<td>0.759</td>
<td>N/A</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>0.866</td>
<td>0.348</td>
<td>N/A</td>
</tr>
<tr>
<td>1RM squat (kg)</td>
<td>0.014*</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>1RM leg press (kg)</td>
<td>0.035*</td>
<td>&lt;0.001***</td>
<td>0.046*</td>
</tr>
<tr>
<td>5RM deadlift (kg)</td>
<td>0.101</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>CS (μmol·g dry wt\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>0.330</td>
<td>0.068</td>
<td>0.913</td>
</tr>
<tr>
<td>HAD (μmol·g dry wt\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>0.044*</td>
<td>0.037*</td>
<td>0.789</td>
</tr>
<tr>
<td>PFK (μmol·g dry wt\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>0.001**</td>
<td>0.859</td>
<td>0.657</td>
</tr>
</tbody>
</table>

CS, citrate synthase; HAD, β-hydroxyacyl-CoA-dehydrogenase; PFK, phosphofructokinase. Main effect: *P < 0.05. **P < 0.01. ***P < 0.001.
Fig. 2. Performance of moderately trained endurance runners in a 10-km run (A), a 1,500-m run (B), and in the Yo-Yo Intermittent Recovery test level 2 (IR2) (C) before (Pre), midway (Mid), and after (Post) an 8-wk intervention period with HICT (closed symbols, left, n = 12 except for Yo-Yo IR2, n = 11) or maintained training in a control group (open symbols, right, n = 7). Values are means ± SE. Different from Pre: *P < 0.05, **P < 0.01, ***P < 0.001. Different from Mid: ##P < 0.01. Different from CON: §P < 0.05.

Muscle Proteins

In HICT, NHE1 content was 35% higher (P < 0.001) after compared with before INT (Fig. 5). SERCA1 content was 15% lower (P < 0.05) after 4 wk compared with before INT in HICT, while after 8 wk of INT a nonsignificant (P = 0.059) 16% lower content was observed. SERCA2 content remained unchanged in HICT during INT. Content of the Na⁺-K⁺ pump subunits (α1, α2, β1, FXYD1), MCT4, Akt, PFK, CS, COX4, HAD, and PECAM1 was unaltered with INT in HICT. No change in protein expression was observed in CON, except that the content of SERCA2 was found to be 18% higher (P < 0.05) after compared with before INT.

Muscle Enzymes

The maximal activity of CS, HAD, and PFK was unchanged during INT in both HICT and CON, but a reduction (P < 0.05) in CS was observed from 4 to 8 wk of INT in HICT (Table 3). In HICT, CON, or groups combined, no correlations between muscle enzymes and performance were found.
Muscle Morphology and Capillarization

Capillary density, C:F-ratio, muscle fiber cross-sectional area, fiber type distribution, and mean fiber area was unchanged with HICT (Table 4). No association between performance and measures of muscle morphology and capillarization was found.

Resting Blood Concentrations

In HICT, ferritin was lower ($P < 0.05$) after 4 and 8 wk of HICT compared with before INT, whereas IgA was higher ($P < 0.01$) after 8 wk compared with before INT. All other blood variables remained unchanged in HICT (Table 5) and no changes were observed in CON, except that an increase ($P < 0.05$) in cortisol occurred from 4 to 8 wk of INT.

Recovery and Stress Questionnaire

In HICT no differences were observed in the four “super-scores” during INT. A decline ($P < 0.05$) in “Sport-Specific Recovery” ($2.7 \pm 0.3$ vs. $3.6 \pm 0.3$) and “General Recovery” ($3.6 \pm 0.4$ vs. $4.1 \pm 0.3$) was observed in CON after relative to before INT.

DISCUSSION

The major findings of the present study were that a period of concurrent speed endurance and heavy resistance training, including two weekly sessions of aerobic training, improved 10-km, 1,500-m and Yo-Yo IR2 test performance of moderately trained endurance runners. In addition, the training led to better running economy, elevated maximal dynamic strength, and content of muscle NHE1, whereas V\textsuperscript{\textcircled{C}}O\textsubscript{2max}, muscle morphology, capillarization, and muscle Na\textsuperscript{+}-K\textsuperscript{+} pump \(\alpha1, \alpha2\) and \(\beta1\) subunits remained unaltered.

In HICT, 10-km performance was $44$ min before INT and significantly reduced to $42:30$ min:s after $4$ wk with no further improvement during the last $4$ wk ($42:20$ min:s). A previous study with trained runners found a $3.1\%$ improved 10-km performance with an intervention adding SET to a reduced
amount of endurance training (7). Thus the 4.2% improvement in 10-km performance after 8 wk of HICT in the present study is of a magnitude observed previously and suggests that HRT does not blunt improved 10-km performance with SET. This change occurred without alterations in $\dot{V}_\text{O}_2\text{max}$ and maximal activity of aerobic enzymes, which is in agreement with the study by Bangsbo et al. (7). Furthermore, CS and HAD did not correlate with 10-km performance in either of the studies. In accordance, CS activity was a poor determinant of 40 km time-trial performance in highly trained cyclists (39), and in a study investigating the response of highly trained cyclists to maximal intensity 5-min intervals with normal or low glycogen levels, improved 60-min time-trial performance was of a similar magnitude with only the latter group increasing CS and HAD activity (65). In accordance with studies examining the effect of SET on trained subjects (30, 37), the present study did not find any effect of HICT on capillary density. Collectively, it appears that increases in $\dot{V}_\text{O}_2\text{max}$, capillary density, and CS and HAD activity are not mandatory for improving endurance performance in already trained subjects.

The estimated fractional utilization of $\dot{V}_\text{O}_2\text{max}$ (21) during the 10-km run after compared with before HICT ($75/110\%$ vs. $73/110\%$ $\dot{V}_\text{O}_2\text{max}$) was not different. Instead the better RE may have been the major cause of the improved performance in HICT supported by studies showing that RE is an important determinant for superior performance in world-class runners (47, 56). It has been observed that a period of SET (7, 37) or HRT (60, 61) can improve movement economy when added to the aerobic training of endurance athletes. With the present design we cannot isolate the effect of SET from HRT on RE and evaluate whether there was an additive effect of performing both intense training forms. Nevertheless, it shows that
SET and HRT are compatible when combined in the present order.

In the present study SERCA1 was lowered after 4 wk of HICT and tended to be lower after 8 wk of HICT, where RE was better. This was not caused by fiber conversion since the fiber composition was unchanged with HICT (Table 4). Nevertheless, a lower content of SERCA pumps may be of importance since calcium handling by the ATP-dependent SERCA pumps are reported to be responsible for up to 50% of the energy used during muscle activity (18, 63). In agreement, a study using untrained subjects ($V_{\text{O}_2}\text{max}$ 46 ml·kg$^{-1}$·min$^{-1}$) observed a reduction in SERCA2 and a tendency for a reduction in SERCA1 after 5 wk of moderate endurance training together with improved exercise economy when cycling (66). Likewise, just 6 days (28) or 10 wk of moderate intensity high-volume training (27) resulted in lower SERCA content. Moreover, a study on rat soleus muscle found that the mRNA level of SERCA2 decreased following electrical stimulation protocols mimicking both moderate and intense training, whereas the mRNA level of SERCA1 decreased only with intense training stimulation (48). Taken together the reduced energy requirement for calcium handling after SET in HICT might be linked to the improved RE in the present study. However, it is challenging that the SERCA2 content of CON was higher at the end of INT, yet RE and performance were unchanged. Clearly, studies are needed to explore the role of changes in SERCA content in relation to exercise economy.

HICT improved maximal dynamic strength likely as a result of HRT as shown by others (3, 32, 34, 50, 60, 61). HRT has been suggested to induce increased neuromuscular function by...
increased descending motor drive from higher CNS centers (5, 29), reduced motor neuron inhibition (6), improved maximal firing frequency of the motor units (1, 62), earlier motor-unit activation (62), improved motor neuron excitability (5), a greater number of fibers being recruited (4), increased synchronization of the muscle contraction (25), and reduction in antagonist coactivation (6). This may have reduced the muscle fiber recruitment required to produce the force needed to run at a given velocity. In support, 8 wk of HRT similar to the present study reduced leg blood flow and \( V\dot{O}_2 \) during intense cycling with identical power output (9). Thus increased neuromuscular function could contribute to the improved RE found with HICT. According to Cavagna and Kaneko (16), \( V\dot{O}_2 \) during running might be 30–40% higher without support from elastic energy stored and returned. An increase in the stiffness of the muscle-tendon system means greater exploitation of stored elastic energy (25, 57), which may lower the cost of running (i.e., improved RE), which is backed by an association between reduced hamstring flexibility and high RE (40). Since HRT has been shown to increase patella tendon stiffness and cross-sectional area (43, 59) it may be that the subjects in HICT improved RE due to tendon specific adaptations. In association, it should also be considered if SET contributed to increased tendon stiffness since this complex is expected to be highly taxed during maximal sprinting supported by the finding of stiffer patella tendons in the lead/dominant leg of fencers and badminton players (20). It has been shown that following HRT, similar to the present study, both 1RM and the ability to rapidly produce force increased in endurance athletes (34, 35, 60, 61). Although not measured, the marked increase in 1RM in HICT in the present study might be associated with an improved rate of force development. This may have enabled the subjects to more rapidly produce the force required for a given running speed, thereby allowing a more prolonged relaxation phase for each muscle contraction cycle. This in turn may have increased the time of muscle perfusion, thereby increasing mean transit time and hence increasing removal of ions, metabolites, and delivery of oxygen, as suggested by others (2). Taken together, a combination of SET and HRT improved RE in moderately trained endurance runners, and future studies should address the mechanisms responsible for the change in RE.

HICT demonstrated improved performance of the 1,500-m run and the Yo-Yo IR2 test after INT. This does not appear to be linked with faster \( V\dot{O}_2 \) kinetics since this parameter was unchanged with HICT, possibly because of an initial low \( \tau \) value as observed in previous studies on soccer players (17) and moderately trained runners (15). Although RE was not measured at Mid, the better RE might have contributed to the improved performance since \( V\dot{O}_2\max \) and estimated fractional utilization of \( V\dot{O}_2\max \) during the 1,500-m run after compared with before INT (92 ± 1 vs. 89 ± 1% \( V\dot{O}_2\max \)) was unchanged. However, this was based on the assumption that RE determined during 12 km/h treadmill running corresponded to running at the 1,500-m pace and without taking the anaerobic contribution into account (23). A larger anaerobic contribution could also promote a faster running speed during these tests, since both a 1,500-m run (12) and the Yo-Yo IR2 (45) leads to a high anaerobic energy turnover with accumulation of muscle lactate and a marked reduction in muscle pH, which may limit performance (8). The finding of an increased content of NHE1 in HICT after compared with before INT suggests that the hydrogen ion release during exercise was elevated and thereby the rate of lowering of muscle pH may not have been larger during the 1,500-m run despite the higher running speed after INT. Thus the effect of intracellular acidification (24) on muscular function may have been the same before and after INT. In accordance with the present study, a 30% increased content of NHE1 was found in association with improved performance in moderately trained runners in the Yo-Yo IR2 test and during repeated intervals at a running speed corresponding to 130% of \( V\dot{O}_2\max \) following a period of SET (38). Furthermore, a higher level of NHE1 after a period of high-intensity training consisting of repeated 1-min intervals at ~150% of \( V\dot{O}_2\max \) was observed together with an increased time to exhaustion and lactate release during an incremental test (41). On the other hand, a combination of SET and aerobic high-intensity training in runners did not alter NHE1 but improved intense exercise capacity (7). Thus elevated NHE1 may contribute but is not mandatory to improve short-term and intense endurance performance.

### Table 3. Maximal activity of CS, HAD, and PFK of moderately trained endurance runners before (Pre), midway (Mid), and after (Post) an 8-week intervention period with high intensity concurrent training (HICT; \( n = 11 \)) or maintained training in a control group (CON; \( n = 7 \))

<table>
<thead>
<tr>
<th></th>
<th>HICT</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Mid</td>
</tr>
<tr>
<td>CS, ( \mu \text{mol·g dry wt}^{-1}·\text{min}^{-1} )</td>
<td>25 ± 1</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>HAD, ( \mu \text{mol·g dry wt}^{-1}·\text{min}^{-1} )</td>
<td>22 ± 1§</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>PFK, ( \mu \text{mol·g dry wt}^{-1}·\text{min}^{-1} )</td>
<td>102 ± 10</td>
<td>106 ± 11§</td>
</tr>
</tbody>
</table>

Values are means ± SE. Different from Mid: \#P < 0.05. Different from CON: §P < 0.05.

### Table 4. Muscle capillarization, fiber type distribution, and fiber type area of moderately trained endurance runners before (Pre), midway (Mid), and after (Post) an 8-wk intervention period with high intensity concurrent training (HICT; \( n = 10 \))

<table>
<thead>
<tr>
<th></th>
<th>HICT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>Capillary density, cap/mm²</td>
<td>335 ± 25</td>
</tr>
<tr>
<td>Capillary to fiber ratio (C:F)</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Type I fibers</td>
<td>64.3 ± 4.4</td>
</tr>
<tr>
<td>Type IIA fibers</td>
<td>32.9 ± 4.0</td>
</tr>
<tr>
<td>Type IX fibers</td>
<td>2.8 ± 2.3</td>
</tr>
<tr>
<td>Area type I fibers, mm²</td>
<td>6,365 ± 416</td>
</tr>
<tr>
<td>Area type IIA fibers, mm²</td>
<td>6,901 ± 569</td>
</tr>
<tr>
<td>Area type IX fibers, mm²</td>
<td>5,404 ± 782</td>
</tr>
<tr>
<td>Mean fiber area, mm²</td>
<td>6,521 ± 441</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Table 5. Ferritin, hemoglobin, leukocytes, reticulocytes, transferrin, immunoglobulin A (IgA), creatine kinase (CK), lactate dehydrogenase (LDH), cortisol and testosterone of moderately trained endurance runners before (Pre), midway (Mid) and after (Post) an 8-week intervention period with high intensity concurrent training (HICT; n = 12) or maintained training in a control group (CON; n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Ref. Level</th>
<th>Pre</th>
<th>Mid</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin, µg/l</td>
<td>12–300</td>
<td>123 ± 198</td>
<td>104 ± 14**</td>
<td>106 ± 20*</td>
</tr>
<tr>
<td>Hemoglobin, mmol/l</td>
<td>8.3–10.5</td>
<td>9.2 ± 0.1</td>
<td>9.0 ± 0.1</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Leukocytes, × 10^9/l</td>
<td>3.5–8.8</td>
<td>5.5 ± 0.3</td>
<td>5.3 ± 0.3</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>Reticulocytes, × 10^9/l</td>
<td>25–99</td>
<td>38 ± 6</td>
<td>37 ± 6</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Transferrin, µmol/l</td>
<td>24–41</td>
<td>30 ± 1</td>
<td>31 ± 1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>IgA, g/l</td>
<td>0.70–4.30</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.3**</td>
</tr>
<tr>
<td>CK, U/l</td>
<td>50–400</td>
<td>192 ± 25</td>
<td>284 ± 56</td>
<td>160 ± 27</td>
</tr>
<tr>
<td>LDH, U/l</td>
<td>105–205</td>
<td>189 ± 14</td>
<td>163 ± 9</td>
<td>166 ± 5</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>170–530</td>
<td>403 ± 45</td>
<td>424 ± 42</td>
<td>456 ± 36</td>
</tr>
<tr>
<td>Testosterone, nmol/l</td>
<td>7.6–31</td>
<td>17 ± 2</td>
<td>21 ± 2</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Reference values are 95% coefficient values generated from a representative normal Nordic population (52). Different from Pre: *P < 0.05, **P < 0.01. Different from Mid: #P < 0.05. Different from CON: §P < 0.05.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.S., P.M.C., S.L., and J.B. conception and design of experiments; C.S. and P.M.C. prepared figures; C.S., P.M.C., and M.T. analyzed data; C.S., P.M.C., S.L., T.R.A., M.T., and J.B. interpreted results of experiments; C.S. and P.M.C. prepared figures; C.S., P.M.C., and J.B. drafted manuscript; C.S., P.M.C., S.L., T.R.A., M.T., and J.B. edited and revised manuscript; C.S., P.M.C., S.L., T.R.A., M.T., and J.B. approved final version of manuscript.

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