Reduced volume but increased training intensity elevates muscle Na\(^{+}\)-K\(^{+}\) pump \(\alpha_1\)-subunit and NHE1 expression as well as short-term work capacity in humans

F. Marcello Iaia, Martin Thomassen, Helle Kolding, Thomas Gunnarsson, Jesper Wendell, Thomas Rostgaard, Nikolai Nordsborg, Peter Krstrup, Lars Nybo, Ylva Hellsten, and Jens Bangsbo

Department of Exercise and Sport Sciences, Section of Human Physiology, Copenhagen Muscle Research Center, University of Copenhagen, Copenhagen, Denmark; and Faculty of Exercise Sciences, State University of Milan, Milan, Italy

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Fatigue during intense exercise has been proposed to be a multifactorial phenomenon. Factors such as sarcolemmal depolarization especially caused by accumulation of extracellular K\(^{+}\) (36), as well as lowered muscle pH and creatine phosphate (CP), have been suggested to be contributing agents in the development of fatigue during repeated intense exercise (12). One way to study the importance of such factors is to change their regulatory systems by performing exercise training and then examine the physiological response and work capacity during various types of exercise. However, exercise training of untrained subjects leads to adaptation in a high number of physiological variables; hence, it is difficult to evaluate the cause of changes in protein expression and the improvement in the work capacity observed after a period of training. By changing the type of training of already trained subjects, new information may be provided.

The muscle ion transport proteins are important when discussing fatigue development. The Na\(^{+}\)-K\(^{+}\) pump is pivotal in maintaining the muscle membrane potential during exercise (7), and the Na\(^{+}\)-K\(^{+}\)2Cl\(^{-}\)-1 (NKCC1) protein cotransporters, primarily located in the sarcolemma, may also be important for maintenance of muscle function during intense exercise, possibly by adding to the K\(^{+}\) reuptake (39). In untrained subjects, the Na\(^{+}\)-K\(^{+}\) pump has been shown to be upregulated by different types of exercise training (7, 8, 28, 29, 31). In addition, Nielsen et al. (31) observed that the elevated level of Na\(^{+}\)-K\(^{+}\) pump \(\alpha_1\)- and \(\alpha_2\)-subunits after 8 wk of knee-extensor training at supramaximal exercise intensities was associated with a reduced muscle interstitial K\(^{+}\) concentration ([K\(^{+}\)]) during exercise as well as better performance during intense exercise. Aughey et al. (1) did not find changes in the abundance of any of the Na\(^{+}\)-K\(^{+}\) pump \(\alpha_1\)- and \(\beta\)-isoforms when already trained subjects performed a period of intensified training. However, the lack of effect may have been a result of the exercise intensity being below the one corresponding to pulmonary maximum oxygen uptake (VO\(_2\)max). The effect of training on NKCC1 has been investigated in rats (15), but there is no information available in humans.

Lowered muscle pH is one of the potential factors involved in the fatigue processes during intense short-term exercise (2, 12). Therefore, the proteins controlling the H\(^{+}\) efflux from the muscle cell may be of importance for the work capacity of a contracting muscle. The Na\(^{+}\)/H\(^{+}\) exchanger isoform 1 (NHE1) has been reported to increase as a result of high-intensity exercise training in rats and in humans (21), but no data are available for trained individuals. In human skeletal muscle, two monocarboxylate transporters (MCT), MCT1 and MCT4, facilitate the lactate and H\(^{+}\) exchange across the muscle membrane (20). A number of studies, including either endurance or high-intensity training programs of untrained subjects, have observed higher MCT1 and some also MCT4 protein density (6, 21, 29). In addition, Bickham et al. (4) reported change in the MCT1 transport proteins in endurance-trained subjects after a period of sprint training, but in this study the participants had maintained a great portion of their weekly endurance training volume, and thus the combined effect of endurance and high-intensity training was studied. Thus, although a num-
A total of studies have shown improvements in specific performance tests after a period with elevated training intensity of already trained individuals (4, 10, 25), little is known about the effect of such training regimes on muscle ion transport proteins and their relationship with muscle fatigue development during intense exercise.

Therefore, the aim of the present study was to examine the effect of a change from endurance training to sprint training on the adaptations of skeletal muscle ion transport proteins and their relation to the physiological response to exercise and work capacity to provide information about the importance of these muscle proteins for performance. Eight endurance trained subjects were studied before and after a 4-wk period with regular sessions of sprint training and reduced volume of training.

### METHODS

#### Subjects

Fifteen moderately trained male endurance runners took part in the study. All subjects were healthy nonsmokers, and none were on regular medication. Age, height, weight, and VO2max were 33.4 ± 1.3 yr, 179.7 ± 2.1 cm, 73.1 ± 2.5 kg, and 55.8 ± 1.3 ml·kg⁻¹·min⁻¹ (means ± SE). The subjects had been training and competing on a regular basis for a minimum of 4 yr, and, before the study, they were all running 3–5 days/wk with an average weekly distance around 45 km (Table 1). All participants were fully informed of any possible risks and discomforts associated with the experimental procedures before giving their written informed consent to participate. The study was designed according to the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

#### Intervention Period and Training

An intervention (IT) period lasting for 4 wk was carried out in the INC group. Experimental sprint training group (ST) following the CON group (see Table 1 for details). The subjects were familiarized with the testing procedures.

#### Exercise Protocol

Before and after the IT period, the participants completed 1) a 30-s sprint test (only ST); 2) an incremental treadmill test (INC test) to determine VO2max; 3) two exhaustive supramaximal treadmill runs (EX1 and EX2) at a speed of ~130% VO2max, separated by 2 min of rest (RS test); 4) the Yo-Yo intermittent recovery test level 2 (Yo-Yo IR2) (24); and 5) a 10,000-m race on a 400-m running track (10 k). All subjects had prior to the experiment a high experience level 2 (Yo-Yo IR2) and 30-s sprint test were preceded by pretests to familiarize the subjects with the testing procedures.

### Table 1. Description of endurance training before the intervention period for the CON and ST groups and during the 4-wk sprint training period for the ST group

<table>
<thead>
<tr>
<th>Type of Training</th>
<th>CON Group</th>
<th>ST Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endurance Training</td>
<td>Endurance Training</td>
</tr>
<tr>
<td>Exercise protocol</td>
<td>Continuous moderate intensity</td>
<td>Continuous moderate intensity</td>
</tr>
<tr>
<td>No. of sessions</td>
<td>4.0±0.3</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>Per week</td>
<td>16.0±1.8</td>
<td>15.6±2.2</td>
</tr>
<tr>
<td>No. running bouts</td>
<td>9.1±0.2</td>
<td>30.9±3.7</td>
</tr>
<tr>
<td>Per session</td>
<td>123.8±10.4</td>
<td></td>
</tr>
<tr>
<td>Per week</td>
<td>12.7±0.8</td>
<td>12.9±0.6</td>
</tr>
<tr>
<td>Distance run, km</td>
<td>11.1±0.4</td>
<td>11.2±0.5</td>
</tr>
<tr>
<td>Per week</td>
<td>44.4±4.5</td>
<td>43.7±5.7</td>
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<tr>
<td>Total</td>
<td>177.6±19.8</td>
<td>174.7±21.4</td>
</tr>
<tr>
<td>Duration, min</td>
<td>52.6±2.7</td>
<td>52.2±2.6</td>
</tr>
<tr>
<td>Per week</td>
<td>210.4±23.2</td>
<td>203.6±20.7</td>
</tr>
<tr>
<td>Total</td>
<td>841.6±92.9</td>
<td>814.3±80.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 CON; n = 8 ST. CON, controls; ST, sprint training; V<sub>max</sub>, velocity at which 30-s all-out sprint was run. *Significantly different (P < 0.05) from the endurance training period. *Warm up and cool down activities are not included. **Passive recovery between bouts is not included.
All tests were carried out at least 2 days apart, and the order of tests was the same for CON and ST. After the IT period, the 30-s sprint test was performed at the start of the last training session, whereas the INC test was performed 48 h after the last training session. Next, the subjects performed an additional training session, 48 h later a muscle biopsy was obtained at rest, and the RS test carried out immediately after. Next, 24 h later, the subjects underwent another additional training session, and 48 h later they performed the Yo-Yo IR2. After another 48 h of recovery, they completed the 10-k test. CON and ST performed the same number of training session between the tests.

**Testing Procedures**

On the day of testing, subjects reported to the laboratory 3 h after consuming a light meal. Subjects refrained from strenuous physical activity in the 48 h before testing and abstained from alcohol and caffeine consumption 24 h before testing. To minimize the effect of diet on muscle metabolism and performance, 2 days before any experimental testing the participants were also required to follow a nutritional strategy designed to ensure an adequate carbohydrate intake (~60% of total energy intake) and to record and replicate their individual dietary pattern during the 48 h before each testing day. All tests were preceded by 15 min of standardized warm up.

A 30-s sprint test was carried out on a 400-m running track, and the distance was recorded (only ST). Next, subjects completed the INC and RS test on a motorized treadmill under standard laboratory conditions. The calibration of the treadmill was checked before each testing session. The INC test started with a 3-min run at a preset speed (12–14 km/h) after which the speed was increased by 1 km/h every minute until volitional fatigue. Pulmonary oxygen uptake (VO₂) was measured throughout the whole protocol by a breath-by-breath gas analyzing system (CPX/D; MedGraphics, Saint Paul, MN). The analyzer was calibrated before each test with two gases of known O₂ and CO₂ concentrations as well as by the use of a 3-liter syringe for the tube flowmeter calibration (35). VO₂max was determined as the highest value achieved over a 20-s period. A plateau in oxygen uptake, despite an increased power output, and a respiratory exchange ratio >1.15 were used as criteria for VO₂max achievement.

In the RS test, the subjects performed two supramaximal exhaustive runs (EX1 and EX2) at a speed corresponding to ~130% pretraining VO₂max, separated by a 2-min rest period. The speed was determined by a linear extrapolation established from the individual relationship between exercise intensity and VO₂ obtained during the INC test. After 15 min of rest in the supine position, the subjects had a catheter (18 G, 32 mm) inserted in an antecubital vein. In preparation for the muscle biopsies, two small incisions through the skin and fascia over the vastus lateralis muscle were made under local anesthesia (1 ml; 20 mg/l lidocaine without epinephrine) and covered by sterile band aid strips and a thigh bandage. The subject’s left or right leg was randomly selected. The same leg was used for pre- and post-IT period biopsies. After warm up, the subjects rested for 5 min before starting EX1. The exercises were terminated when the subject failed to maintain the speed. Subjects were not given any feedback. VO₂ was measured as previously described. Blood samples were taken before and at the end of each exercise bout as well as 1 min after EX1 and 1.5 and 3 min after EX2. All blood samples were collected in 2-ml heparinized syringes. A muscle biopsy (3) was taken from the vastus lateralis muscle before and immediately after both EX1 and EX2. Tissue samples were immediately frozen in liquid nitrogen and subsequently stored at −80°C.

The Yo-Yo IR2 test was performed on an artificial grass surface using 2 m × 20 m lanes (24). The Yo-Yo IR2 test consists of 2 × 20 m shuttle runs at increasing speeds, interspersed with a 10-s period of active recovery (controlled by audio signals from a compact disc). The test was terminated when an individual was no longer able to maintain the required speed. The distance covered up to the end point represented the test result. As for all the other tests, the exact distance or time achieved was subsequently verified using video recordings. The 10-k trial was carried out on a 400-m running track. To avoid racing tactics and strategies, the test was conducted on an individual basis with participants starting at 1-min intervals in random order.

**Blood Analysis**

Immediately after sampling, a part of the blood was rapidly centrifuged at 20,000 g for 30 s. Thereafter, the plasma was transferred to Eppendorf tubes and placed in ice-cold water until stored at −20°C. Samples were subsequently analyzed for [K+] by an ion-selective electrode using a Hitachi 912 Automatic Analyzer (Roche Diagnostic). Another part of the blood sample (100 µl) was hemo-

lized using a 1:1 dilution with a buffer solution (Yellow Spring Instruments, Yellow Springs, OH) to which 20 g/l Triton X-100 were added (13) for analysis of lactate concentration ([Lac−]) (model 23; Yellow Spring Instruments).

**Muscle Analysis**

The frozen muscle biopsies were weighed before and after freeze-drying to determine the water content. After freeze-drying all connective tissue, visible fat, and blood were carefully dissected away under a stereomicroscope in a room with a temperature of 18°C and a relative humidity <30%.

Muscle metabolites, pH, and buffer capacity. About 2 mg dry wt of the muscle tissue obtained during the RS test were extracted in a solution of 0.6 M perchloric acid and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO₃, and stored at −80°C until analyzed for lactate, creatine, and CP contents by a fluorometric assay (26). An additional 1 mg dry wt muscle tissue was extracted in 1 M HCl and hydrolyzed at 100°C for 3 h, and the glycogen content was determined by the hexokinase method (26).

Muscle pH was measured by a small glass electrode (XC 161; Radiometer-analytical) after homogenization of ~1 mg freeze-dried samples in a nonbuffered solution containing 145 mM KCl, 10 mM NaCl, and 5 mM sodium fluoride (27). The muscle buffer capacity was measured in a sample collected at rest. After having adjusted pH of the sample to 7.1 with 0.01 M NaOH, the sample was titrated to pH 6.0 by serial additions of 0.01 M HCl followed by titration back to pH 7.1 by serial additions of 0.01 M NaOH. The pH was measured after each addition. The non-HCO₃ physiochemical buffer capacity was determined from the number of moles of H⁺ required to change pH from 7.1 to 6.5 and was expressed as micromoles H⁺ per kilogram dry weight per unit of pH (27). The in vivo muscle buffer capacity was calculated as the change in muscle lactate from rest to exhaustion divided by the change in muscle pH (37).
Muscle ion transport proteins. Approximately 4–5 mg dry wt of muscle tissue taken at rest were homogenized on ice with a Polytron 3100 [7-mm diameter (Kinematica) 1,300 revolutions/min] in a fresh batch of buffer (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM phenylmethyl fluoride, 1 mM EDTA and EGTA, aprotinin, leupeptin, and benzamidine) for not more than 30 s. After rotation end over end for 1 h, the samples were centrifuged for 30 min at 17,500 g at 4°C, and the lysate was collected as the supernatant. Protein concentrations were determined in the lysates using BSA standards (Pierce Reagents).

The lysates were diluted to appropriate protein concentrations in a 6× sample buffer (0.5 M Tris base, dithiothreitol, SDS, glycerol, and bromphenol blue) and then boiled for 3 min at 96°C to denature the proteins. An equal amount of total protein was loaded for each sample in different wells on a 5% (NKCC1) or a 10% Tris-HCl gel (Bio-Rad Laboratories): 5 μg (Na⁺-K⁺-pump α₁-isoform), 12 μg (NHE1 and NKCC1), and 15 μg (MCT1, MCT4, Na⁺-K⁺ pump α₁- and α₂-isoforms). For comparisons, samples from the same subject were always loaded on the same gel. The gel electrophoresis was done with 150 volts and 55 mA/gel in 80–100 min; after wards, the proteins were blotted to a polyvinylidene difluoride membrane using 25 volts and 70 mA/gel in 2 h. The membrane was incubated with ~8–10 ml of primary antibody overnight and then washed for 5 min in TBS-Tween 20 before incubation with secondary antibody for 1 h. The primary antibodies were diluted in either 2% nonfat milk [monoclonal Na⁺-K⁺-pump α₁- 1:500 (o6F; Iowa Hybridoma Bank), monoclonal α₁ 1:200 (McB2 kindly donated by K. J. Sweadner to H. Bundgaard), monoclonal β₁ 1:1,000 (MA3-930; Affinity BioReagents), and polyclonal NKCC1 1:200 (Sc-21545; Santa Cruz Biotechnology)] or 3% BSA [monoclonal NHE1 1:500, polyclonal MCT1 1:1,000, and polyclonal MCT4 1:1,000 (MAB3140, AB3538P, and AB3316P; Chemicon)]. The secondary horseradish peroxidase (HRP)-conjugated antibody (P-0447, P-0448, and P-0449; DakoCytagomation) was diluted 1:5,000 in 2% nonfat milk or 3% BSA depending on the primary antibody. The membrane staining was visualized by incubation with a chemiluminescent HRP substrate (Millipore) for 5 min. The gel image was digitalized (KODAK Image Station 2000MM), and the net band intensities were quantified as the total minus background intensity.

Muscle enzymes. For the determination of enzymatic activity, ~2 mg dry wt of muscle tissue taken at rest were homogenized (1:400) in a 0.3 M phosphate buffer adjusted to pH 7.7 containing 0.5 mg/ml BSA. Creatine kinase (CK) activity was determined fluorometrically on whole muscle homogenized in a tetraethylammonium-BSA buffer (26). Homogenates for phosphofructokinase (PFK) were prepared in 100 mM of potassium buffer phosphate (pH 8.2) containing 10 mM of glutathione, 0.5 mM of ATP, 5 mM of MgSO₄, and 30 mM of NaF.

Statistics

Student’s unpaired t-tests were used to compare subjects’ characteristics between the two training groups before the IT period. Blood variables, VO₂max, body mass, exercise performance, and muscle enzymes were analyzed using a two-factor repeated-measures ANOVA (RM ANOVA), with one between factor (group: ST vs. CON) and one within factor (training status: pre vs. post). Likewise, performance, muscle, and blood metabolites during the two repeated supramaximal exhaustive runs were evaluated using a two-factor RM ANOVA with the training status (pre vs. post) as between factor and the exercise time or sampling time as within factor. If a significant interaction was detected, a Newman-Keul’s post hoc test was subsequently applied to locate the differences.

In ST, possible differences in training load and in vivo buffer capacity between before and after the IT period were assessed by using a Student’s paired t-test. Training-induced changes on muscle protein expression were examined by log transforming the post-to-preexpression ratio and applying a one-sample t-test (H₀: μ = 0). Possible differences between ST and CON were evaluated using a t-test on the log-transformed post- to preratios for CON and ST. Correlation coefficients were determined and tested for significance using the Pearson’s regression test. The level of statistical significance was set for all analyses at P ≤ 0.05. Data are presented as means ± SE except for muscle protein transporters, which are geometric means ± 95% confidence intervals.

RESULTS

Performance, Body Mass, and VO₂max

In ST, the performance of the Yo-Yo IR2 test was improved (P < 0.01) by 19.0 ± 4.5% after the IT period, whereas no difference was observed for CON (Fig. 2 and Table 2). Similarly, the time to exhaustion in EX1 and EX2 was 26.5 ± 5.5 and 19.4 ± 4.6%, respectively, longer in ST, whereas CON had no change in performance. The time to exhaustion during the INC test increased by 5.4 ± 0.8% in ST (P < 0.001) and 3.0 ± 1.0% in CON (P < 0.05). The 10-k time was not changed in either ST or CON. For ST, the distance covered in the 30-s sprint test was 6.9 ± 1.6% longer (P < 0.05) after the IT period.

The body mass remained unaltered during the IT period in both ST (72.8 ± 2.5 vs. 72.9 ± 2.5 kg) and CON (73.3 ± 2.8 vs. 73.5 ± 2.9 kg). VO₂max was also the same before and after the IT period in both ST (55.3 ± 1.8 and 54.1 ± 1.9 ml/min·kg⁻¹) and CON (56.4 ± 2.0 and 56.8 ± 2.1 ml/min·kg⁻¹).

Muscle Ion Transport Proteins, Enzymes, and In Vitro Buffer Capacity

In ST, the expressions of the muscle Na⁺-K⁺-pump α₁-subunit and NHE1 were ~29 and ~30%, respectively, higher (P < 0.05) after compared with before the IT period, whereas the Na⁺-K⁺-pump α₂-subunit (P = 0.08) and NKCC1 channel (P = 0.1) levels tended to be higher. No significant changes were observed for the expression of the other investigated transport proteins (Fig. 3). In CON, the muscle protein expressions were unaltered (Fig. 3). In both ST and CON, the activity of muscle CK (4,352 ± 81 vs. 4,339 ± 241 and 3,949 ± 157

![Graph](Fig. 2. Training-induced changes in performance (expressed in percentage) for ST (●) and CON (○). Values are means ± SE. Significantly different from preintervention (IT) period value: *P < 0.05, **P < 0.01, and ***P < 0.001. Significantly different (P < 0.05) from CON group.)
vs. 4.004 ± 165 μmol·min⁻¹·g dry wt⁻¹, respectively) and PFK (128 ± 7 vs. 129 ± 11 and 123 ± 17 vs. 130 ± 20 μmol·min⁻¹·g dry wt⁻¹, respectively) after remained the same as before the IT period. In ST, the in vitro muscle buffer capacity was the same before and after the IT period (184 ± 10 and 178 ± 7 μmol·g dry wt⁻¹·pH unit⁻¹, respectively).

After the IT period, the muscle Na⁺-K⁺ pump β₁-subunit expression in ST was correlated (P < 0.05) to the performance of the Yo-Yo IR2 test (r = 0.94), to the distance covered in the 30-s sprint test (r = 0.79), and to the maximal treadmill speed reached in the INC test (r = 0.89), whereas no significant correlations were observed before the IT period.

**Physiological Response in the Repeated Intense Exercise Test**

Muscle metabolites, pH, and in vivo buffer capacity (only ST). In ST, before the IT period, muscle CP content decreased by −58% during EX1, and it was still lowered (P < 0.05) before EX2, whereas after the IT period muscle CP content before EX2 was not significantly different from before EX1. During EX2, it decreased to the level seen after EX1 both before and after the IT period (Table 3). Muscle creatine levels followed inversely the changes in CP, and total creatine was constant both before and after the IT period (Table 3).

Muscle glycogen content was 402 ± 35 and 388 ± 31 mmol/kg dry wt at rest before and after the IT period, respectively, and it decreased during EX1 to 325 ± 30 and 324 ± 29 mmol/kg dry wt, respectively, being unaltered during recovery from EX1 after the IT period and reaching 314 ± 34 and 293 ± 32 mmol/kg dry wt, respectively, at the end of EX2 (Table 3). The mean rate of net glycogen utilization during EX2 was lower (P < 0.05) after compared with before the IT period (38.3 ± 8.8 vs. 58.2 ± 19.7 mmol·kg⁻¹·min⁻¹ dry wt⁻¹).

Muscle lactate levels increased about threefold during EX1 and remained high during recovery with a further increase during EX2 with the same level after the IT period (Table 3). The muscle H⁺ concentration ([H⁺]) at the end of EX1 tended to be higher (P = 0.09) after compared with before the IT period (123 ± 12 vs. 103 ± 11 nM), but it was the same before and at the end of EX2 (Table 3). The net rate of muscle H⁺ accumulation during EX1 (26.1 ± 9.9 vs. 26.6 ± 4.6 nM/min) and EX2 (18.1 ± 5.0 vs. and 18.7 ± 3.1 nM/min) was not changed by the sprint training. The net rate of H⁺ removal in recovery from EX1 was higher (P < 0.05) after compared with before the IT period (11.5 ± 5.7 vs. −4.9 ± 5.0 nM/min, respectively).

The estimated in vivo muscle buffer capacity for EX1 was 96.5 ± 33.1 and 88.1 ± 15.7 mmol·kg⁻¹·min⁻¹·pH⁻¹ before and after the IT period, respectively, and it was also the same for EX2 (135.9 ± 40.5 vs. 140.2 ± 37.8 mmol·kg⁻¹·dry wt⁻¹·pH⁻¹).

**Venous Blood Lactate and Plasma K⁺**

In ST, blood [Lac⁻] reached 10.0 ± 0.8 mmol/l during EX1 and did not change during recovery from EX1, and increased to 12.1 ± 1.0 mmol/l during EX2 (Fig. 4A). After the IT period, blood [Lac⁻] at the end of EX1 and EX2 was the same as

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**Table 2. Various performance variables before (pre) and after (post) the 4-wk IT period for ST and CON groups**

<table>
<thead>
<tr>
<th>Performance</th>
<th>ST (n = 8)</th>
<th>CON (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprint test</td>
<td>30 s, distance covered, m</td>
<td>201±5</td>
</tr>
<tr>
<td>Incremental treadmill test maximum speed, km/h</td>
<td>18.6±0.5</td>
<td>19.6±0.5‡</td>
</tr>
</tbody>
</table>
| Exhaustive supramaximal treadmill running
  First bout, s | 101±13 | 126±62§ |
  Second bout, s | 58±5 | 69±6§ |
| Yo-Yo IR2 test running distance, m | 440±58 | 520±88‡ |
| 10-km time, min:s | 40:52±1:09 | 40:59±1:30 |

Values are means ± SE; n, no. of subjects. Yo-Yo IR2, intermittent Yo-Yo 2. *Running speed corresponding to approximately 130% VO₂max before the intervention recovery test level (IT) period. †P < 0.05, ‡P < 0.01, and §P < 0.001, significantly different from pre-IT period. §Significantly different (P < 0.05) from CON.
The major findings of the present study were that a change in training from regular endurance to sprint training resulted in increased levels of muscle NHE1 and Na\(^{+}\)-K\(^{+}\) pump α1-subunit. These alterations were associated with significant improvements in performance during repeated intense exercise while the time for 10 k was unaltered despite a reduction in the total distance of training by 64% (Fig. 2). Specifically, the K\(^{+}\) level in the blood at exhaustion of EX1 was lower than before the IT period, despite a longer duration of the exercise.

The amount of the Na\(^{+}\)-K\(^{+}\) pump α1-isoforms was elevated after the sprint training period, which may have increased the number of functional pumps causing a lower accumulation of K\(^{+}\) in muscle interstitium during and in recovery from exercise (31). Consequently, the reduction in muscle membrane potential may have been lowered and cell excitability preserved, and thereby the time to fatigue during the supramaximal exercises was prolonged. There is some evidence to support this hypothesis. After the sprint training period, the rate of increase in venous plasma [K\(^{+}\)] during EX1 was lower, and the lowering of plasma [K\(^{+}\)] 1 min after EX1 was correlated with the level of muscle Na\(^{+}\)-K\(^{+}\) pump α1-subunits. Neither the level of α2-nor β1-isoform was changed with sprint training. The Na\(^{+}\)-K\(^{+}\) pump α2- and β1-isoforms are probably the most abundant

Table 3. Muscle metabolites and [H\(^{+}\)]

<table>
<thead>
<tr>
<th></th>
<th>EX1 Pre</th>
<th>EX1 Post</th>
<th>EX2 Pre</th>
<th>EX2 Post</th>
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<tr>
<td>CP, mmol/kg dry wt</td>
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<tr>
<td>Pre-IT period</td>
<td>83.6±7.8</td>
<td>34.8±8.2</td>
<td>63.7±6.4*</td>
<td>32.8±4.7</td>
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<tr>
<td>Post-IT period</td>
<td>72.1±8.7</td>
<td>28.0±3.7</td>
<td>60.9±4.2</td>
<td>27.9±3.6</td>
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<td>Cr, mmol/kg dry wt</td>
<td></td>
<td></td>
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<tr>
<td>Pre-IT period</td>
<td>39.2±9.9</td>
<td>84.7±11.6</td>
<td>58.0±7.3*</td>
<td>89.2±8.7</td>
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<tr>
<td>Post-IT period</td>
<td>41.5±4.2</td>
<td>83.9±7.5</td>
<td>53.3±2.9</td>
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<td>Post-IT period</td>
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<tr>
<td>Pre-IT period</td>
<td>8.7±1.6</td>
<td>31.4±4.6</td>
<td>29.6±2.9</td>
<td>38.1±3.3</td>
</tr>
<tr>
<td>Post-IT period</td>
<td>11.3±1.6</td>
<td>30.6±2.8</td>
<td>30.2±2.3</td>
<td>39.8±3.4</td>
</tr>
<tr>
<td>Glycogen, mmol/kg dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-IT period</td>
<td>402±35</td>
<td>325±30</td>
<td>365±29†</td>
<td>314±34†</td>
</tr>
<tr>
<td>Post-IT period</td>
<td>388±31</td>
<td>324±29</td>
<td>331±34</td>
<td>293±32</td>
</tr>
<tr>
<td>[H(^{+})], nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-IT period</td>
<td>61±5</td>
<td>103±11</td>
<td>116±9</td>
<td>124±9</td>
</tr>
<tr>
<td>Post-IT period</td>
<td>69±5</td>
<td>123±12</td>
<td>107±7</td>
<td>126±7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects ST. CP, creatine phosphate; Cr, creatine; [H\(^{+}\)], H\(^{+}\) concentration. Data are for muscle metabolites and [H\(^{+}\)] before and immediately after two repeated supramaximal (~130% VO\(_{2\text{max}}\) before the IT period) treadmill runs to exhaustion (EX1 and EX2) separated by 2 min of passive rest before (pre-IT period) and after (post-IT period) 4 wk of sprint training for ST. *Significant difference (P < 0.05) from the level of CP and pre-EX1. †Significant difference (P < 0.05) from the level of glycogen post-EX1.

The difference in plasma [K\(^{+}\)] after the IT period (10.6 ± 0.6 and 13.8 ± 0.8 mmol/l; Fig. 4A). In CON, blood [Lac\(^{-}\)] at the end of EX1 and EX2 was 8.6 ± 0.5 and 11.0 ± 0.8 mmol/l, respectively, and it was not changed after the IT period (Fig. 4B). In ST, blood [Lac\(^{-}\)] at the end of EX1 and EX2 after the IT period was higher (P < 0.05) compared with CON (7.2 ± 1.0 and 9.5 ± 1.4 mmol/l, respectively).

In ST, plasma [K\(^{+}\)] at the end of EX1 and during recovery from EX1 was lower (P < 0.05) after than before the IT period, whereas no differences were observed during and after EX2 (Fig. 5A). In CON, the plasma K\(^{+}\) levels were the same before and after the IT period (Fig. 5B). In ST, the rate of K\(^{+}\) accumulation in venous blood during EX1 was lower (P < 0.05) after compared with before the IT period (0.78 ± 0.14 vs. 1.30 ± 0.25 mmol·l\(^{-1}\)·min\(^{-1}\)), whereas in CON remained unchanged (1.46 ± 0.27 vs. 1.28 ± 0.18 mmol·l\(^{-1}\)·min\(^{-1}\)). The difference in plasma [K\(^{+}\)] after 1 min of recovery from EX1 between pre- and posttraining was related (r = 0.98; P < 0.05) to the level of muscle Na\(^{+}\)-K\(^{+}\) pump α1-subunit after the IT period.

**DISCUSSION**

The major findings of the present study were that a change in training from regular endurance to sprint training resulted in increased levels of muscle NHE1 and Na\(^{+}\)-K\(^{+}\) pump α1-subunit. These alterations were associated with significant improvements in performance during repeated intense exercise while the time for 10 k was unaltered despite a reduction in the
subunits in muscle (23, 32), and it may be that, in the present study, the amount of these isoforms was already high in the subjects before the sprint training, since endurance training has been shown to increase the Na\(^{+}\)-K\(^{+}\) pump \(\alpha_2\)- and \(\beta_1\)-isoform protein expression (17). Likewise, in two studies of sedentary people performing repeated high-intensity training, elevated levels of the \(\alpha_2\)- and \(\beta_1\)-subunits were found (29, 31), but in only one of the studies the level of the \(\alpha_1\)-subunit was higher after training (31). It may be speculated that the elevated level of \(\alpha_1\)-isoforms after the sprint period may have made the \(\beta\)-units limiting for the formation of Na\(^{+}\)-K\(^{+}\) pumps during exercise. This notion is supported by the finding that the Na\(^{+}\)-K\(^{+}\) pump \(\beta_1\)-subunit after, and not before, the IT period was correlated \((P < 0.05)\) to the performance of the Yo-Yo IR2 test, 30-s sprint test, and maximal speed reached in the incremental test. A closer coupling between the \(\alpha\)- and \(\beta\)-subunits after the sprint training period is also supported by the finding of a relationship between the improvement in Yo-Yo test performance and the amount of Na\(^{+}\)-K\(^{+}\) pump \(\alpha_2\)-subunits after the sprint training period \((r = 0.78;\) data not shown). Together these findings support a role of the muscle Na\(^{+}\)-K\(^{+}\) pumps in the control of extracellular K\(^{+}\) and fatigue development during intense exercise.

To our knowledge, this is the first human study to examine training-induced effects on NKCC1. Although we only found a tendency \((P = 0.1)\) to a higher amount of NKCC1 after the IT period, the increase \((-14\%)\) was of similar magnitude to that observed in rats \((14–29\%)\) (15), suggesting that the increased density of NKCC1 proteins may have contributed to increased K\(^{+}\) reuptake and thereby maintenance of a higher muscle cell excitability. On the other hand, the simultaneous increase in intracellular Cl\(^{-}\) concentration would tend to depolarize the sarcolemma, and thus the effect of a potential increase in NKCC1 expression on the development of muscle fatigue is unclear.

The amount of muscle NHE1 was elevated after the sprint training period, which is in agreement with studies where untrained subjects performed a period of sprint training (22, 29). The higher level of NHE1 may explain why, in ST, the increase in muscle pH after EX1 was higher after the IT period. This is consistent with the suggestion that NHE1 is the main pH regulator at rest (21). A greater H\(^{+}\) transport by NHE1 may also have contributed to a greater release of H\(^{+}\) during exercise, but it appeared to have a minimal effect, since the rate of muscle H\(^{+}\) accumulation during EX1 or EX2 was not altered by the sprint training period. In accordance, neither in vitro nor in vivo muscle buffer capacity was changed, which is in agreement with some (18, 30, 34) but not all studies of sprint training of untrained subjects (14, 37). Muscle [H\(^{+}\)] tended to be higher at the end of EX1 after the sprint training period, which may have been an effect of the longer exercise time, whereas no difference was observed for EX2. Nevertheless, it does not appear that the improved performance during the two exhaustive supramaximal running bouts was associated with a change in the handling of H\(^{+}\) and supports the suggestion that low muscle pH is not the single cause of fatigue (2).

In the present study, neither the expression of MCT1 nor of MCT4 increased after the sprint training period. The finding of unaltered MCT4 levels is consistent with the majority of the other studies (4, 11, 22, 29), but, in contrast to the present finding, most other studies using high-intensity intermittent training have shown a higher amount of MCT1 transporters (4, 6, 22, 29, 34). It may be that the subjects in the present study already had an elevated MCT1 protein content before the IT period since endurance training has been shown to increase the MCT1 density (5, 9, 16) and the lactate transport capacity has been observed to be higher in trained compared with untrained subjects (33). One study has reported sprint training-induced changes in MCT1 transport proteins in endurance-trained subjects (4). In that study, the subjects maintained a high volume of training \((\sim 50 \text{ km/wk})\), whereas in the present study the participants underwent a severe 64\% reduction in training distance \((\sim 15 \text{ km/wk})\). Taken together, the present study and the studies by Bickham et al. (4) and Pilegaard et al. (33) may suggest that, for trained athletes, a basic volume of training, including frequent sessions of high-intensity exercise, is necessary to change the muscle MCT1 protein content and the lactate transport capacity. However, further studies are needed to test this hypothesis. The unaltered MCT protein expression...
observed after the IT period in the ST group is consistent with the finding of an unchanged muscle and blood [Lac−] as well as muscle H+ accumulation found during the repeated supra-maximal exercise.

The present study showed that performance during intense exercise was elevated despite a significant reduction in the amount of training. A number of other studies have demonstrated that short-term performance can be improved after a period including high-intensity exercise training (4, 10, 19, 25, 38). For example, Bickham et al. (4) reported a longer time to exhaustion (11%) during a supramaximal treadmill run at an intensity of ~110% VO2max in response to a 6-wk short-sprint training program. However, in contrast to the other studies, the amount of training was reduced significantly in the present study, which suggests that the intensity, rather than the duration, of the training is the main factor in performance improvement after a period of sprint training. In addition, the finding of an unchanged 10-k performance showed that long-term performance can be maintained with sprint training despite a marked reduction in the total amount of training.

**Perspectives and Significance**

The present study examined the muscular effects and performance aspects in relation to a change from regular endurance training to sprint training. Significant changes were observed in some key muscle ion transport proteins and in venous K+ levels during exercise, which were related to changes in the capacity to perform repeated intense exercise. The findings indicate a role of the Na+−K+ pump in the control of K+ homeostasis and in the development of fatigue during short-term exhaustive and repeated high-intensity exercise. The study also has important practical implications, since it suggests that, in already trained subjects, muscle adaptations can occur and performance can be improved despite a drastic reduction in training volume, indicating that exercise at very high intensity is a powerful stimulus for adaptation. Endurance runners, in some periods, may therefore benefit from replacing overall volume of training with sessions of high-intensity exercise. This information is clearly of great interest not only for elite athletes but also for people participating in recreational activities. Further studies should focus on muscle adaptations and changes in working capacity when endurance training is combined with sprint training.

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