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

Casper Skovgaard,1,2 Peter M. Christensen,1,2 Sonni Larsen,1 Thomas Rostgaard Andersen,1 Martin Thomassen,1 and Jens Bangsbo1

1Department of Nutrition, Exercise and Sports, Section of Integrated Physiology, University of Copenhagen, Copenhagen, Denmark; and 2Team Danmark (Danish Elite Sport Organization), Copenhagen, Denmark

Submitted 6 November 2013; accepted in final form 2 September 2014

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

The present study demonstrates that SET and HRT, when performed in succession together with improved running economy as well as increased dynamic muscle strength and capacity for muscular H+ transport in moderately trained endurance runners.

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% VO2max) 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
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 VO2max 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⁻¹kg⁻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, 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).

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, 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, 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, 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, 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).

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 (min + recovery (intensity))</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
<td>4 × 4 + 2 min (~85% HRmax)</td>
</tr>
<tr>
<td>AMI: Sat (time (intensity))</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</td>
<td>~50 min (75%–85% HRmax)</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-wk period with training days listed in italic.

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% HRmax 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% HRmax 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 HRT training were performed with an average of 86.1 ± 0.9% HRmax, whereas the AHI training had an average duration of 51.9 ± 4.8 min performed with an average of 82.1 ± 1.3% HRmax. 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,
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 VO2 kinetics, running economy (RE), and VO2max, 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.

VO2 kinetics, running economy, and VO2max. 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: 1) a moderate running at 12 km/h separated by 20 min of rest to quantify VO2 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 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 anesthesia (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 in a fresh batch of ice-cold modified GSK3 buffer (MG-buffer) [80 μM Mg] 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 benza-
dine, 10 μg/ml leupeptin, 2 mM Na2VO3] for 2 rounds of 30 s using a TissueLyser II (Retch, Germany). After being rotated end over end for 1 h 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 were 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, β,β-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 Protean 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, Singapore) until the desired separation was achieved between 80 and 180 min.

The desired areas of the gels were placed on a polyvinylidene difluoride (PVDF) membrane (Immobilon P Transfer Membranes, Millipore) and sandwiched between two layers of filter paper soaked in transfer-buffer [5.8 g Tris base, 2.9 g glycin, 1.5 ml SDS (of a 10% Millipore) and sandwiched between two layers of filter paper soaked in 96% ethanol, 1 liter ddH2O]. The proteins were blotted to an activated and calibrated membrane (activated in 96% trifluoride (PVDF) membrane (Immobilon P Transfer Membranes, Millipore); NHE1, 100 –110 kDa, mouse monoclonal (MA3-930, Thermo Fischer Scientific); SERCA1, 110 kDa, mouse monoclonal (MA3-930, Thermo Fischer Scientific); SERCA2, 100 kDa, goat polyclonal (Sc-8095, Santa Cruz Biotechnology).

After a brief wash in TBST (2 times 5 min), membranes were incubated with horseradish peroxidase (HRP) substrate antibody for 1 h at room temperature. The secondary HRP-conjugated antibodies were used diluted 1:5,000 in 2% nonfat milk or 3% BSA depending on the primary antibody (P-0447, P-0448 and P-0449, Dako/Cytomation). After three repetitions of membrane-washing with TBST over a period of 45 min, staining of the membranes was 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 KH2PO4 Merck 4873 in 250 ml H2O) and was titrated 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 PFK was determined fluorometrically with NAD-NADH (NADP-NADPH) coupled reactions (46) on a Fluoroskan 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 europeaus agglutinin 1 lectin (VECTB-1065, VWR, Bie and Bernten, 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, 1:1,000) 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, A21202, 1:1,000), Alexa-350 goat anti-rabbit (Invitrogen, P0994, 1:1000) and Alexa-488 donkey anti-mouse (Invitrogen, A21202, 1:1,000)] 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 Image). 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 (CF-ratio) and capillary density (cap/mm²).

Mean fiber area was assessed by manual drawing of the perimeter of fibres 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 europeaus agglutinin 1 lectin (VECTB-1065, VWR, Bie and Bernten, 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, 1:1,000) 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, A21202, 1:1,000), Alexa-350 goat anti-rabbit (Invitrogen, P0994, 1:1000) and Alexa-488 donkey anti-mouse (Invitrogen, A21202, 1:1,000)] 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 Image). 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 (CF-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, Neulii sur Seine, France).
Statistics

Student’s unpaired t-tests were used to compare subjects’ characteristics (age, height, body mass, training during lead-in, and \( V\dot{O}_{2\max} \)) in HICT and CON before INT. A two-way ANOVA for repeated measurements was used to determine the effect of INT on body mass, \( V\dot{O}_{2\max} \), performance tests, \( V\dot{O}_{2} \) 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 \( [V\dot{O}_{2\max} \text{ (ml·min}^{-1} \text{·kg}^{-1})] \), RE, \( \tau \), 1RM squat, 1RM leg press, 5RM deadlift, maximal enzymatic activity of CS, PFK and HAD, capillary density (cap/mm²), 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 higher 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).

\( V\dot{O}_{2} \) Kinetics, Running Economy, and \( V\dot{O}_{2\max} \)

In HICT, the speed of the rise in \( V\dot{O}_{2} \) (time constant, \( \tau \)) in the transition from walking to running was not changed after compared with before INT (222 ± 21 vs. 21 ± 2 s). Steady-state \( V\dot{O}_{2} \) at 12 km/h was lower \( (P < 0.01) \) after compared with before INT (2.89 ± 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\(^{-1} \text{·km}^{-1}\)) (Fig. 3). \( V\dot{O}_{2\max} \) was the same before and after INT in both HICT (60.7 ± 1.2 vs. 59.5 ± 0.8 ml·min\(^{-1} \text{·kg}^{-1}\)) and CON (58.9 ± 2.1 vs. 58.2 ± 2.3 ml·min\(^{-1} \text{·kg}^{-1}\)). No difference in \( \tau \) (19 ± 1 vs. 19 ± 2 s), steady-state \( V\dot{O}_{2} \) 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 \( V\dot{O}_{2\max} \) (ml·min\(^{-1} \text{·kg}^{-1}\)) \((n = 18, r^2 = 0.20, \tau (n = 18, r^2 = 0.47) \) and RE \((n = 18, r^2 = 0.35)\). The 1,500-m performance correlated with \( V\dot{O}_{2\max} \) (ml·min\(^{-1} \text{·kg}^{-1}\)) \((n = 18, r^2 = 0.50)\). And last, Yo-Yo IR2 performance correlated with \( V\dot{O}_{2\max} \) (ml·min\(^{-1} \text{·kg}^{-1}\)) \((n = 17, r^2 = 0.22)\). After 8 wk of HICT, \( V\dot{O}_{2\max} \) (ml·min\(^{-1} \text{·kg}^{-1}\)) correlated \( (P < 0.05) \) with 1,500-m \((n = 12, r^2 = 0.40)\) and Yo-Yo IR2 \((n = 12, r^2 = 0.80)\) performance. No other correlations were found between performance and \( \tau \), RE, or \( V\dot{O}_{2\max} \) 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.

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)

| Group | 10 km (min:s) | 1,500 m (min:s) | Yo-Yo IR2 (m) | \( \tau \) (s) | RE (ml·kg\(^{-1} \text{·km}^{-1}\)) | \( V\dot{O}_{2\max} \) (ml·min\(^{-1} \text{·kg}^{-1}\)) | Body mass (kg) | 1RM squat (kg) | 1RM leg press (kg) | 5RM deadlift (kg) | CS (\( \mu \text{mol·g} \) dry wt \(^{-1} \text{·min}^{-1}\)) | HAD (\( \mu \text{mol·g} \) dry wt \(^{-1} \text{·min}^{-1}\)) | PFK (\( \mu \text{mol·g} \) dry wt \(^{-1} \text{·min}^{-1}\)) |
|-------|--------------|----------------|--------------|--------------|----------------|----------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| HICT vs. CON | 0.609 | 0.594 | 0.165 | 0.295 | 0.121 | 0.450 | 0.866 | 0.014* | 0.035* | 0.101 | 0.330 | 0.044* | 0.001** |
| Pre vs. post | 0.398 | 0.095 | 0.018* | 0.939 | 0.001** | N/A | N/A | <0.001*** | <0.001*** | <0.001*** | N/A | N/A | 0.018* | 0.012* |
| Pre vs. mid | 0.475 | 0.218 | 0.205 | N/A | N/A | N/A | N/A | 0.046* | 0.046* | 0.018* | 0.068 | 0.789 | 0.657 | 0.939 |
| Mid vs. post | 0.570 | 0.414 | 0.129 | 0.525 | 0.012* | 0.018* | 0.018* | 0.054 | 0.054 | 0.018* | 0.037* | 0.054 | 0.724 |

**CS**, citrate synthase; **HAD**, β-hydroxyacyl-CoA-dehydrogenase; **PFK**, phosphofructokinase. Main effect: *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).
by 12% (126 ± 6 kg), leg press by 18% (271 ± 16 kg), and
deadlift by 22% (122 ± 6 kg) corresponding to an unchanged
1RM squat, 9% improved (P < 0.01) 1RM leg press, and 7%
Improved (P < 0.01) 5RM deadlift during the last 4 wk. 1RM
Squat and leg press as well as 5RM deadlift remained un-
changed in CON. Before INT, Yo-Yo IR2 performance corre-
lated (P < 0.05) with 1RM squat (n = 18, r^2 = 0.38), 1RM leg
press (n = 18, r^2 = 0.21), and 5RM deadlift (n = 17, r^2 =
0.25). In HICT, 1RM squat correlated (P < 0.05 and P < 0.01)
with Yo-Yo IR2 performance (n = 10, r^2 = 0.48 and n = 11,
 r^2 = 0.57) after 4 and 8 wk, respectively. Furthermore, 5RM
deadlift correlated (P < 0.01) after 8 wk of HICT with Yo-Yo
IR2 performance (n = 10, r^2 = 0.57).

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 ± 0.3 vs. 3.6 ± 0.3) and “General Recovery” (3.6 ± 0.4 vs. 4.1 ± 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 $\dot{V}O_2\max$, muscle morphology, capillarization, and muscle Na$^+$-K$^+$ pump $\alpha_1$, $\alpha_2$ and $\beta_1$ 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...
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 $V\dot{O}_{2\max}$ 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 $V\dot{O}_{2\max}$, capillary density, and CS and HAD activity are not mandatory for improving endurance performance in already trained subjects.

Fig. 4. Maximal strength of moderately trained endurance runners in 1 repetition maximum (1RM) squat (A), 1RM leg press (B), and 5RM deadlift (C) before (Pre), midway (Mid), and after (Post) an 8-wk intervention period with HICT (closed symbols, left; squat and leg press, $n = 12$; deadlift, $n = 11$) or maintained training in a control group (CON; open symbols, right; $n = 8$). Values are means ± SE. Different from Pre: **P < 0.01, ***P < 0.001. Different from Mid: ##P < 0.01. Different from CON: $\S$P < 0.05, §§P < 0.01.

The estimated fractional utilization of $V\dot{O}_{2\max}$ (21) during the 10-km run after compared with before HICT (75 ± 1% vs. 73 ± 1% $V\dot{O}_{2\max}$) 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 (VO2max 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

---

**Fig. 5.** Protein content of NHE1 (A), SERCA1 (B), and SERCA2 (C) in moderately trained endurance runners before (Pre), midway (Mid), and after (Post) an 8-wk intervention period with HICT (closed symbols, left; NHE1 n = 9; SERCA1 n = 12; SERCA2, n = 11) or maintained training in a control group (open symbols, right; NHE1 n = 7; SERCA1 n = 6; SERCA2 n = 7). Values are geometric means ± 95% confidence interval (CI) (Mid and Post relative to Pre). Different from Pre: *P < 0.05, ***P < 0.001.
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 \( \text{V}^{\text{\text{\text{-}}}2} \text{O}_{2} \) 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), \( \text{V}^{\text{\text{\text{-}}}2} \text{O}_{2} \) 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, stiffness of the tendons was increased by 10.2 ± 3.6 on April 30, 2017 http://jap.physiology.org/ Downloaded from
Studies adding SET to the normal training of runners have shown to result in increased content of Na\(^+\)/K\(^+\) pump subunits following training (7, 38). However, in the present study no increase was observed in muscle proteins involved in potassium handling following HICT, and similarly, in a recent study on cyclists performing SET and aerobic high-intensity training no changes were found in Na\(^+\)/K\(^+\) pump subunits despite an improved ability to perform intense exercise (31). Thus these latter studies suggest that improvements in intense exercise performance can occur without changes in the amount of Na\(^+\)/K\(^+\) pumps.

Based on blood markers and questionnaires, no signs of overtraining were apparent in HICT, which fits well with the improvements seen in performance. This may relate to the long recovery (days) between the intense training sessions. In support, it has been reported that intense sprint capacity was not elevated when SET was performed daily for a 2-wk period whereas performance was improved when 2-days of recovery separated the training sessions (53). It should be noted, however, that in HICT, ferritin was lowered after 4 wk of INT and remained lowered after 8 wk, although there may not have been a functional significance of the lowered ferritin, since a decrease in ferritin will not affect performance without other signs of anemia (54). Taken together, it seems prudent to include recovery day(s) after intense training such as HICT, but it remains to be investigated if subjects, as in the present study, could train more additional aerobic training and gain from this or whether this would impair the adaptations.

In summary, 8 wk of concurrent SET and HRT along with a reduced volume of aerobic training improved performance of moderately trained endurance runners during long (10-km), short (1,500-m) and intense (Yo-Yo IR2) tests, which was associated with improvement in RE and dynamic muscle strength as well as a higher content of muscle NHE1. Future studies are needed to address if the order of the SET and HRT influences the adaptations, and it remains to be elucidated if the use of both SET and HRT provide larger gains in RE and performance relative to only using one of the training forms as well as the relationship between intensified training and the effect on performance longer than 10 km.

### ACKNOWLEDGMENTS

We thank the subjects for their participation. We also thank F. Ægidius and K. Danielsen for excellent assistance during training and testing. The excellent technical assistance by J. J. Nielsen, L. N. Nielsen, Peter Piil, and T. P. Gunnarsson is highly appreciated.

### GRANTS

The study was supported by Team Danmark.

### 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. conceived and designed the research; C.S. and S.L. performed experiments; C.S., P.M.C., S.L., T.R.A., and M.T. analyzed data; C.S., P.M.C., S.L., T.R.A., M.T., and J.B. interpreted the 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.

### REFERENCES


---

### 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>HICT Pre</th>
<th>HICT Mid</th>
<th>HICT Post</th>
<th>CON Pre</th>
<th>CON Mid</th>
<th>CON 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>
<td>65 ± 16</td>
<td>79 ± 18</td>
<td>88 ± 18</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>
<td>8.8 ± 0.2</td>
<td>8.7 ± 0.2</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td>Leukocytes, × 10(^9)/liter</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>
<td>5.4 ± 0.8</td>
<td>5.3 ± 0.4</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Reticulocytes, × 10(^9)/liter</td>
<td>25–99</td>
<td>38 ± 6</td>
<td>37 ± 6</td>
<td>36 ± 4</td>
<td>33 ± 3</td>
<td>31 ± 3</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>Transferrin, µmol/l</td>
<td>24–41</td>
<td>30 ± 1</td>
<td>31 ± 1</td>
<td>32 ± 1</td>
<td>34 ± 2</td>
<td>35 ± 2</td>
<td>35 ± 2</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>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 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>
<td>277 ± 85</td>
<td>172 ± 22</td>
<td>224 ± 46</td>
</tr>
<tr>
<td>LDH, U/l</td>
<td>105–205</td>
<td>189 ± 14</td>
<td>163 ± 9</td>
<td>166 ± 5</td>
<td>163 ± 11</td>
<td>172 ± 11</td>
<td>175 ± 13</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>170–530</td>
<td>403 ± 45</td>
<td>424 ± 42</td>
<td>456 ± 36</td>
<td>504 ± 42</td>
<td>425 ± 53</td>
<td>526 ± 41#</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>
<td>16 ± 2</td>
<td>16 ± 2</td>
<td>16 ± 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.


