Effect of two different intense training regimens on skeletal muscle ion transport proteins and fatigue development

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Submitted 11 April 2006; accepted in final form 22 December 2006

Mohr M, Krstrup P, Nielsen JJ, Nybo L, Rasmussen MK, Juel C, Bangsbo J. Effect of two different intense training regimens on skeletal muscle ion transport proteins and fatigue development. Am J Physiol Regul Integr Comp Physiol 292: R1594–R1602, 2007. First published December 28, 2006; doi:10.1152/ajpregu.00251.2006.—This study examined the effect of two different intense exercise training regimens on skeletal muscle ion transport systems, performance, and metabolic response to exercise. Thirteen subjects performed either sprint training [ST; 6-s sprints (n = 6)], or speed endurance training [SET; 30-s runs ∼130% VO2max, n = 7]. Training in the SET group provoked higher (P < 0.05) plasma K+ levels and muscle lactate/H+ accumulation. Only in the SET group was the amount of the Na+/H+ exchanger isoform 1 (31%) and Na+/K+-ATPase isoform α2 (68%) elevated (P < 0.05) after training. Both groups had higher (P < 0.05) levels of Na+/K+-ATPase β1-isoform and monocarboxylate transporter 1 (MCT1), but no change in MCT4 and Na+/K+-ATPase α1-isoform. Both groups had greater (P < 0.05) accumulation of lactate during exhaustive exercise and higher (P < 0.05) rates of muscle lactate decrease after exercise. The ST group improved (P < 0.05) sprint performance, whereas the SET group elevated (P < 0.05) performance during exhaustive continuous treadmill running. Improvement in the Yo-Yo intermittent recovery test was larger (P < 0.05) in the SET than ST group (29% vs. 10%). Only the SET group had a decrease (P < 0.05) in fatigue index during a repeated sprint test. In conclusion, turnover of lactate/H+ and K+ in muscle during exercise does affect the adaptations of some but not all related muscle ion transport proteins with training. Adaptations with training do have an effect on the metabolic response to exercise and specific improvement in work capacity.

SKELETAL MUSCLES HAVE A HIGH capacity to adapt to increases in loading, such as that which occurs during exercise training. Thus, a high number of studies have demonstrated that variables such as the number of capillaries, activity of oxidative enzymes, and amount of mitochondria are elevated in the activated muscles after a period of interval and endurance training (41, 44). Such adaptations lead to a greater fat and lower carbohydrate oxidation at submaximal work loads and increased endurance performance (26, 41). Several studies have also been conducted to examine the effect of intense training with an exercise load above the intensity eliciting the maximum oxygen uptake (VO2max) (29, 34, 42). It is a common finding that the activity of creatine kinase (CK), glycolytic enzymes, e.g., phosphofructokinase (PFK), the relative number of fast-twitch-a fibers and muscle buffer capacity are elevated after a period of high-intensity training (21, 22, 24). Some studies have also demonstrated that intense training leads to a higher lactate-H+ transport capacity and amount of both monocarboxylate transporters (MCT) and Na+/H+ exchanger isoform 1 (NHE1) (2, 23, 25, 36, 37), as well as a larger Na+/K+ pump activity and amount of Na+/K+ pumps (18, 19, 29, 31, 34). These changes are among other adaptations associated with a lowering of muscle interstitial potassium concentration and delayed development of fatigue during intense exercise (34). However, little is known about what causes these muscle adaptations and how they are related to the improvement in different kinds of muscle performance.

It is a common belief that the flux through a metabolic pathway or a transport system is a crucial factor in the adaptation of the contracting muscle, but no clear evidence has been provided. One way to study factors that may be of importance for the adaptation to training may be to carry out various training protocols that result in different metabolic responses in the contracting muscle and then examine the changes in the muscle variables. One study has compared the effect of a period with either 6-s or 30-s strength training bouts and found a different response in PFK and CK with the two training regimens (11). However, in that study the physiological response, such as changes in muscle lactate, pH, and creatine phosphate (CP), to the different training protocols was not examined; and the variables, assumed to be of importance for changes in exercise performance during intense exercise, such as the MCT isoforms, NHE1, and Na+/K+ pump isoforms, were not determined.

Thus, the aim of the present study was to use two different high-intensity exercise training regimens, expected to provide a different physiological response, to examine the adaptations in muscle ion transport proteins, and relate those to the metabolic response, as well as performance in different types of exercise, to evaluate to what extent the adaptations may be important for the work capacity of the muscle.

MATERIALS AND METHODS

Subjects

Thirteen healthy male subjects participated in the study. They were normally active, and no one trained for competition. They were randomly separated into two different training groups. One group (n = 6) was assigned to perform intermittent 6-s runs at near-maximal speed (sprint training group; ST), while the other subjects (n = 7)
carried out 30-s runs at a lower-intensity (speed endurance training group; SET). The age, body mass, height, and \( V\dot{O}_2 \) max of subjects in the ST group was 26.7 ± 1.7 (mean ± SE) yr, 77.5 ± 3.8 kg, 182.3 ± 2.9 cm, and 50.2 ± 1.5 ml \( O_2 \)-kg\(^{-1}\)-min\(^{-1}\), respectively, and not different from the subjects in SET (24.6 ± 0.6 yr, 78.2 ± 3.1 kg, 182.6 ± 2.6 cm, and 49.0 ± 1.6 ml \( O_2 \)-kg\(^{-1}\)-min\(^{-1}\), respectively). All subjects were informed of any risks and discomforts associated with the experiment before giving their written consent to participate. The study conforms with the code of Ethics of World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

**Design and Experimental Procedures**

Before and after the training period, both ST and SET groups carried out a number of performance tests (see below): 1) Yo-Yo intermittent recovery test level 2 (Yo-Yo IR-2), 2) repeated sprint test consisting of five 30-m sprints interspersed by 25 s of active recovery, 3) two 50-m sprints separated by 5 min of recovery, and 4) incremental treadmill test until exhaustion. Besides the posttraining Yo-Yo IR-2 test to exhaustion, an additional test was carried out in which the Yo-Yo IR-2 test was terminated, at the exact point where the subjects became exhausted in the pretraining test, to study the physiological response at this specific point.

All tests were preceded by pretests to familiarize the subjects with the test procedure. The tests were performed on separate days with at least 48 h of recovery between tests, except the two sprint tests that were performed on the same day (first the 50-m sprint test and then the 30-m repeated sprint test after 60 min of recovery). After training, the Yo-Yo IR-2 test was the first test that the subjects completed (48 h after the last training session). The day after the Yo-Yo IR-2 test, the subjects had one extra training session, and then, after 48 h of recovery, they performed the sprint tests in the same order as before training. After the sprint tests, the subjects had yet another training session, and 48 h later, they performed the incremental treadmill test. After another 48 h of recovery, they finally completed the last Yo-Yo IR-2 test, where they ran until the point where fatigue occurred before the training was initiated.

**Test Procedures**

The Yo-Yo IR-2 was performed indoors on a wooden surface on running lanes having a width of 2 m and a length of 20 m. The test was performed after a 10-min warm-up period. The Yo-Yo IR-2 has been described and evaluated elsewhere (3). Briefly, it consists of repeated 20-m runs at a progressively increased speed controlled by audio bleeps from a tape recorder. Between each running bout the subjects carried out 5 min of the Yo-Yo IR-1 test to raise muscle temperature to a level above 38°C (27). Thereafter, the subjects rested for 4 min before they performed the Yo-Yo IR-2 test (3). Blood was sampled at rest and prior to the test, as well as during the 10-s recovery period after 80, 160, 280, 360, 440, 520, 600, 680, 760, 840, 920 m, etc. In addition, blood was collected at the point of fatigue, as well as 1, 3, 5, 10, and 15 min after the test. A muscle biopsy was taken (7) immediately after the last interval in both the first and the last training session.

**Experimental Protocol**

The study included three experimental days with invasive measurements before, during, and after the Yo-Yo IR-2 test. On an experimental day the subjects reported to the laboratory in the morning after consuming a light meal. Intake of caffeine on the day of the experiment, as well as alcohol consumption and heavy physical activity on the day prior to the experiment, was avoided. A heart rate monitor was placed and after resting for ~30 min, a catheter (18 gauge, 32 mm) was inserted in an antecubital vein and covered by a wrist bandage. In preparation for obtainment of biopsy in the musculus vastus lateralis, at exhaustion an incision was made under local anesthesia (20 mg/l lidocaine without adrenalin), which was covered by sterile Band-Aid strips and a thigh bandage during exercise. For the warm-up, the subjects carried out 5 min of the Yo-Yo IR-1 test to raise muscle temperature to a level above 38°C (27). Thereafter, the subjects rested for 4 min before they performed the Yo-Yo IR-2 test (3). Blood was sampled at rest and prior to the test, as well as during the 10-s recovery period after 80, 160, 280, 360, 440, 520, 600, 680, 760, 840, 920 m, etc. In addition, blood was collected at the point of fatigue, as well as 1, 3, 5, 10, and 15 min after the test. A muscle biopsy was obtained at rest, at exhaustion, and 3 min into recovery. In addition, heart rate was recorded during the test. This experimental procedure was carried out both before and after the training period. In addition, the subjects took part in a third supplementary experimental day after training, where the experimental procedure was the same, except that the subjects only ran to the point of fatigue prior to training (submaximal Yo-Yo IR-2 test). A muscle biopsy was collected at the end of the test.
Blood Analysis

Immediately after sampling, 100 μl of blood was hemolyzed in an ice-cold 100 μl Triton X-100 buffer solution, and was later analyzed for lactate using an YSI 2300 lactate analyzer (Yellow Spring Instruments, Yellow Springs, OH) (15). The rest of the sample was rapidly centrifuged for 30 s. From this, plasma was collected and stored at −20°C until subsequent analysis. Plasma potassium concentration was measured using an electrometric method (model FLM3; Radiometer, Copenhagen, Denmark) with lithium as internal standard.

Muscle Analysis

The muscle tissue was immediately frozen in liquid N2 and stored at −80°C. The frozen sample was weighed before and after freeze drying to determine water content. After freeze drying, the muscle tissue was dissected free of blood, fat, and connective tissue and stored at −80°C until analyzed for CP and lactate by a fluorometric assay (25, 28). Another 1–2 mg dry wt muscle tissue was extracted in a nonbuffering solution containing 145 mM KCl, 10 mM NaCl, and 5 mM iodoacetic acid (25). CK activity was determined fluorometrically on whole muscle homogenized in a tetra ethyl ammonium-Tris acid buffer (28).

A nonbuffering solution containing 145 mM KCl, 10 mM NaCl, and 5 mM iodoacetic acid (25). CK activity was determined fluorometrically on whole muscle homogenized in a tetra ethyl ammonium-Tris acid buffer (28).

Muscle Fiber Type Analysis

A part of the resting biopsy obtained from the subjects before and after training was mounted in an embedded medium (OCT Compound Tissue-Tek; Sakura Finetek, Zoeterwoude, The Netherlands) and stored at −80°C until analyzed for fiber type distribution by histochemical analysis (8). Five serial 10-μm thick sections were cut at −20°C and incubated for myofibrillar ATPase histochemical analysis at pH 9.4, after preincubation at pH 4.3, 4.6, and 10.3. Based on the myofibrillar ATPase staining, individual fibers were classified under light microscopy as slow-twitch or fast-twitch fibers (8).

Determination of Muscle Transporters

About 20–50 mg of each muscle sample were homogenized in sucrose buffer (in mM: 250 sucrose, 30 HEPES, 2 EGTA, 40 NaCl, and 2 PMSF, pH 7.4) with a Polytron 2100 and centrifuged at 1,000 g for 5 min. This procedure removes heavy material, including a fraction of the mitochondria. The pellet was then homogenized and centrifuged once more. The supernatant was then spun at 190,000 g and the resulting protein content was determined with a BSA standard (DC protein assay; Bio-Rad). Samples were mixed 1:1 with sample buffer (1 mM Tris·HCl, 0.1 mM EDTA, 10 mM DTT, 2% SDS, 5% glycerol, 0.02% Bromphenol Blue, pH 8.0) and heated 30 min at 37°C. Protein (11.25 μg) from each sample was subjected to SDS-PAGE (Excell 8 –18% gradient gel) and electroblotted to a Millipore Immobilon-P polyvinylidene difluoride membrane. The membrane was blocked by 5% BSA, 0.1% Tween 20, and 1% low-fat milk and was incubated with the primary antibody diluted in a BSA-containing buffer. After treatment with the horseradish peroxidase-coupled secondary antibody (DAKO), it was repeatedly washed with distilled water, 0.05% Tween-20 and 1 mM NaCl (see also Refs. 25 and 38). The membrane was incubated with enhanced chemiluminescence reagent (Amer sham) and visualized on film. The quantification of protein was performed by scanning the film and analyzing band intensities with SigmaGel software (SigmaGel analysis software version 1.0). Samples from the same subject were always run on the same gel and with identical amounts of protein per lane.

NHE1 was detected by the antibody MAB3140 (Chemicon). The antibodies to the human MCT1 were a gift from Professor A. Halestrap (Bristol, UK). MCT4 was detected by the antibody Sc-14934 (Santa Cruz Biotechnology, Santa Cruz, CA). Na+/K+-ATPase α1-isoform, was detected by the antibody α6F (Iowa Hybridoma Bank), whereas Na+/K+-ATPase α2- and β1-antibodies were gifts from Professor P. A. Pedersen (IMBF, Copenhagen, Denmark).

Statistics

A two-factor (time × training) repeated-measures ANOVA was used to evaluate possible differences in the physiological response to the Yo-Yo IR-2 test for each of the two training groups (ST and SET). Likewise, a two-factor repeated-measures ANOVA was used to evaluate possible differences in the physiological response to the first and last training session within each of the two groups. A one-factor ANOVA was used to evaluate possible differences in the physiological response to training and testing between ST and SET. Possible changes in muscle characteristics and performance as a result of training were tested by a one-factor repeated-measures ANOVA. When a significant interaction was detected, data were subsequently analyzed by using a Newman-Keuls post hoc test. Possible between-group differences in changes in muscle characteristics and performance were tested by Student’s unpaired t-test. The significance level was set to P < 0.05. Values are presented as means ± SE.

RESULTS

Physiological Response to the Training Sessions

Mean heart rate during the first training in SET was 162 ± 3 beats/min or 84.3 ± 1.1% of the maximal heart rate, which was higher (P < 0.05) than the corresponding values in ST (159 ± 6 beats/min and 79.9 ± 5.1%; Fig. 1A). Peak heart rate reached in SET during the 15 intervals was 97.0 ± 1.1% of maximal heart rate, which also was higher (P < 0.05) than in ST (88.2 ± 0.6%). Similar heart rate values were obtained in the last training session (Fig. 1A).

During the first training session in SET, the blood lactate concentration rose from 1.1 ± 0.1 to 14.6 ± 1.0 mmol/l, which was lower (P < 0.05) than during the final training session (16.7 ± 1.1 mmol/l; Fig. 1B). In SET, peak blood lactate concentration for the first and last training sessions was similar (8.3 ± 0.6 and 8.7 ± 0.8 mmol/l, respectively) and lower (P < 0.05) than in SET (Fig. 1B). Plasma potassium was not different when the first and the final training session were compared in either ST or SET (Fig. 1C). In SET, plasma potassium peaked at 6.4 mmol/l, both during the first and final training session, which was higher (P < 0.05) than the corresponding values in ST (~5.5 mmol/l; Fig. 1C).

Muscle lactate concentration at the end of the first and last training sessions was the same in both SET and ST, but higher (P < 0.05) in SET than in ST (Table 1). Correspondingly, muscle pH at the end of both the first and last training session was lower (P < 0.05) in SET compared with ST (6.98 ± 0.03 and 6.98 ± 0.04 vs. 7.06 ± 0.03 and 7.07 ± 0.03 −log [H+]). Muscle CP after the first and last training session was not significantly different either in SET (49.3 ± 6.7 and 41.8 ± 4.7 mmol/kg dry wt, respectively) or in ST (53.7 ± 6.3 and 44.7 ± 4.7 mmol/kg dry wt; Table 1). Muscle glycogen after the first and last training session in SET was 400 ± 39 and 353 ± 23...
mmol/kg dry wt, respectively, and 370/H11006 362/H11006 17 mmol/kg dry wt in ST, respectively (Table 1).

Effect of Training on Body Mass and Muscle Characteristics

The training period lowered (P < 0.05) the body mass from 78.2/H11006 3.1 to 77.2/H11006 3.0 kg in SET and from 77.5/H11006 3.8 to 76.4/H11006 3.5 kg in ST with no differences between groups. In SET, the amount of NHE1 was 31/H11006 12% higher (P < 0.05) after, compared with before, the training period, whereas it was unchanged in ST (Fig. 2). The level of MCT1 was higher (P < 0.05) after the training period in both SET and ST (28/H11006 12 and 30/H11006 9%, respectively), whereas MCT4 did not change significantly in either group. The Na\(^+\)-K\(^+\)-ATPase 2-isoform was elevated (P < 0.05) after the training period by 68/H11006 26% in SET, and not in ST, whereas the 1-isoform was higher (P < 0.05) in both SET and ST, and the 1-isoform was not significantly changed with training in either groups.

Muscle fiber type distribution was the same before and after the training period in both SET and ST (Table 2). In SET, muscle CK was not changed, whereas in ST, it was higher (P < 0.05) after, compared with before, the training period (4,265/H11006 127 vs. 4,656/H11006 186 mmol·g\(^{-1}\)·dry wt\(^{-1}\)·min\(^{-1}\); Table 2).

Table 1. Muscle CP, lactate, pH, and glycogen after the first and last training session in the sprint training group and the speed endurance training group

<table>
<thead>
<tr>
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<th>ST First Training</th>
<th>ST Last Training</th>
<th>SET First Training</th>
<th>SET Last Training</th>
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</thead>
<tbody>
<tr>
<td>Muscle CP, mmol/kg dw</td>
<td>53.7±6.3</td>
<td>44.7±4.5</td>
<td>49.3±6.7</td>
<td>41.8±4.7</td>
</tr>
<tr>
<td>Muscle lactate, mmol/kg dw</td>
<td>30.0±4.7</td>
<td>31.1±5.2</td>
<td>47.1±6.0*</td>
<td>44.8±3.9*</td>
</tr>
<tr>
<td>Muscle pH, log [H(^+)]</td>
<td>7.06±0.03</td>
<td>7.07±0.03</td>
<td>6.98±0.03*</td>
<td>6.98±0.04*</td>
</tr>
<tr>
<td>Muscle glycogen, mmol/kg dw</td>
<td>370±48</td>
<td>362±17</td>
<td>400±39</td>
<td>353±23</td>
</tr>
</tbody>
</table>

Values are means ± SE. CP, creatine phosphate; ST, sprint training; SET, speed endurance training, dw, dry weight. *Significant difference from ST group.

mmol/kg dry wt, respectively, and 370 ± 48 and 362 ± 17 mmol/kg dry wt in ST, respectively (Table 1).

Effect of Training on Body Mass and Muscle Characteristics

The training period lowered (P < 0.05) the body mass from 78.2 ± 3.1 to 77.2 ± 3.0 kg in SET and from 77.5 ± 3.8 to 76.4 ± 3.5 kg in ST with no differences between groups.

In SET, the amount of NHE1 was 31 ± 12% higher (P < 0.05) after, compared with before, the training period, whereas it was unchanged in ST (Fig. 2). The level of MCT1 was higher (P < 0.05) after the training period in both SET and ST (28 ± 12 and 30 ± 9%, respectively), whereas MCT4 did not change significantly in either group. The Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\)-isoform was elevated (P < 0.05) after the training period by 68 ± 26% in SET, and not in ST, whereas the \(\beta_1\)-isoform was higher (P < 0.05) in both SET and ST, and the \(\alpha_1\)-isoform was not significantly changed with training in either groups.

Muscle fiber type distribution was the same before and after the training period in both SET and ST (Table 2). In SET, muscle CK was not changed, whereas in ST, it was higher (P < 0.05) after, compared with before, the training period (4,265 ± 127 vs. 4,656 ± 186 mmol·g\(^{-1}\)·dry wt\(^{-1}\)·min\(^{-1}\); Table 2).

Fig. 1. Heart rate (A), blood lactate (B), and plasma potassium (C) during the first and final training session for sprint training (ST) and speed endurance training (SET). #Significant (P < 0.05) difference between ST and SET (P < 0.05); §significant (P < 0.05) difference between first and last training session.

Fig. 2. Training-induced change in muscle monocarboxylate transporters (MCT1, MCT4, Na\(^+\)/H\(^+\) exchanger isofor 1 (NHE1) and Na\(^+\)-K\(^+\)-ATPase \(\alpha_1\), \(\alpha_2\), \(\beta_1\)-isoforms. #Significant difference between ST and SET (P < 0.05); §significant (P < 0.05) difference between before and after the training period.
Effect of Training on Performance

In SET, performance in the Yo-Yo IR-2 was 28.7 ± 7.5% better (606 ± 72 vs. 483 ± 61 m; P < 0.05) after, compared with before, the training period, which was a greater (P < 0.05) improvement than observed in ST (573 ± 62 m vs. 520 ± 54; 9.9 ± 2.4%, Fig. 3).

In SET, the 50-m sprint time was not changed with training (7.26 ± 0.16 vs. 7.15 ± 0.16 s), whereas in ST, it was 5.8% better (7.25 ± 0.22 vs. 6.99 ± 0.18 s; P < 0.05; Fig. 3). No difference was observed in sprint performance between SET and ST either before or after the training period. After the training period, the mean sprint time for the five sprints was lower (P < 0.05) than before training (4.78 ± 0.11 vs. 4.58 ± 0.10 and 4.90 ± 0.13 vs. 4.78 ± 0.11 s, respectively; Fig. 3). As a result of training, in SET the fatigue index was 53.8 ± 9.9% lower (P < 0.05), whereas it remained the same in ST (Fig. 3).

In SET, time to exhaustion in the incremental treadmill was longer (P < 0.05) after, compared with before, the training period (4.77 ± 0.31 vs. 4.16 ± 0.41 min), whereas it was not changed significantly in ST (Fig. 3).

Effect of Training on Physiological Response to the Yo-Yo Intermittent Recovery Test

Plasma potassium. Plasma potassium was 3.9 – 4.1 mmol/l at rest in both groups before and after training. During the Yo-Yo IR-2 test, plasma potassium increased gradually and peaked at exhaustion at 6.0 – 6.4 mmol/l without differences between ST and SET (Fig. 4). In both ST and SET groups, plasma potassium was the same during the Yo-Yo IR-2 before and after the training period, except in the SET group where plasma potassium at exhaustion before training was higher (P < 0.05) compared with the same time point after the training period (6.2 ± 0.2 vs. 5.2 ± 0.1 mmol/l) revealed by the submaximal Yo-Yo IR-2 test.

Blood and muscle lactate. In SET, the blood lactate concentration at 80, 160, 280, and 360 m of the Yo-Yo IR-2 test was lower (P < 0.05) after the training period, while no differences were observed in ST (Fig. 5). After the training period, the blood lactate level at exhaustion was higher (P < 0.05) in both SET (11.6 ± 0.9 vs. 9.8 ± 1.0 mmol/l) and ST (11.4 ± 0.6 vs. 9.6 ± 1.2 mmol/l), and remained higher (P < 0.05) during the first 5 min of recovery. In addition, the peak blood lactate concentration increased (P < 0.05) from 12.3 ± 0.8 and 11.0 ± 1.1 mmol/l before training to 14.1 ± 0.6 and 13.5 ± 0.7 mmol/l after training in SET and ST, respectively. After training, the blood lactate level was 20% lower (P < 0.05) at the point where the subjects fatigued prior to training in SET, but was the same in ST (Fig. 5).

After the training period, the muscle lactate concentration in SET rose (P < 0.05) to 71.4 ± 13.9 mmol/kg dry wt, which was higher (P < 0.05) than before the training (45.3 ± 6.9 mmol/kg dry wt; Table 3). Also in ST, muscle lactate was higher after the training period (64.7 ± 5.7 vs. 47.4 ± 4.7 mmol/kg dry wt; Table 3). When the subjects after training ran to the point where fatigue occurred prior to the training period, the muscle lactate level in SET tended to be lower (P = 0.091) than before training (33.8 ± 7.4 vs. 45.3 ± 6.9 mmol/kg dry wt). The rate of muscle lactate decrease during the first 3 min of recovery was higher (P < 0.05) after than before the training period in both SET (108.2 ± 25.3 vs. 30.4 ± 15.3 μmol·kg⁻¹·dry wt·s⁻¹) and ST (108.8 ± 31.4 vs. 52.2 ± 23.7 μmol·kg⁻¹·dry wt·s⁻¹).

Muscle pH and CP. Muscle pH at exhaustion was the same before and after training in both SET (6.79 ± 0.04 and 6.78 ± 0.06 −log [H⁺]) and ST (6.82 ± 0.04 and 6.80 ± 0.05 −log [H⁺]). In SET, muscle pH at the point where fatigue occurred before training tended (P = 0.068) to be higher after training, whereas there was no difference in ST (Table 3). In SET, the increase in muscle pH during the first 3 min of recovery was higher (P < 0.05) after, compared with before, the training period (0.16 ± 0.02 vs. 0.07 ± 0.02 −log [H⁺]), whereas no changes were observed in ST.

In both groups, muscle CP utilization during the test and CP resynthesis during the first 3 min of recovery before and after the training period were not significantly different (Table 3).

DISCUSSION

The major findings of the present study were that NHE1 and the Na⁺-K⁺-ATPase α2-isoform were significantly elevated in the SET group and not changed in the ST group after the training period. Furthermore, only in ST was a significant elevation of CK activity observed, whereas both groups had
higher levels of MCT1 and the Na\(^+\)-K\(^+\)-ATPase \(\beta_1\)-isoform after the training period. Performance of a 50-m sprint was improved only in the ST group, whereas the time to fatigue during incremental treadmill running was only elevated in the SET group. The improvement in the Yo-Yo IR-2 test was significantly larger in SET than in ST. In SET the blood lactate concentration during the Yo-Yo test was lower after the training period, but higher at exhaustion, and the decrease in muscle lactate and increase in muscle pH were more pronounced during the first 3 min of recovery after training, whereas no difference was observed in the change in muscle pH during recovery in ST. It is clear that there were some similarities in the muscle adaptations and metabolic response to exercise, as well as improvements in performance, with the two training forms, but also that there were major differences. Thus, these findings allow a discussion about the importance of factors that may have influenced the muscle adaptations observed and the effect of the adaptations for the work capacity. It should be emphasized, however, that it is possible that the training also has affected other muscle variables, as well as the central nervous system, which may have contributed to the improvements in performance.

The speed endurance training compared with the speed training led to a significantly greater accumulation of lactate both in the muscle and blood, as well as a more marked decrease in muscle pH. Despite these differences and that the exercise time and the distance covered for each training bout was longer in SET compared with ST [4 min vs. 90 s and 1,264–1,360 (range) m vs. 600–630 m], MCT1, increased significantly in both SET and ST, while no change was observed in MCT4. Previous studies have shown that both MCT1 and MCT4 can be elevated by high-intensity training (2, 25, 36, 37). Nevertheless, the finding that SET did not have a greater response in MCT1 than ST despite the differences in the physiological training response suggests that it is not the degree of lactate and H\(^+\) accumulation that is the main factor in stimulating the synthesis of these proteins. On the other hand, NHE1 increased only in SET. The observation that muscle pH was lowered to 6.98 in SET compared with 7.07 in ST during the training could lead to the proposal that a

Fig. 4. Plasma potassium before, during, and after the Yo-Yo IR-2 test before and after the training period in ST (A) and SET (B). §Significant (\(P < 0.05\)) difference between before and after the training period.

Fig. 5. Blood lactate before, during and after the Yo-Yo IR-2 test before and after the training period in ST (A) and SET (B). §Significant (\(P < 0.05\)) difference between before and after the training period.
Table 3. **Muscle CP, lactate, pH, and glycogen at rest, at exhaustion, and 3 min after the Yo-Yo intermittent recovery test (level 2) before and after training period for the ST and SET**

<table>
<thead>
<tr>
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<th>ST Posttraining</th>
<th>SET Pretraining</th>
<th>SET Posttraining</th>
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<td><strong>Muscle CP, mmol/kg dw</strong></td>
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<tr>
<td>Rest</td>
<td>77.3±1.8</td>
<td>81.5±4.6</td>
<td>83.5±2.1</td>
<td>74.2±3.1</td>
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<tr>
<td>Exhaustion</td>
<td>32.7±3.6</td>
<td>28.2±1.2</td>
<td>33.7±2.2</td>
<td>30.5±9.4</td>
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<tr>
<td>3-min Recovery</td>
<td>63.6±4.6</td>
<td>68.2±5.1</td>
<td>65.4±4.2</td>
<td>65.4±4.2</td>
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<tr>
<td><strong>Muscle lactate, mmol/kg dw</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rest</td>
<td>4.8±1.0</td>
<td>6.0±1.0</td>
<td>5.5±1.1</td>
<td>5.1±0.8</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>47.4±4.7</td>
<td>64.7±5.7</td>
<td>45.3±6.9</td>
<td>71.4±13.9*</td>
</tr>
<tr>
<td>3-min Recovery</td>
<td>38.0±6.0</td>
<td>45.0±5.5</td>
<td>41.3±5.7</td>
<td>52.2±16.9</td>
</tr>
<tr>
<td><strong>Muscle pH, log H^+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>6.94±0.03</td>
<td>7.06±0.02</td>
<td>7.02±0.03</td>
<td>7.08±0.02</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>6.82±0.04</td>
<td>6.80±0.05</td>
<td>6.79±0.04</td>
<td>6.78±0.06</td>
</tr>
<tr>
<td>3-min Recovery</td>
<td>6.88±0.04</td>
<td>6.91±0.03</td>
<td>6.86±0.04</td>
<td>6.94±0.05†</td>
</tr>
<tr>
<td><strong>Muscle glycogen, mmol/kg dw</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>480±33</td>
<td>385±46*</td>
<td>460±40</td>
<td>411±23</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>418±34</td>
<td>346±44*</td>
<td>408±35</td>
<td>377±32</td>
</tr>
<tr>
<td>3-min Recovery</td>
<td>444±35</td>
<td>362±40*</td>
<td>453±53</td>
<td>343±34*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from pretraining level; †significant difference from ST group.

Possible that the adaptations in the Na⁺/K⁺ pump isoforms have led to a greater activity of the pump during exercise. In support, in both ST and SET the plasma potassium concentrations were the same during and after the first and last training bout, despite that the subjects performed significantly (P < 0.05) more work in the last training session (SET, ~8% and ST, ~6%), but it cannot be excluded that a difference in plasma potassium was too small to be detected. In accordance, Nielsen et al. (34) observed that an increase in the Na⁺-K⁺-ATPase subunits was associated with lower interstitial potassium concentrations during exercise at the same intensity.

The muscle CK activity was only elevated in ST after the training period, although muscle CP was the same in SET and ST at the end of a training session. In contrast, Costill et al. (11) observed that the CK activity was higher in a group that had performed strength training with 30-s bouts, whereas no change was observed for a group that had used 6-s exercise periods. In ST, the subjects performed fifteen 6-s maximal runs separated by 1 min of recovery, whereas in SET, the subjects performed eight 30-s runs at a mean running speed of ~19–20.5 km/h (130% of the maximal oxygen uptake) separated by a 1.5-min recovery. In both cases, it should be expected that CP had almost recovered completely prior to the following exercise period. Thus, it may be that it is a high rate of CP utilization rather than the total net utilization of CP that is important for the adaptation of CK to training, although the results of Costill et al. (11) do not support this suggestion.

There were clear differences between the two groups in terms of improvements in the work capacity. Only the ST group increased performance on a 50-m sprint, whereas only the SET group performed better on the treadmill running test to exhaustion. These findings suggest that the adaptations occurring are specific to the way of training. The improvements in the 50-m run may be related to adaptations in the neural system (1) and perhaps the increased levels of CK, which was not observed in SET, since no change in muscle fiber area (data not shown) and fiber-type distribution was observed in ST. On the other hand, the better performance in the treadmill test in SET and the greater improvement in the Yo-Yo IR-2 test compared with ST could be due to a number of factors. SET led to an elevated level of NHE1 level, which may have caused a more pronounced release of H⁺ and thus, a lower rate of decrease in muscle pH. This may have contributed to the delay in the development of fatigue during the treadmill test and the Yo-Yo IR-2 test (6). Accumulation of potassium in the muscle interstitium has been suggested to cause fatigue during intense exercise (14) and muscle acidosis (4) seems to increase the potassium efflux from the muscle to the interstitium (5, 32, 35, 43) perhaps due to an elevated opening probability of the KATP channels (12). Thus, an elevated level of NHE1 together with a possible higher activity of the Na⁺/K⁺ pump due to the higher level of the α2 and β1 subunits may also have lowered the extracellular potassium accumulation and thereby also contributed to the delayed fatigue development. However, since the cause of fatigue during intense exercise is likely to be complex (6, 14) several other factors may have contributed to the improved performance.

When performing repeated intense exercise, which combines the ability to perform intensely and the capacity to recover from intense exercise, both groups had improvements. In the repeated sprint test both groups had a lower mean sprint

Table S2. **Muscle CP, lactate, pH, and glycogen at rest, at exhaustion, and 3 min after the Yo-Yo intermittent recovery test (level 2) before and after training period for the ST and SET**
time, whereas only the SET group had a lowering of the fatigue index, suggesting that by performing speed endurance training, the ability to recover is enhanced. In line, the improvement in performance in the Yo-Yo IR-2 test was significantly greater in the SET-group compared with the ST-group. In both groups, muscle and blood lactate concentrations were higher at the point of fatigue, suggesting continuing the exercise for a longer time allowed for a greater lactate production and release, which could be related to recruitment of additional motor units after training. Similarly, Nevill et al. (33) observed that muscle lactate was higher at exhaustion after a period with intense training. On the other hand, muscle pH at exhaustion was the same both for SET and ST, which may have been due to a greater transport of $\text{H}^+$ out of the contracting muscle after training as observed previously (37). This effect was more pronounced in the SET group, which to some extent can be explained by the higher amount of Na$^+$/$\text{H}^+$ transporters after the training period. The greater capacity in the SET group for $\text{H}^+$ release was also observed in the first 3 min of recovery from the Yo-Yo IR-2 test, since the rate of increase in pH was greater after the training period. Both groups had a more pronounced rate of decrease in muscle lactate after the test, which may be explained by the elevated levels of MCT1 and the higher muscle lactate concentrations at the end of the test.

An interesting finding was that the SET-training caused a reduction in the blood lactate response during the Yo-Yo IR-2 test. This is a typical observation after a period of endurance training and is a result of both a greater removal of lactate from the blood and lower production of lactate, which has been associated with an increase in the activities of oxidative enzymes and an elevated fat oxidation (9). However, in the present study, no change in V$\text{O}_2$ max and respiratory exchange ratio during submaximal exercise was observed (data not shown). Neither was the rate of oxidation elevated in the initial phase of exercise at different running speed on the treadmill (data not presented). In the muscle biopsy taken after the training period at the time where the subjects became exhausted before the training period, the lactate concentration tended to be lower ($P = 0.094$; 33.8 vs. 45.0 mmol/kg dry wt), suggesting that the lowering of the blood lactate concentration to some extent was a result of a reduced rate of lactate production. Apparently, the rate of glycolysis can be lowered or pyruvate uptake in mitochondria can be elevated after a period of training without any notable change in the oxidative capacity of the muscle (data not shown) or V$\text{O}_2$ max. Similarly, Green et al. (17) observed that after a week of endurance training, muscle glycogen utilization and lactate accumulation during submaximal exercise were reduced without changes in enzymes representative of the citric acid cycle. Apparently, intense training can reduce the rate of lactate production at a given work intensity independently of the mitochondrial activity.

In summary, the present study showed that high intensity training leading to significant disturbance of muscle ion homeostasis caused adaptations in muscle ion transport proteins, with the changes in MCT1 and Na$^+$-$\text{K}^+$-ATPase $\beta_1$-isoform being independent of the duration and intensity level of the training, whereas NHE1 and the $\alpha_2$-isoform were only elevated after a period with speed endurance training. Moreover, the speed endurance training led, in contrast to sprint training, to an improvement in performance during continuous intense exercise and to greater improvements in the ability to perform high-intensity intermittent exercise, indicating that NHE1 and the Na$^+$-$\text{K}^+$-ATPase $\alpha_2$-isoform may play a role in controlling muscle ion homeostasis during intense intermittent exercise.

Sprint training at nearly maximal intensity elevated the muscle CK levels and peak sprint performance in contrast to the speed endurance group, indicating that the exercise intensity was important for adaptations in muscle CK and that this enzyme may be important for the maximal sprint performance.

**Practical Implications**

The present study showed that the improvements in performance were closely related to the type of training performed, suggesting that it is important to be specific in the training in relation to the demands in a sport. Furthermore, it was shown that SET would be useful in the high number of sports, such as soccer and basketball, where the ability to carry out high-intensity intermittent exercise is essential for high-level performance.

**ACKNOWLEDGMENTS**

We thank the subjects for their committed participation in the training and testing procedures. The excellent technical assistance of Rasmus Fischer Smidt, Matthias Zangenberg, Jim West, Ingelise Kring, the late Merete Vannby, Winnie Taagerup, and Nikolai Nordborg is greatly appreciated. In addition, assistance was provided by Carsten Lundby, Rasmus Damsgaard, and Christian Fischer in the biopsy procedure.

**GRANTS**

This study was supported by Team Denmark, The Danish Natural Science Research Council, and the Ministry of Culture (Kulturministeriets Udvalg for Idrætsforskning).

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