Effect of 2-wk intensified training and inactivity on muscle Na\(^+\)-K\(^+\) pump expression, phospholemman (FXYD1) phosphorylation, and performance in soccer players

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Thomassen M, Christensen PM, Gunnarsson TP, Nybo L, Bangsbo J. Effect of 2-wk intensified training and inactivity on muscle Na\(^+\)-K\(^+\) pump expression, phospholemman (FXYD1) phosphorylation, and performance in soccer players. J Appl Physiol 108: 898–905, 2010. First published February 4, 2010; doi:10.1152/japplphysiol.01015.2009.—The present study examined muscle adaptations and alterations in performance of highly trained soccer players with intensified training or training cessation. Eighteen elite soccer players were, for a 2-wk period, assigned to either a group that performed high-intensity training with a reduction in the amount of training (HI, \(n = 7\)), or an inactivity group without training (IN, \(n = 11\)). HI improved 48 to 654 m. The Yo-Yo intermittent recovery level 1 test performance of IN was lowered from 845 ± 48 to 654 ± 30 m. In HI, the protein expression of the Na\(^+\)-K\(^+\) pump \(\alpha_2\)-isoform was 15% higher (\(P < 0.05\)) after the intervention period, whereas no changes were observed in \(\alpha_1\)- and \(\beta_2\)-isoform expression. In IN, Na\(^+\)-K\(^+\) pump expression was not changed. In HI, the FXYD1ser68-to-FXYD1 ratio was 27% higher (\(P < 0.01\)) after the intervention period, and, in IN, the AB_FXYD1ser68 signal was 18% lower (\(P < 0.05\)) after inactivity. The change in FXYD1ser68-to-FXYD1 ratio was correlated (\(r^2 = 0.35; P < 0.05\)) with change in performance in repeated sprint test. The present data suggest that short-term intensified training, even for trained soccer players, can increase muscle Na\(^+\)-K\(^+\) pump \(\alpha_2\)-isoform expression, and that cessation of training for 2 wk does not affect the expression of Na\(^+\)-K\(^+\) pump isoforms. Resting phosphorylation status of the Na\(^+\)-K\(^+\) pump is changed by training and inactivity and may play a role in performance during repeated, intense exercise.

fatigue; training cessation; performance; monocarboxylate transporter; Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter isoform 1 (NHE1) and the monocarboxylate transporter 1 (MCT1) and 4 (MCT4) controlling H\(^+\) and lactate efflux from the muscle cell may well be of importance for the work capacity of contracting muscles (4, 15).

The expression of proteins involved in muscle ion homeostasis and maintenance of sarcolemmal excitability can be altered by exercise training in untrained subjects (9, 11, 13, 18, 21, 25, 26, 28). Also, changes in trained subjects are observed. In endurance-trained athletes, the Na\(^+\)-K\(^+\) pump \(\alpha_1\)-subunit and NHE1 protein expression was increased after 4 wk of altered training (20), and Na\(^+\)-K\(^+\) pump \(\alpha_2\)-subunit was increased after 6–9 wk of intensified training (5), which were associated with marked improvements in short-term performance. On the other hand, Aughey et al. (1) found that the abundance of the Na\(^+\)-K\(^+\) pump \(\alpha\)- and \(\beta\)-isoforms was not altered after a period of intensified, but submaximal, exercise training in endurance trained subjects (1). Nevertheless, it is unclear whether changes occur in the Na\(^+\)-K\(^+\) pump subunits after a shorter period of intense training and with subjects used to perform repeated intense exercise, like soccer players. Not only is the expression of the Na\(^+\)-K\(^+\) pump of interest, but also the degree of FXYD1 phosphorylation, which, in rats, has been shown to be altered with exercise training (16), but not yet studied in humans. The response in trained subjects of other muscle ion transport proteins to changes in training is also not clear. For example, a study on endurance-trained subjects showed that sprint training induced changes in the expression of MCT1 (8), whereas Ilaia et al. (20) and Bangsbo et al. (5) did not find any changes in MCT1 in endurance-trained subjects after a period of speed endurance training (SET).

Several studies have shown that training cessation reduced performance and muscular variables, such as oxidative enzymes, mitochondrial content, and capillary density (27), but few studies have examined the effect of inactivity on transport protein expression. Detraining of a duration of 6 and 10 days lowered the muscle glucose transporter (GLUT)-4 protein concentration by 17% (36) and 33% (24), respectively. In contrast, a study by Burgomaster et al. (9) reports no effect on GLUT-4 protein expression after 1 wk of detraining, but only an effect after 6 wk of detraining. Likewise, the MCT4 content decreased only after 6 wk of detraining (9). In different tissues and species, long-term immobilization, denervation, and tenotomy are reported to reduce the content of Na\(^+\)-K\(^+\) pumps by ~25% (11). However, little is known about how fast degradation of proteins involved in ion transport across the muscle membrane occurs with detraining in human skeletal muscle.

Thus the aim of the present study was to examine whether intensified training with reduced volume and training cessation
METHODS

Subjects

Eighteen male elite soccer players participated in the study. All subjects were healthy, and none was on medication. Age, height, weight, and pulmonary maximum oxygen uptake (V\(\text{O}_2\text{max}\)) were 23.4 ± 0.8 yr, 181.9 ± 1.6 cm, 78.5 ± 2.0 kg, and 55.0 ± 0.7 ml O\(_2\) kg\(^{-1}\) min\(^{-1}\), respectively (means ± SE). The subjects had been training and competing at the elite level on a regular basis for at least two seasons. After receiving oral and written information about any possible risks and discomforts associated with the experimental procedures, all participants gave their written, informed consent to participate. The study conformed to the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksborg communities.

Intervention Period and Training

The players were all members of an elite soccer squad and performed three to four training sessions and one match per week. The 2-wk intervention period was carried out immediately after the last match of the season. The players were randomly assigned either to a high-intensity training group (HI, \(n = 7\)) or an inactivity control group (IN, \(n = 11\)). Protocols were performed in a 3-wk period before the intervention, and posttests in the days following the intervention period.

During the intervention period, HI had 10 training sessions on an artificial grass surface, consisting of five aerobic high-intensity (AHI) sessions and five SET sessions. In every training session, heart rate (HR) was recorded at 5-s intervals (Polar team system, Polar Electro Oy, Kempele, Finland) and afterwards downloaded to a personal computer for further analysis. Each training session included a 20- to 25-min warm-up program. The AHI sessions were performed as small-sided (4 vs. 4) soccer drills, with eight repetitions of 2 min of exercise, interspersed by 1 min of recovery, and always the day before SET. Mean HR during the 2-min exercise periods in AHI was 87.7 ± 1.2% of maximal HR (HR\(_{\text{max}}\)), and the total exercise duration of the AHI training sessions was 75.4 ± 7.8 min. Four of the SET sessions consisted of 10–12 × 25–30 s all-out running bouts, including changes of directions and parts with ball contacts. Peak HR was 88.4 ± 1.9% of HR\(_{\text{max}}\) during these sessions, and total exercise duration was 19.9 ± 0.6 min. In one of the SET sessions, the players performed 16 exercise bouts each, lasting 40–60 s, with an equal recovery time between the exercise bouts. Mean HR reached 84.4 ± 1.7% of HR\(_{\text{max}}\), and total exercise duration was 14 min. No training was performed the day after a SET session. Overall, the players in HI reduced total training time during the 2-wk training intervention period. For both HI and IN, a muscle biopsy was obtained from m. vastus lateralis with the Bergstrom technique (6) at rest, before and after the intervention period and 36 h after a training session. For IN, an additional biopsy was taken 72 h after the last match to determine the time cause of changes with inactivity. The biopsies were immediately frozen in liquid nitrogen and subsequently stored at −80°C.

Testing Procedures

On the days of testing, subjects reported to the laboratory at least 2 h after consuming a light meal. Subjects refrained from strenuous physical activity in the last 32 h before testing and abstained from alcohol and caffeine consumption 24 h before the testing.

\(V\text{O}_2\text{max}\). The subjects completed an incremental running protocol, starting with 5 min at 10 km/h, continuing with 4 min at 14 km/h, and then the running speed was increased by 1 km/h every minute until exhaustion. Throughout the test, pulmonary oxygen uptake was measured by a breath-by-breath gas analyzing system (MedGraphics CPX/D, St. Paul, Minneapolis). The analyzer was calibrated before each test with two gases of known O\(_2\) and CO\(_2\) concentrations, as well as by the use of a 3-liter syringe for the tube flowmeter calibration (31). \(V\text{O}_2\text{max}\) was determined as the highest values achieved over a 20-s period. A plateau in oxygen uptake, despite an increased power output, and a respiratory exchange ratio > 1.10 were used as criteria for \(V\text{O}_2\text{max}\) achievement.

\(R\text{ST}\). Two minutes following a standardized 15-min warm-up procedure, including two 20-m sprints, RST was performed indoors on a wooden floor. The subjects carried out ten 20-m sprints from a standing position, interspersed by 15 s of active recovery. In the recovery period, the subjects ran slowly back to the starting point before the next sprint. Time to cover the 20 m was measured with ports of light censors (Newtest Powertimers, Newtest Oy, Oulu, Finland). Fastest sprint time (FST) and total sprint time (TST) for all 10 sprints were determined. In addition, a sprint fatigue index (SFI) was calculated as SFI = \(1 − (FST\text{-10/TST}^{-1})\)·100%. Furthermore, the reliability of RST was determined in six subjects, who performed the RST twice, separated by 4 days, and the test retest variability was determined as the coefficients of variance. The coefficients of variance for TST, FST, and SFI were 0.7 ± 0.2, 0.6 ± 0.2, and 7.7 ± 3.0%, respectively.

\(Yo-Yo IR2\). The Yo-Yo IR2 test was performed indoors on a wooden floor, after a 15-min standardized warm-up procedure. The Yo-Yo IR2 test consists of 2 × 20-m shuttle runs at increasing speeds, interspersed with 10 s of active recovery, controlled by audio signals from a compact disk (3). The test was terminated when the subject was no longer able to maintain the required speed. The distance covered up to the end point represented the test result.

Muscle Analysis

In the muscle samples, protein expression was determined by Western blotting. Approximately 3 mg dry weight of muscle tissue taken at rest were homogenized 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 PMSF, 1 mM each EDTA and EGTA, 10 µg/ml each aprotinin and leupeptin and 3 mM benzamidine) two times for 30 s (Qiagen TissueLyser II, Retsch, Haan, Germany). 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,DTT, SDS, glycerol, and bromphenol blue), and equal amount of total protein were loaded for each sample in different wells on precasted gels (Bio-Rad Laboratories). For comparisons, samples from the same subject were always loaded on the same gel. After gel electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane, which was incubated with ~10 ml of primary antibody over night and then...
washed for 5 min in Tris-buffered saline-Tween before incubation with secondary antibody for 1 h.

The primary antibodies targeting the different ion transport proteins were diluted in either 2% nonfat milk [monoclonal Na\(^{+}\)-K\(^{+}\) pump α1 (C464.6, no. 05–369, Millipore), polyclonal α2 (AB9094; Chemicon and 07–674; Millipore), monoclonal β1 (MA3–930, Affinity BioReagents), and polyclonal NKCCC (Sc-21545, Santa Cruz Biotechnology)] or 3% BSA [monoclonal NHE1, polyclonal MCT1 and polyclonal MCT4 (AB3538P, and AB3316P, Chemicon)]. In addition, two polyclonal rabbit antibodies (kindly provided by Dr. J. Randall Moorman, University of Virginia, and Dr. D. Bers, Loyola University, respectively), targeting either unphosphorylated FX1D1 proteins (AB_FXYD1) or FX1D1 proteins phosphorylated at the serine 68 site (AB_FXYD1ser68) were used (32, 33). The secondary HRP-conjugated antibody (P-0447, P-0448, and P-0449, DakoCytomation) was diluted 1:5,000 in 2% nonfat milk or 3% BSA, depending on the primary antibody. The mean ratio was calculated from the signal intensity of each sample in the same analysis. This calculation was done to set the mean Pre value to 1, and concomitant include a variation of the samples in the same analysis. This calculation was used twice on separate days for Western blotting with the different antibodies. For HI, the mean ratio [postintervention (Post) vs. preintervention (Pre)] was used as the result. For IN, the signal intensity for the individual time points. In addition, IN changes in Yo-Yo IR2 perfor-

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**RESULTS**

**Performance**

HI improved (P < 0.05) performance of the 4th, 6th, and 10th sprint in RST during the 2-wk period (Table 1). After 2 wk of inactivity, IN reduced (P < 0.05) performance in the 5th to the 10th sprint during RST (Table 1). In HI, TST was lower (P < 0.05) from 33.44 ± 0.44 to 32.81 ± 0.38 s (Fig. 1). In IN, TST was longer (P < 0.01) after 2 wk of inactivity (34.31 ± 0.32 vs. 34.11 ± 0.30 s; Fig. 1). The 20-m FST was not changed, neither in HI (Pre: 3.15 ± 0.03 s vs. Post: 3.15 ± 0.05 s), nor in IN (3.14 ± 0.03 vs. 3.15 ± 0.05 s). SFI was not changed in either HI (5.75 ± 1.04 vs. 3.88 ± 0.62%) or IN (5.92 ± 0.78 vs. 7.56 ± 0.92%), but a tendency for an effect on group (P = 0.052) and interaction (group × time) (P = 0.051) was observed.

In HI, Yo-Yo IR2 performance was not changed during the intervention period (937 ± 56 vs. 994 ± 72 m). The Yo-Yo IR2 test performance of IN was 845 ± 48 m before and 801 ± 57 m 72 h into the inactivity period, with both values being higher (P < 0.01) than after 2 wk of inactivity (654 ± 30 m; Fig. 2).

**Protein Expression**

In HI, the expression of the Na\(^{+}\)-K\(^{+}\) pump α2-isofrom was 14.5 ± 4.9% higher (P < 0.05) after the intervention period, whereas no changes were observed in IN. The Na\(^{+}\)-K\(^{+}\) pump α1- and β1-isofrom protein expression was not changed in either HI or IN (Fig. 3).

There was a nonsignificant elevation of AB_FXYD1ser68 signal (6.8 ± 8.7%) after the intervention period in HI. In IN, the AB_FXYD1ser68 signal was lowered (P < 0.05) by 19.4 ± 4.2 and 17.7 ± 6.3% after 72 h and 2 wk of inactivity, respectively. Neither of the interventions changed the intensity of AB_FXYD1 (Fig. 3). Thus in HI, the FXYD1ser68-to-FXYD1 ratio was 27.3 ± 7.6% higher (P < 0.01) after the intervention period (0.29 ± 0.03 vs. 0.36 ± 0.04). In IN, the ratio was lowered nonsignificantly by 15.4 ± 8.4 and 13.5 ± 3.8% after 72 h (0.31 ± 0.02 vs. 0.26 ± 0.03) and 2 wk (0.31 ± 0.02 vs. 0.27 ± 0.02) of inactivity, respectively (Fig. 3). The change in FXYD1ser68-to-FXYD1 ratio

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**Table 1. Duration of each 20-m sprint in the repeated sprint test performed before and after 2 wk of intensified training or inactivity**

<table>
<thead>
<tr>
<th>Sprint No.</th>
<th>HI Pre</th>
<th>HI Post</th>
<th>IN Pre</th>
<th>IN Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.18 ± 0.04</td>
<td>3.18 ± 0.05</td>
<td>3.15 ± 0.03</td>
<td>3.15 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>3.25 ± 0.04</td>
<td>3.24 ± 0.05</td>
<td>3.23 ± 0.04</td>
<td>3.28 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>3.30 ± 0.04</td>
<td>3.28 ± 0.04</td>
<td>3.28 ± 0.03</td>
<td>3.31 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>3.35 ± 0.05</td>
<td>3.26 ± 0.05*</td>
<td>3.37 ± 0.03</td>
<td>3.40 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>3.37 ± 0.04</td>
<td>3.28 ± 0.03</td>
<td>3.38 ± 0.04</td>
<td>3.46 ± 0.05*</td>
</tr>
<tr>
<td>6</td>
<td>3.43 ± 0.08</td>
<td>3.32 ± 0.04*</td>
<td>3.41 ± 0.05</td>
<td>3.48 ± 0.04*</td>
</tr>
<tr>
<td>7</td>
<td>3.38 ± 0.03</td>
<td>3.32 ± 0.03</td>
<td>3.41 ± 0.04</td>
<td>3.50 ± 0.04*</td>
</tr>
<tr>
<td>8</td>
<td>3.40 ± 0.06</td>
<td>3.32 ± 0.04</td>
<td>3.42 ± 0.04</td>
<td>3.50 ± 0.04*</td>
</tr>
<tr>
<td>9</td>
<td>3.40 ± 0.05</td>
<td>3.33 ± 0.06</td>
<td>3.42 ± 0.03</td>
<td>3.56 ± 0.03*</td>
</tr>
<tr>
<td>10</td>
<td>3.39 ± 0.08</td>
<td>3.29 ± 0.04*</td>
<td>3.35 ± 0.04</td>
<td>3.46 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE in seconds; n = 7 and 9 subjects in the high-intensity training (HI) and inactivity (IN) group, respectively. Pre, before training intervention; Post, after training intervention. *Significant (P < 0.05) different from before the intervention period.

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**Statistics**

Changes for HI and IN in the RST performance was examined by a two-way ANOVA for repeated measures with sprint number and time (Pre vs. Post) as factors. Changes in TST, SFI, and Yo-Yo IR2 performance were tested by a two-way ANOVA for repeated measurements (one-factor repetition) with group (HI vs. IN) and time (Pre vs. Post) as factors. In addition, IN changes in Yo-Yo IR2 performance was examined by a one-way ANOVA for repeated measurements (Pre, 72 h, Post).

A one-sample t-test on the signal intensity ratio (Post vs. Pre) was used for HI to examine changes in protein expression. For IN, the signal intensity for each sample was related to the mean Pre signal intensity, before changes in protein expression were examined by a one-way ANOVA for repeated measurements (Pre, 72 h, Post). Changes in protein expression are presented as mean of the individual ratios (Post vs. Pre and 72 h vs. Pre values) with standard error of the mean.

When a significant main effect was detected in the one- or two-way ANOVA for repeated measures, a Student-Newman-Keuls post hoc analysis was performed for pairwise multiple comparison. All of the statistical analysis was made in Sigmagplot version 11.0, and the level of statistical significance was set at P < 0.05. Data are expressed as means ± SE.
was correlated (n = 15; r² = 0.35; P < 0.05) with the change in RST performance (Fig. 4).

MCT1 protein expression tended (P = 0.088) to be higher (13.3 ± 6.5%) in HI, while it was not changed for IN. The MCT4, NHE1, and NKCC1 expressions were not changed in either HI or IN (Fig. 5).

**DISCUSSION**

The major findings in the present study were that only 2 wk of intensified training of well-trained soccer players lead to an increase in Na⁺-K⁺ pump α₂-isoform protein expression and change in the resting phosphorylation status of the accessory and regulatory FXYD1 protein. Furthermore, 2 wk of cessation of training resulted in a lowering of the resting FXYD1ser68 phosphorylation level. The elevated protein expression and phosphorylation status in the HI group were associated with improved performance in a RST, whereas, in the IN group, the phosphorylation status was reduced, and performance in the RST and Yo-Yo IR2 test was lowered.

The content of Na⁺-K⁺ pump α₂-isoforms was elevated by 15% after just 2 wk of intensified training, despite the subjects being soccer players used to perform repeated bouts of high-intensity exercise (2). In accordance, Bangsbo et al. (5) observed that the amount of the Na⁺-K⁺ pump α₂-isoform was 68% higher when a group of endurance-trained runners performed a 6- to 9-wk period with SET and reduced volume of training, and, as in the present study, the α₁- and β₁-subunits were not changed. Iaia et al. (20) observed that a change in training from regular endurance running to SET resulted in an increased level of muscle Na⁺-K⁺ pump α₁-isoforms, but they only found a tendency (P = 0.08) to an elevated (18%) level of the α₂-isoform and no change in β₁ (20). The difference from the present study may be related to the difference in the amount of aerobic training performed, since it has been shown to increase the Na⁺-K⁺ pump α₂-isoform expression (17). In the study by Iaia et al. (20), the runners had a 64% reduction in the volume of training and only performed SET (20), whereas the subjects in the present study also carried out a significant amount of AH1 training. An interesting finding in the present study was also that 2 wk without training did not lead to any changes in the Na⁺-K⁺ pump isoforms, suggesting that reducing the training for 2 wk has little effect on the net synthesis of these proteins. In contrast to the above-mentioned studies, Aughey et al. (1) found no changes in the abundance of any of the Na⁺-K⁺ pump α- and β-isoforms, when already-trained subjects performed a period of intensified training. It may be explained by the exercise intensity being below the one corresponding to VO2max in that study (1). Taken together, SET with repeated bouts of 30-s near-maximal exercise in well-trained subjects can even, within 2 wk, lead to further adaptations in the muscle Na⁺-K⁺ pump α-isoforms, which may be due to recruitment of fibers that are not used or not utilized to the same extent in the normal soccer or endurance training. One of the reasons for the Na⁺-K⁺ pump α-isoform upregulation may have been a higher intracellular Na⁺ concentration during the high-intensity training compared with the normal training (34), since intracellular Na⁺ is a possible activator of signaling pathways, leading to increases in transcription factors relevant for the Na⁺-K⁺ pump isoforms. Thus Ladka and Ng (23) used the Na⁺ channel activator, veratridine, to modulate intracellular Na⁺ and observed increased α₂-protein expression in C2C12 skeletal muscle cells, and increased intracellular Na⁺ was related to an upregulation of Na⁺-K⁺-ATPase molecules in cultured chick skeletal muscles (37).

In the present study, performance in the 4th, 6th, and 10th sprint in RST was improved after the intensified training period in HI, whereas 2 wk of cessation of training resulted in an impaired performance in the 5th to the 10th sprint, as well as reduced performance in the Yo-Yo IR2 test. An association between an elevated content of Na⁺-K⁺ pump α-isoforms and performance during intense continuous (26, 28, 29) and repeated intense (25, 26) exercise has been observed in untrained, as well as in well-trained, subjects (20) after a period of intense training. It may be speculated that the elevated level of α₂-isoforms in HI after the intensified training period had increased the number of functional pumps, causing a lower

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**Fig. 1.** Mean and individual values of total sprint time (TST) in repeated sprint test (RST) for the high-intensity training (HI; n = 7, left) and inactivity (IN; n = 9, right) groups, before (Pre; hatched bars) and after the 2-wk intervention period (Post; open bars). Values are expressed as means ± SE. *Significantly (P < 0.05) different from Pre. **Significantly (P < 0.01) different from Pre.

**Fig. 2.** Mean and individual values of Yo-Yo intermittent recover level 2 (IR2) performance for the HI (n = 7, left) and the IN (n = 11, right) groups, Pre (hatched bars), 72 h into the 2-wk intervention period (checkered bar), and Post (open bars). Values are expressed as means ± SE. **Significantly (P < 0.01) different from Pre. #Significantly (P < 0.01) different from Post.
accumulation of $K^+$ in the muscle interstitium during and in recovery from exercise (28, 29). Thereby, the reduction in muscle membrane potential may have been lowered, and cell excitability preserved (10). Consequently, the degree of fatigue was reduced. On the other hand, performance in the Yo-Yo IR2 test was not improved in HI after the 2 wk of intensified training, although the level of $Na^+/K^+$-pump isoforms, $Na^+/K^+$-pump, was elevated. It may have been that the change in $Na^+/K^+$-pump isoforms was too small to cause any significant change in performance in these well-trained soccer players. They performed 940 m on the Yo-Yo IR2 test before the training, in comparisons to 440 m for the endurance-trained athletes in the study by Iaia et al. (20), who observed a 19% improvement after 4 wk of SET (20). Nevertheless, performance in both RST and in Yo-Yo IR2 test was reduced in IN, despite no changes in the $Na^+/K^+$-pump isoforms, suggesting that the amount of $Na^+/K^+$-pump isoforms per se is not determining performance after detraining.

The present study is the first to show a change in the resting phosphorylation status of the $Na^+/K^+$ pump accessory and regulatory FXYD1 protein in human skeletal muscle. Intensi-fied training in HI induced an alteration in the resting FXYD1 phosphorylation status, determined as the $\text{AB}_\text{FXYD1}_{\text{ser68}}$-to-$\text{AB}_\text{FXYD1}$ signal ratio, which is in accordance with a recent study in rats, where 5 days of swim training induces an increased $\text{FXYD1}_{\text{ser68}}$ phosphorylation (16). Likewise, in IN, the 2 wk of training cessation lowered the $\text{AB}_\text{FXYD1}_{\text{ser68}}$ signal in the resting muscle. Furthermore, although weak, a significant relationship between the change in $\text{AB}_\text{FXYD1}_{\text{ser68}}$-to-$\text{AB}_\text{FXYD1}$ signal ratio and change in performance in RST was observed (Fig. 5). $\text{FXYD1}_{\text{ser68}}$ phosphorylation has been shown to increase the apparent $Na^+$ affinity of $Na^+/K^+$ pump $\alpha_2$ and $\alpha_2/\beta$-isozymes, as well as the maximal activity of the $Na^+/K^+$ pump $\alpha_2/\beta$-isozymes in an oocyte expression system (7). Thus it is possible that the altered $\text{FXYD1}_{\text{ser68}}$ phosphorylation status with the intensified training and inactivity has affected the $Na^+/K^+$ activity at the onset of exercise.

There seems to be a time lag in the activation and increase of the $Na^+/K^+$ pump activity at the beginning of exercise (35), contributing to the initial rapid increase in $K^+$ release from contracting muscles at the onset of exercise (19, 29). Therefore, it may be that a more prepared pool of $Na^+/K^+$ pumps
could explain part of the better short-term performance in HI, and, correspondingly, a less ready pool of Na\(^+\)-K\(^+\) pumps could have reduced performance in IN. However, further studies are needed to explore these possibilities.

The expression of NKCC1, MCT1, and MCT4 did not change significantly with 2 wk of intensified training, which is in accordance with the observations by Iaia et al. (20) and Bangsbo et al. (5) after 4 and 6–9 wk, respectively, of SET with reduced volume (20). On the other hand, most studies with untrained subjects have shown a higher amount of MCT1 transporters after a period of high-intensity training (8, 9, 22, 26, 30), and, in the present study, MCT1 tended to increase \((P = 0.088)\) in HI. One study has reported sprint training-induced changes in MCT1 transport proteins in endurance-trained subjects (8), where the subjects maintained a high volume of training (~50 km/wk), so the MCT1 response of trained subjects to SET appears to depend on the total amount of training performed. The finding of unaltered MCT4 levels is consistent with the majority of the other studies (8, 14, 22, 26). The 2 wk of cessation of training did not show a decrease in NKCC1, MCT1, and MCT4, indicating that these proteins are not very sensitive to lowered activity, and it can be one of the explanations that a marked reduction in volume of training in the study by Iaia et al. (20) did not reduce the levels of these proteins. NHE1 was unaltered in HI, which is in agreement with Bangsbo et al. (5), but NHE1 tended to be elevated by 2 wk of inactivity. Iaia et al. (20) found that NHE1 was higher after a 4-wk period of SET, with a 64% reduction in total volume. In the present study, the nonsignificant higher expression of NHE1 was associated with impaired performance.

**Fig. 4.** Individual \((n = 15)\) relationship \((P < 0.05)\) between changes in \(AB\_FXYD1\text{ser68-to-AB\_FXYD1}\) Western blotting signal intensity ratio and changes in performance of the RST determined as TST after 2 wk of intervention, including both the HI and IN groups.

**Fig. 5.** A: representative Western blot bands are shown for HI and IN. B: percent changes in the Western blot signal intensity of monocarboxylate transporter 1 (MCT1), monocarboxylate transporter 4 (MCT4), Na\(^+\)/H\(^+\) exchanger isoform 1 (NHE1), and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter isoform 1 (NKCC1) for the HI group \((n = 7)\) after the 2-wk intervention period (open bars) and for the IN group \((n = 9)\) 72 h into (light shaded bars) and 2 wk of the intervention (dark shaded bars). Values are expressed as means ± SE.
during repeated intense exercise, whereas it was the opposite in the study by Iaia et al. (20) and in a number of studies in which untrained subjects performed a period of sprint training (22, 26). Thus it is not clear which role, if any, NHE1 does play, but it is obvious from the present data that changes in short-term performance can occur without alterations in some of the key H^+/K^+ transport proteins.

In summary, 2 wk of intensified training of well-trained soccer players did elevate muscle Na^+–K^+ pump α2-isozyme protein expression, whereas 2 wk of cessation of training did not significantly affect the level of any of the ion transport proteins. On the other hand, resting FXYD1ser68 phosphorylation level was lowered by the inactivity period, and the phosphorylation status of the FXYD1 protein was elevated in the IN group. The findings may suggest that the resting phosphorylation status of the Na^-K^+ pump plays a role for performance during repeated intense exercise, but further studies are needed to examine this issue.

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