Exercise-induced regulation of muscular Na\(^+\)-K\(^+\) pump, FXYD1, and NHE1 mRNA and protein expression: importance of training status, intensity, and muscle type

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Rasmussen MK, Juel C, Nordsborg NB. Exercise-induced regulation of muscular Na\(^+\)-K\(^+\) pump, FXYD1, and NHE1 mRNA and protein expression: importance of training status, intensity, and muscle type. Am J Physiol Regul Integr Comp Physiol 300: R1209–R1220, 2011. First published February 16, 2011; doi:10.1152/ajpregu.00635.2010.—It is investigated if exercise-induced mRNA changes cause similar protein expression changes of Na\(^+\)-K\(^+\) pump isoforms (\(\alpha_1, \alpha_2, \beta_1, \beta_2\)), FXYD1, and Na\(^+\)/K\(^+\) exchanger (NHE1) in rat skeletal muscle. Expression was evaluated (n = 8 per group) in soleus and extensor digitorum longus after 1 day, 3 days, and 3 wk (5 sessions/wk) of either sprint (4 × 3-min sprint + 1-min rest) or endurance (20 min) running. Two hours after exercise on day 1, no change in protein expression was apparent in either training group or muscle, whereas sprint exercise increased the mRNA of soleus \(\alpha_2 (4.9 \pm 0.8\)-fold; \(P < 0.05)\), \(\beta_2 (13.2 \pm 4.4\)-fold; \(P < 0.001)\), and NHE1 (12.0 ± 3.1-fold; \(P < 0.01)\). Two hours after sprint exercise, protein expression normalized to control samples was higher on day 3 than day 1 for soleus \(\alpha_1 (41 \pm 18\%-increase vs. 15 \pm 8\%-reduction; P < 0.05)\), \(\alpha_2 (64 \pm 35\%-increase vs. 37 \pm 12\%-reduction; P < 0.05)\), \(\beta_1 (17 \pm 21\%-increase vs. 14 \pm 29\%-reduction; P < 0.05)\), and FXYD1 (35 ± 16\%-increase vs. 13 ± 10\%-reduction; \(P < 0.05)\). In contrast, on day 3, soleus \(\alpha_1 (0.1 \pm 0.1\%-fold; P < 0.001)\), \(\beta_2 (0.2 \pm 0.1\%-fold; P < 0.001)\), \(\beta_1 (0.4 \pm 0.1\%-fold; P < 0.05)\), and \(\beta_2\)-mRNA (2.9 ± 1.7-fold; \(P < 0.001)\) expression was lower than after exercise on day 1. After 3 wk of training, no change in protein expression relative to control existed. In conclusion, increased expression of Na\(^+\)-K\(^+\) pump subunits, FXYD1 and NHE1 after 3 days of sprint exercise does not appear to be an effect of increased constitutive mRNA levels. Importantly, sprint exercise can reduce mRNA expression concomitant with increased protein expression.

EXERCISE IS ASSOCIATED WITH an intensity-dependent muscular net efflux of K\(^+\) and H\(^+\) ions (2, 44, 46), reflecting K\(^+\) accumulation in the muscle interstitium and intramuscular acidosis, both suggested as primary agents causing muscle fatigue (10, 43). The exercise-induced disturbance of ion homeostasis is counteracted by several transport systems, one being the ATP demanding Na\(^+\)-K\(^+\) pump that transports two K\(^+\) ions into and three Na\(^+\) ions out of the muscle fiber (3, 5, 18). The functional Na\(^+\)-K\(^+\) pump consists of one of four different catalytic α-subunits (\(\alpha_1–\alpha_4\)) and one of three different structural β-subunits (\(\beta_1–\beta_3\)) (3). In addition, the Na\(^+\)-K\(^+\) pump is associated with the regulatory protein FXYD1, which moderately reduces external K\(^+\) affinity and severely reduces internal Na\(^+\) affinity of \(\alpha_1–\beta_1\) and \(\alpha_2–\beta_3\) complexes (9). During exercise, Na\(^+\)-K\(^+\) pump activity is increased by up to 20-fold, while regular exercise training causes increased muscular Na\(^+\)-K\(^+\) pump content, as determined by [\(^3\)H]ouabain binding (5), and reduced K\(^+\) perturbations during exercise (17, 33). It has previously been demonstrated that exercise is associated with an increase in both Na\(^+\)-K\(^+\) pump \(\alpha\)- and \(\beta\)-mRNA levels within the first few hours after exercise (31, 34, 36, 45), indicating that transcriptional regulation is a determining factor of Na\(^+\)-K\(^+\) pump subunit expression. However, no changes in Na\(^+\)-K\(^+\) pump subunit protein expression could be detected within 24 h after an intense exercise bout lasting for a few minutes (31), whereas 16 h of repeated intermittent cycling, as well as 3 and 6 days of less demanding training, have proved sufficient to increase total Na\(^+\)-K\(^+\) pump protein expression by up to 13% and \(\alpha\)-subunit expression by up to 29% (12–14). Thus increased Na\(^+\)-K\(^+\) pump expression after exercise training may be caused by rapid initial increases in Na\(^+\)-K\(^+\) pump \(\alpha\)- or \(\beta\)-mRNA expression, followed by a slower de novo protein synthesis. It is often assumed that increased mRNA expression causes increased protein de novo synthesis. However, numerous other factors also affect the relation between mRNA and protein expression, such as protein degradation rate, posttranscriptional mRNA modification, mRNA stability, etc. The relation between Na\(^+\)-K\(^+\) pump and FXYD1 mRNA and protein expression has never been addressed at the same time during the initial part of an exercise training period, as well as after several weeks of training. Thus one aim of the present study was to investigate whether regular exercise training causes rapid initial increases in Na\(^+\)-K\(^+\) pump \(\alpha\)- or \(\beta\)-mRNA expression, followed by a slower increase in protein expression.

In general, exercise intensity is decisive for the adaptations to exercise (7). In example, strength training may cause activation of specific molecular signaling pathways, such as the insulin-like growth factor I-induced activation of the mammalian target of rapamycin. Endurance training apparently activates other signaling pathways, including peroxisome proliferator-activated receptor-γ coactivator-1α activation (7), which is expected to result in elevated mitochondrial biogenesis (16, 40). Moreover, it has recently been demonstrated that the degree of activation of key regulators for cellular adaptation can depend on the imposed exercise stimuli, as found for increases in proliferator-activated receptor-γ coactivator-1α mRNA (38). It has also been demonstrated that the exercise-induced increase in Na\(^+\)-K\(^+\) pump mRNA expression is dependent on exercise intensity, with a high relative exercise intensity causing the largest stimuli (37), and that intensified training further increases Na\(^+\)-K\(^+\) pump protein expression of moderately trained runners (21). Moreover, the intensity sensitivity of the Na\(^+\)-K\(^+\) pump mRNA response...
appears to be subunit specific, with α1 demonstrating the most pronounced sensitivity (37). In contrast to studies showing the largest increases in Na\(^{+}\)-K\(^{+}\) pump content with intense exercise, it has also been demonstrated that a high volume of submaximal training results in a faster and larger increase in Na\(^{+}\)-K\(^{+}\) pump expression than high-intensity resistance training (11). With regard to FXYD1, one study has shown that long-term endurance training increases the expression of FXYD1 in old rats (42). The possible intensity dependence of changes in FXYD1 expression is currently unknown, but can be hypothesized to follow the changes in Na\(^{+}\)-K\(^{+}\) pump expression. When investigating the effect of different training intensities, it is of importance to match the training stimuli between groups. Otherwise, it is not possible to state whether differences are related to the difference in intensity or volume. No studies have investigated the importance of training intensity for simultaneous contraction-induced changes in both mRNA and protein expression of Na\(^{+}\)-K\(^{+}\) pump and FXYD1 expression. Moreover, it appears likely that not only training volume, but also training status, is of importance for adaptation to an exercise regime. For example, a trained subject exercising at the same absolute, and, therefore, at a lower relative, workload experiences a less pronounced change in mRNA expression (37). The importance of relative training intensity for protein and mRNA expression during prolonged training can be studied by keeping the absolute intensities constant during a training period, which causes a gradual reduction of relative intensity. Thus a second aim of the present study was to investigate if exercise intensity and training status are of importance for the association between changes in Na\(^{+}\)-K\(^{+}\) pump mRNA and protein expression.

The cellular signals leading to fluctuations in mRNA and protein expression are likely to be muscle-type specific, because the constitutive expression of subunit-specific mRNA is highly dependent on muscle type (20). This assumption is supported by the finding that 1 h of exercise increased α1-mRNA expression exclusively in oxidative rat muscle and β2 only in glycolytic muscle (45). Furthermore, as little as 3 × 10 s of electrical stimulation of excised glycolytic rat muscle [extensor digitorum longus (EDL)] elevates α- but not β-mRNA expression (29). Recently, it was reported that electrical in vitro stimulation of rat soleus and EDL muscle yields pronounced differences with reductions of mRNA levels observed for α1, α2, and β2 levels in EDL, whereas α1, β1, and β2 levels were increased in soleus (37). As such, it appears that pronounced fiber-type differences exist with regard to regulation of Na\(^{+}\)-K\(^{+}\) pump expression, but divergent results have been obtained from in vivo and in vitro studies. The importance of muscle type for exercise induced changes in FXYD1 mRNA and protein expression is largely unknown. Thus, a third aim was to investigate if changes in mRNA and protein expression during a training period with different exercise intensities are dependent on the investigated muscle group.

In addition, exercise training also improves the capacity for extrusion of intramuscular protons that accumulate during intense exercise (25). The primary H\(^{+}\) transport systems in the muscle membrane is the lactate-H\(^{+}\) cotransporters (monocarboxylate transporter 1 and 4) and the Na\(^{+}\)/K\(^{+}\) exchanger (NHE1) (22). Previously, intensified training of moderately trained runners has been shown to increase the NHE1 expression (21), indicating that the regulation of this transport system is dependent on training intensity. This is in line with the observation that only high-intensity training, but not endurance training, causes increased NHE1 activity (23). Moreover, a fiber-type-specific expression with more NHE1 in glycolytic than oxidative fibers has been reported (24). Thus a fourth aim of the present study was to investigate if NHE1 mRNA, as well as protein expression, is altered by intense and moderate exercise training in a muscle group-specific way.

The overall primary research hypothesis of the present study was that 1) one bout of exercise increases Na\(^{+}\)-K\(^{+}\) pump isoform, FXYD1, and NHE1 mRNA expression within hours after cessation of exercise; and 2) a corresponding increases in protein expression can be observed after 3 days of regular exercise training. Furthermore, it was hypothesized 3) that one bout of exercise subsequent to 3 wk of regular exercise training would still cause elevated mRNA and protein levels. Moreover, it was expected that high-intensity exercise would induce the greatest increase in mRNA and protein expression and primarily so in glycolytic muscle.

**METHODS**

The protocol was approved by The Danish Animal Experimental Inspectorate and complied with the European Convention for the Protection of Vertebrates Animals used for Experiments and Other Scientific Purpose (Council of Europe 123, Strasbourg, France, 1985). Male Wistar rats (~100 g) were used for the 1- and 3-day exercise training study, while male Sprague-Dawley rats (~100 g) were used for the 3-wk exercise training study. All training groups were compared with strain and age-matched controls. Animals were housed in groups of four to five per box cage. The temperature was 22 ± 1°C and a 12:12-h light-dark cycle was used (light 0800–2000). The rats were provided unrestricted access to food and water. All rats were killed by cervical dislocation.

**One- and Three-Day Exercise Study**

Forty rats were randomly assigned to five groups consisting of eight rats each. Two groups performed sprint exercise (four 3-min runs at 33 m/min, 10% incline, separated by 1-min rest), two groups performed endurance exercise (20-min running at 21 m/min, 10% incline), and one group acted as control animals. The sprint intensity was chosen as the highest speed at which most animals could complete the protocol in pilot studies. The intensity of the endurance group was chosen to be ~60% of that of the sprint groups, and the total running distance was matched between groups. However, due to a recalibration of the treadmill, the distances are not exactly the same (sprint 396 m vs. endurance 420 m).

Of the two sprint and two endurance exercise groups, one group was killed 2 h after a single exercise bout (1-day group), while the other group (3-day group) was killed 2 h after the training session completed on the 3rd consecutive training day. Control animals were killed on day 3 and served as controls for both day 1 and day 3. Muscles were excised 2 h after exercise, because human studies have demonstrated substantial increases of Na\(^{+}\)-K\(^{+}\) pump isoform mRNA expression between 1 and 3 h after exercise (36).

**Three-Week Training Study**

Twenty-four rats were randomly assigned to three groups consisting of eight rats each. Two groups completed a 3-wk training period with exercise 5 days/wk. One of these groups performed sprint exercise (four 3-min runs at 33 m/min, 10% incline, separated by 1-min rest), and the other group performed endurance exercise (20-min running at 21 m/min, 10% incline). One group acted as age-
matched control animals. To investigate if frequent training at the same absolute intensity is sufficient to cause initial and thereafter sustained changes of the investigated proteins expression, the exercise intensity was kept constant during the training period. This protocol will have caused a gradual reduction of the relative exercise intensity as the training period progressed. In accordance with the 1- and 3-day training groups, the animals were killed, and muscles were removed 2 h after the last training session.

Muscle Handling

EDL and soleus muscles were rapidly removed and frozen in liquid nitrogen and stored at −80°C for later analysis.

RNA Isolation and Reverse Transcription

Total RNA from the muscles was isolated using Trizol reagent following the manufacturer’s guidelines, and the final pellets were resuspended in 50-μl diethyl pyrocarbonate-treated H2O containing 0.1 mM EDTA. RNA was quantified by measuring the absorbance at 260 nm, and the purity of the samples was assessed from the 260-nm-to-280-nm ratio, which was always >1.9. The integrity of the RNA was confirmed by visual inspection of the 18S and 28S RNA bands on an ethidium bromide-stained formaldehyde agarose gel. Reverse transcription (RT) of total RNA (3 μg) was performed using the Superscript II RNase H− system (Invitrogen, Carlsbad, CA). Each RT sample was diluted in nuclease-free water to a total volume of 170 μl.

Normalization of mRNA Expression

For normalization of mRNA expression, the single-stranded DNA (ssDNA) content was assessed in each sample using OligGreen reagent (Molecular Probes, Leiden, The Netherlands), as previously described (27). Thus the mRNA expression is expressed relative to the sample ssDNA content.

PCR

The mRNA content of selected genes was determined by real-time PCR (ABI PRISM 7900 Sequence Detection System, Applied Biosystems, Foster City, CA). Forward and reverse primers and TaqMan probes were designed from rat-specific sequence databases (Entrez-NIH and Ensembl, Sanger Institute) using computer software (Primer Express, Applied Biosystems). Sequences are given in Table 1. For each of the genes, a Blast Search revealed that sequence homology was obtained only for the target gene. All TaqMan probes were 5′-6-carboxyfluorescein (FAM) and 3′-6-carboxy-N',N',N',N'-tetramethylrhodamine (TAMRA) labeled. Optimization and PCR were performed as previously described (27). Samples were analyzed in triplicate, and the mean interassay coefficient of variation was <2% for all target genes. The threshold cycle, reflecting the initial target mRNA content in the sample, was converted to a relative amount using a standard curve obtained by running a serial dilution of a pooled RT sample, together with the samples.

Determination of Specific Subunit Protein Content

Approximately 30 mg of muscle tissue were minced and homogenized in a sucrose buffer [in mM: 250 sucrose, 30 HEPES, 2 EGTA, 40 NaCl, and 2 PMSF (phenylmethylsulfonyl fluoride), pH 7.4] using a Polytron-2100 and centrifuged 5 min at 1,000 g (4°C). The resulting supernatant was then centrifuged at 190,000 g for 90 min (4°C), and the pellet (the membrane fraction) was dissolved in TS-SDS (10 mM Tris base, 1 mM EDTA, 4% SDS, 2 mM PMSF, pH 7.4). The initial spinning (1,000 g) removes connective tissue and other dense material, but it has previously been demonstrated that the main part of both sarcolemmal and T-tubuli Na+–K+ pump subunits is preserved (41). Total protein concentrations were

Table 1. Sequences of primers and probes used for analysis of Na+–K+ pump mRNA expression in rats

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<th>Target</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
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| Na+–K+ PUMP | 5′-ACG GGA GGA GCA GAC TCC-3′ | 5′-GGA GGA GGA GCA GAC TCC-3′ | TAMRA-
| FXYD1 | 5′-GGA GGA GGA GCA GAC TCC-3′ | 5′-GGA GGA GGA GCA GAC TCC-3′ | TAMRA-
| NHE1 | 5′-GGA GGA GGA GCA GAC TCC-3′ | 5′-GGA GGA GGA GCA GAC TCC-3′ | TAMRA-
measured with a DC protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as standard.

The relative amount of the Na⁺-K⁺-pump subunits, FXYD1, and NHE1 protein was determined by Western blotting, as previously described (41). Briefly, equal amounts of protein were loaded and separated by 8–18% or 12% SDS-PAGE and transferred to a polyvinylidene difluoride membrane and exposed to a specific antibody (see Fig. 1). Efficient and uniform transfer was verified by visual inspection of the membrane protein content by Ponceau staining. After treatment with secondary antibody and repeated washing, the membrane was visualized on film by use of enhanced chemiluminescence. The relative protein concentration was quantified by scanning the film and analyzing with UN-SCAN-IT (Silk Scientific, Orem, UT). All samples were analyzed in duplicates, and the linearity of the signal was verified by analysis of diluted and concentrated loading controls.

In skeletal muscle, the Na⁺-K⁺-pump α₁-, α₂-, β₁-, and β₂-subunits appear to be the most abundant (20, 36), even though mRNA expression of α₃, α₄, and β₅ has been detected (29). Thus only α₁, α₂, β₁, and β₂ expression was investigated in the present study. The α₁-subunit was detected with the α6F antibody (Iowa Hybridoma Bank, Iowa City, IA), while antibodies against the α₂-(McB2) and β₁-subunits were generously provided by Dr. P. A. Pedersen (University of Copenhagen, Denmark). The β₂-subunit was detected with an antibody from Upstate Biotechnology (06–171). FXYD1 was detected with an antibody generously provided by Dr. S. Karlish (Weizmann Institute, Israel). For detection of the NHE1, the MAB3140 antibody (Chemicon) was used.

Statistics and Calculations

The mRNA expression was calculated as target gene mRNA content normalized to the amount of ssDNA in the sample (27) and related to the time-matched control sample. For mRNA data, the reported values are fold changes and SEs of the mean. Protein expression densitometry values are expressed as sample values in percentage of the time-matched control value (mean ± SE).

The isoform-specific mRNA and protein expression data were investigated statistically using a mixed-model procedure (6) (SPSS 17.0). For the day 1 and day 3 data points, “group” (3-day control, endurance, sprint), “day” (day 1, day 3), and “muscle” (soleus, EDL) were included as fixed factors. The 3-wk data point was investigated by including “group” (3-wk control, 3-wk endurance, 3-wk sprint) and “muscle” (soleus, EDL) as fixed factors. Significant effects on each of the fixed factors and interaction between factors were investigated further by application of Bonferroni adjusted multiple pairwise comparisons. The significance level was set at $P < 0.05$.

![Fig. 1. Examples of quantified bands for the analyses of protein expression by Western blotting. Soleus and extensor digitorum longus (EDL) muscles were excised 2 h after exercise from five different groups of animals ($n = 8$ in each group). Sprint exercise (SP) and endurance (END) exercise were performed by separate groups (see METHODS). A control (CON) group was killed at day 3 and after 3 wk. For each muscle and specific time point, samples from the CON, SP, and END groups were run on the same gel (for example, EDL, Day 1). Analyses were performed in duplicates on separate gels. The intensity of a specific band from the exercise group was normalized to the average intensity of the CON group. Thus the provided example blots can only be compared between the SP, END, and CON samples within a specific muscle and time point. Because the example blots have been randomly selected and no normalization is possible, the visual impression may deviate from the quantified data. However, the blots are provided as examples of the applied analytic procedure.](http://ajpregu.physiology.org/)
RESULTS

\textbf{Na}⁺-K⁺ Pump Isoform-Specific Changes in mRNA Expression Relative to Muscle Type, Exercise Intensity, and Training Status

See Fig. 2.

\( \alpha _1 \). In soleus, no change was detected in \( \alpha _1 \)-mRNA expression 2 h after the exercise bout at any time point during the endurance training period, whereas sprint exercise reduced \( \alpha _1 \)-mRNA expression after one exercise bout on \textit{day 3} (\( P < 0.05 \)) to 0.1 ± 0.1-fold of the control level. Moreover, the response of soleus \( \alpha _1 \)-mRNA expression to sprint exercise on \textit{day 3} was lower (\( P < 0.001 \)) than after \textit{day 1}. The \( \alpha _1 \)-mRNA expression was lower (\( P < 0.05 \)) after sprint exercise than after endurance exercise on \textit{day 3} in soleus. In EDL, no effect of endurance exercise was detected on \( \alpha _1 \)-mRNA expression, but, after one bout of sprint exercise subsequent to 3 wk of training, \( \alpha _1 \)-mRNA expression was reduced (\( P < 0.05 \)) to 0.4 ± 0.1-fold. In addition, the EDL \( \alpha _1 \)-mRNA expression was lower (\( P < 0.01 \)) after sprint exercise on \textit{day 3} compared with \textit{day 1}. Furthermore, the \( \alpha _1 \)-mRNA expression was lower (\( P < 0.05 \)) after 3 wk of sprint exercise than after 3 wk of endurance exercise.

\( \alpha _2 \). One bout of endurance exercise did not induce any changes in soleus \( \alpha _2 \)-mRNA expression at any of the investigated time points. In soleus, one bout of sprint exercise increased (\( P < 0.05 \)) \( \alpha _2 \)-mRNA expression 4.9 ± 0.8- and 5.5 ± 1.6-fold (\( P < 0.01 \)) 2 h subsequent to sprint exercise after 1 day and 3 wk, respectively. However, soleus \( \alpha _2 \)-mRNA expression subsequent to sprint exercise on \textit{day 3} (0.2 ± 0.1-fold) was lower (\( P < 0.001 \)) than after sprinting on \textit{day 1}. Endurance exercise did not alter the \( \alpha _2 \)-mRNA expression in EDL on \textit{day 1} and \textit{day 3}, but, after 3 wk of training, \( \alpha _2 \)-mRNA was elevated (\( P < 0.01 \)) 10.5 ± 2.5-fold after the exercise bout, which was higher (\( P < 0.01 \)) than the expression observed in soleus after endurance exercise. In EDL, sprint training did not affect the mRNA expression compared with the control group. However, the sprint exercise-induced response in EDL \( \alpha _2 \)-mRNA expression after \textit{day 3} was lower (0.4 ± 0.1-fold; \( P < 0.001 \)) than on \textit{day 1} (5.7 ± 2.0-fold).

\( \beta _1 \). Both in soleus and EDL, no changes in \( \beta _1 \)-mRNA expression were detected after one bout of sprint or endurance exercise (\textit{day 1}). However, in soleus, a reduction (\( P < 0.05 \)) in \( \beta _1 \)-mRNA expression was apparent after sprint exercise on \textit{day 3} (0.4 ± 0.1-fold) compared with \textit{day 1} (1.1 ± 0.2-fold). Furthermore, after endurance exercise, subsequent to 3 wk of training, the expression of \( \beta _1 \)-mRNA was higher (\( P < 0.05 \)) in EDL (1.6 ± 0.3-fold) compared with soleus (1.0 ± 0.3-fold).

\( \beta _2 \). Endurance exercise did not alter the expression of \( \beta _2 \)-mRNA in soleus. In contrast, after sprint training, soleus \( \beta _2 \)-mRNA expression was increased 13.2 ± 4.4-fold (\( P < 0.001 \)) on \textit{day 1} and 33.6 ± 5.8-fold (\( P < 0.001 \)) after 3 wk of training. No change was observed after sprint training on \textit{day 3} in soleus \( \beta _2 \)-mRNA, but the expression (2.9 ± 1.7-fold) was lower (\( P < 0.001 \)) than on \textit{day 1}. Endurance exercise did not alter \( \beta _2 \)-mRNA expression in EDL on \textit{day 1} or \textit{day 3}, but increased (\( P < 0.001 \)) the expression 62.2 ± 10.6-fold when exercise was performed after 3 wk of training. Sprint exercise did not alter EDL \( \beta _2 \)-mRNA on \textit{day 1} and \textit{day 3}, but, when performed after 3 wk of training, EDL \( \beta _2 \)-mRNA was increased (\( P < 0.001 \)) to 29.9 ± 5.7-fold. Moreover, the EDL \( \beta _2 \)-mRNA expression was lower (\( P < 0.05 \)) after sprint exercise on \textit{day 3} than on \textit{day 1}. In addition, a difference between the response in soleus and EDL was observed after sprint training on \textit{day 1} (\( P < 0.05 \)) and endurance training after 3 wk of training (\( P < 0.001 \)).

\textbf{Na}⁺-K⁺ Pump Isoform-Specific Changes in Protein Expression Relative to Muscle Type, Exercise Intensity, and Training Status

See Fig. 3.

\( \alpha _1 \). In soleus, the protein expression of \( \alpha _1 \) was not different between the control and endurance training groups at any investigated time point. Likewise, no difference between the control and sprint training group was detected. However, the numerical increase in soleus \( \alpha _1 \) expression of 41 ± 18% after sprint exercise on \textit{day 3} relative to the control group was higher (\( P < 0.05 \)) than the numerical reduction of 15 ± 8% relative to the control group after sprint exercise on \textit{day 1}. In EDL, no difference in \( \alpha _1 \) expression was apparent at any time point in neither the endurance, nor the sprint training group relative to the control group. However, the EDL \( \alpha _1 \) expression in the sprint group relative to the control group on \textit{day 3} was higher (\( P < 0.05 \)) than the expression after sprint exercise on \textit{day 1} (a numerical 41 ± 21% increase vs. 17 ± 12% reduction). Subsequent to 3 wk of endurance training, the \( \alpha _1 \) expression was lower (\( P < 0.05 \)) in EDL (21 ± 7% reduction relative to control) than in soleus (15 ± 9% increase relative to control), but, in both muscles, no difference existed relative to the muscle-specific control.

\( \alpha _2 \). In soleus, no effect of endurance or sprint exercise was apparent on \( \alpha _2 \) expression relative to the control group at all investigated time points. However, the \( \alpha _2 \) expression in soleus after sprint exercise on \textit{day 3} (64 ± 35% relative to control) was higher (\( P < 0.05 \)) than the expression after sprint exercise on \textit{day 1} (37 ± 12% reduction relative to control). In EDL, the applied training protocols had no effect on \( \alpha _2 \)-protein expression.

\( \beta _1 \). In soleus, no effect of endurance or sprint exercise was apparent on \( \beta _1 \) expression relative to the control group at all investigated time points. However, the \( \beta _1 \) expression in soleus after sprint exercise on \textit{day 3} was higher (\( P < 0.05 \)) than after sprint exercise on \textit{day 1} (17 ± 21% increase vs. 14 ± 29% reduction). In EDL, the applied training protocols had no effect on \( \beta _1 \) expression.

\( \beta _2 \). \( \beta _2 \) was not detectable in soleus. In EDL, the \( \beta _2 \) expression after exercise on \textit{day 3} was reduced (\( P < 0.001 \)) in both the endurance and sprint group to 27 ± 9 and 38 ± 9% of the expression in the control group, respectively. Moreover, the \( \beta _2 \) expression was reduced after endurance (\( P < 0.001 \)) and sprint exercise (\( P < 0.01 \)) on \textit{day 3} compared with after exercise on \textit{day 1}. After endurance exercise subsequent to 3 wk of training, the \( \beta _2 \) expression was reduced to 64 ± 8% (\( P < 0.05 \)) of the expression in the control group.

Effect of Muscle Type, Exercise Intensity, and Training Status on Exercise-Induced Changes in FXYD1 mRNA and Protein Expression

See Fig. 4.
Fig. 2. Effect of SP (four 3-min runs at 33 m/min, 10% incline, separated by 1 min; n = 8) and END (20 min at 20 m/min, 10% incline; n = 8) exercise on Na⁺-K⁺ pump mRNA levels in rat soleus and EDL muscle. The mRNA expression was investigated after one training session (Day 1), after 3 consecutive days with exercise training (Day 3), and after 3 wk of exercise training (3 Wks). The mRNA expression is reported as fold changes relative to an age-matched nonexercising CON group (n = 8). Results are means ± SE. Significant difference between the exercise and CON group: *P < 0.05; **P < 0.01; ***P < 0.001. Significant difference between muscle groups: #P < 0.05; ##P < 0.01; ###P < 0.001. Significant difference between the SP and END group: $P < 0.05; $$$P < 0.001. Significant difference between Day 1 and Day 3 within an exercise group: & P < 0.05; &&P < 0.01; &&&P < 0.001.
Fig. 3. Effect of SP (four 3-min runs at 33 m/min, 10% incline, separated by 1 min; •; n = 8) or END (20 min at 20 m/min, 10% incline; ○; n = 8) exercise on Na\(^+\)-K\(^+\) pump protein expression in rat soleus and EDL muscle. For each muscle and protein, an example of the quantified Western blot band and the corresponding molecular weight are shown. The protein expression was investigated 1 h after a training session performed on Day 1, Day 3, and after 3 wk of exercise training. The mRNA expression is reported as fold changes relative to an age-matched nonexercising CON group (n = 8). Results are means ± SE. Significant difference between the exercise and CON group: \(*P < 0.05; \text{***}P < 0.001\). Significant difference between muscle groups: \(#P < 0.05\). Significant difference between the SP and END group: \(\$P < 0.05\). Significant difference between Day 1 and Day 3 within an exercise group: \(\&P < 0.05; \text{&&P} < 0.01; \text{&&&P} < 0.001\).
**FXYD1.** In soleus, neither sprint nor endurance training altered FXYD1 mRNA expression relative to the time-specific control group. In EDL, FXYD1 mRNA expression was not regulated by exercise on days 1 and 3. However, 2 h after endurance and sprint exercise subsequent to 3 wk of training, a reduction \((P < 0.05)\) in FXYD1 mRNA was apparent relative to the time-matched control group \((0.8 \pm 0.1\text{-fold and } 0.5 \pm 0.0\text{-fold}, respectively)\). Also, after the 3-wk training period, FXYD1 mRNA expression was lower after both endurance \((P < 0.01)\) and sprint \((P < 0.05)\) exercise in EDL compared with soleus. The protein expression of FXYD1 in soleus was not different after exercise at any time point relative to the control group. However, the \(35 \pm 16\%\) numerical increase in FXYD1 protein expression after sprint exercise on day 3 was higher \((P < 0.05)\) than the numerical \(13 \pm 10\%\) reduction observed after sprint exercise on day 1. EDL FXYD1 protein expression was unaffected by exercise compared with that of the control group \((day 1)\). However, the FXYD1 protein expression in soleus was higher \((P < 0.05)\) on day 3 than day 1 in the sprint group. In addition, EDL FXYD1 protein expression after sprint exercise on day 3 was lower \((P < 0.01)\) than in soleus.

**Effect of Muscle Type, Exercise Intensity, and Training Status on Exercise-Induced Changes in NHE1 mRNA and Protein Expression**

See Fig. 5.

**NHE1.** In soleus, endurance exercise did not alter NHE1 mRNA expression. However, after sprint exercise on day 1, NHE1 mRNA was elevated \((P < 0.01)\) 12.0 \(\pm 3.1\)-fold relative to the time-matched control group. Moreover, the soleus NHE1 mRNA expression after sprint exercise on day 3 \((1.8 \pm 1.3\text{-fold})\) was lower \((P < 0.001)\) than the expression after sprint exercise on day 1. In EDL, endurance and sprint exercise did not induce alterations in NHE1 mRNA expression relative to the time-matched control group \((day 1)\). Nevertheless, the EDL NHE1 mRNA expression after sprint exercise on day 3 \((0.3 \pm 0.1\text{-fold})\) was lower \((P < 0.01)\) than the expression observed after sprint exercise on day 1 \((2.7 \pm 0.5\text{-fold})\). In addition, after 3 wk of endurance training, the postexercise EDL NHE1 mRNA expression was higher than the soleus NHE1 postexercise mRNA expression.

NHE1 protein expression in soleus was unaffected by exercise at all investigated time points. No exercise-induced changes of EDL NHE1 protein expression were observed compared with the time-matched control group. However, the observed \(40 \pm 21\%\) numerical increase in EDL NHE1 protein expression after sprint exercise on day 3 was higher \((P < 0.01)\) than the numerically \(8 \pm 8\%\) reduction after sprint exercise on day 1.

**DISCUSSION**

The major findings in the present study are as follows. 1) Changes in mRNA and protein expression did not follow each other at the investigated time points for Na\(^+\)-K\(^+\) pump subunits, FXYD1, or NHE1. 2) Sprint training induced the most changes in mRNA and protein expression, including reductions of Na\(^+\)-K\(^+\) pump \(\alpha_1\), \(\alpha_2\), \(\beta_1\), and NHE1 mRNA levels after 3 days relative to 1 day of training in both soleus and EDL. 3) A few muscle group differences were observed in

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**Fig. 4.** Effect of SP (four 3-min runs at 33 m/min, 10% incline, separated by 1 min; \(\bullet; n = 8\)) or END (20 min at 20 m/min, 10% incline; \(\circ; n = 8\)) exercise on FXYD1 mRNA and protein expression in rat soleus and EDL muscle. In relation to the protein expression data, an example of the quantified Western blot band and the corresponding molecular weight are shown for each muscle and protein. The mRNA and protein expression was investigated 1 h after a training session performed on Day 1, Day 3, and after 3 wk of exercise training. The mRNA and protein expression is reported as fold and percent changes relative to an age-matched nonexercising CON group \((n = 8)\), respectively. Results are means \(\pm SE\). Significant difference between the exercise and CON group: \(*P < 0.05\). Significant difference between muscle groups: \(\#P < 0.05\); \(\#\#P < 0.01\). Significant difference between Day 1 and Day 3 within an exercise group: \(\& P < 0.05\).
the changes of mRNA expression with training. This was primarily the case for Na\(^{+}\)-K\(^{+}\) pump \(\alpha_2\)-, \(\beta_1\)-, and \(\beta_2\)-, as well as FXYD1 and NHE1, mRNA after 3 wk of endurance training. These differences in mRNA expression changes were not associated with differences in protein expression changes.

**Na\(^{+}\)-K\(^{+}\) Pump, FXYD1, and NHE1 Expression After One Exercise Bout in the Untrained State**

In the present study, one sprint exercise bout increased soleus Na\(^{+}\)-K\(^{+}\) pump \(\alpha_2\)- and \(\beta_2\)-mRNA expression, whereas no changes were observed in EDL. It may be noted that the mRNA expression of \(\alpha_1\) was increased (\(P < 0.05\)) after sprint training when pooling data for EDL and soleus (not shown). Thus sprint exercise is able to induce increases in \(\alpha_1\)-, \(\alpha_2\)-, and \(\beta_2\)-mRNA levels, which are in accordance with observations in human skeletal muscle (31, 34, 36) and the study research hypothesis. In addition, the current observations show that \(\beta_1\)-mRNA expression is not elevated by the applied exercise protocols. This is in agreement with previous rat (45, 47) and human exercise studies, reporting no change in \(\beta_1\)-mRNA expression after exercise (1, 30, 31, 34, 39). However, exercise-induced increase in \(\beta_1\)-mRNA expression has been reported after intense exercise in humans (36), showing that very intense exercise may elicit changes in \(\beta_1\)-mRNA. Fold changes in \(\beta_1\) mRNA expression may be difficult to detect due to the high constitutive expression (36), and for that reason a high absolute increase is necessary to detect changes. It is also a concern that mRNA expression data often have a high variability (27, 36), causing an increased risk of type II errors. Thus it cannot be excluded that small (i.e., <2-fold) changes in mRNA expression were present but undetected. Moreover, it should be noted that the pronounced fold increase in soleus \(\beta_2\)-mRNA after sprint exercise may be related to a very low constitutive expression of \(\beta_2\)-mRNA as found in human skeletal muscle (36) and as indicated by the present finding that no \(\beta_2\)-protein could be detected, which is in accordance with previous results (20).

It has previously been reported that rats running for 1 h have increased \(\alpha_1\)-mRNA levels in oxidative muscle and increased \(\beta_2\)-mRNA in glycolytic muscle immediately after exercise (45). In the present study, we detected no changes in \(\beta_2\)-mRNA expression in the glycolytic EDL, 2 h after a bout of endurance exercise. The discrepancy between these findings may be related to the differences in protocols. In the present study, continuous exercise was only performed for 20 min to match the total exercise volume between the sprint and endurance groups, whereas the continuous exercise was performed for 1 h in the previous study (45). Also, analyses of mRNA expression were performed 2 h after exercise in the present study, and it cannot be ruled out that undetected changes in mRNA expression occurred immediately after exercise. However, this seems unlikely based on previous finding in human muscle, demonstrating that Na\(^{+}\)-K\(^{+}\) pump mRNA is elevated most around 1 to 3 h postexercise (36).

Because of the few significant changes observed in mRNA expression after day 1 relative to the control group, it is of concern whether the applied training intervention was effective. The training intensities used in the present study are higher than intensities that have previously been observed to cause increased Na\(^{+}\)-K\(^{+}\) pump protein expression (32) and similar to intensities causing increased glycogen content and glucose transporter (GLUT)-4 expression at rest after a period.
of exercise training (26). The training intensities used correspond to ~70 and ~60 ml O₂·kg⁻¹·min⁻¹ (4), which may be higher than 80% (8) or at least 60% of maximal oxygen uptake (19). Also, it has previously been reported that a period of exercise training at 28 m/min for 36 min, 4 times/wk, results in similar increases of muscle glycogen levels and GLUT-4 expression in EDL and soleus (26). Thus the speeds applied in the present study must be expected to have caused several training-induced adaptations in both soleus and EDL.

In line with previous studies of changes in Na⁺-K⁺ pump expression subsequent to a single exercise bout (12–14, 31), no changes were observed in protein expression after exercise on day 1 in this study. However, we cannot preclude that one bout of exercise will regulate the protein expression due to the fact that only one sampling time was analyzed.

This is the first study to investigate changes in FXYD1 mRNA after exercise, and no changes were observed in FXYD1 mRNA or protein expression after a single exercise bout, indicating that the protein expression of FXYD1 is not regulated at the mRNA level by acute exercise.

Sprint exercise caused a large increase in soleus NHE1 mRNA expression with no changes in protein expression. Possibly, severe metabolic perturbations (e.g., disturbance of the proton homeostasis) are required for NHE1 expression to be altered, as indicated by the increased NHE1 expression with intensified training in runners (21) and selective increase after high-intensity training (22). Thus the endurance type of exercise may not have caused metabolic perturbations large enough to induce NHE1 mRNA increases. Moreover, the higher constitutive expression of NHE1 in glycolytic fiber types (24) is likely to be associated with higher constitutive NHE1 mRNA expression. A higher constitutive expression of either mRNA or protein in glycolytic compared with oxidative fibers would reduce the fold or percent changes associated with a given increase in absolute expression and, therefore, make it more difficult to detect the changes. Thus the analysis of expression changes may be more sensitive in oxidative fibers with a lower constitutive expression.

Taken together, the results show that a single bout of sprint exercise is sufficient to induce an elevated mRNA level for the Na⁺-K⁺ pump subunits α₁ (when pooling data from EDL and soleus), α₂, β₂, and NHE1, whereas no effect of endurance exercise was observed. These observations are in line with the study hypothesis that sprint exercise yields a more powerful adaptive stimulus than endurance exercise. Moreover, corresponding mRNA expression changes occurred in soleus and EDL, except for β₂, in which a large increase was evident in soleus, but not EDL. Thus exercise intensity appears to be a more important regulator for changes in mRNA levels than muscle type. A single sprint or endurance bout of exercise is insufficient to regulate Na⁺-K⁺ pump, FXYD1, and NHE1 protein expression within the first 2 h after exercise.

Na⁺-K⁺ Pump, FXYD1, and NHE1 Expression After 3 Consecutive Days of Exercise

One single bout of exercise on day 3 of the training period reduced α₁-mRNA in soleus compared with the control group. Furthermore, sprint exercise decreased mRNA expression of α₁ and α₂ on day 3 relative to day 1. Moreover, soleus β₁-mRNA and β₂-mRNA in both muscles were decreased on day 3 compared with day 1. Taken together, these findings demonstrate that exercise not only causes increased mRNA levels, as seen on day 1, but also can cause reduced mRNA expression, as seen for α₁-mRNA expression after 3 consecutive days of training. Moreover, the mRNA expression after exercise on day 3 was lower than after exercise on day 1 for α₁, β₁, and β₂. These observations are contrary to the proposed hypothesis that consecutive days of exercise training would cause a general increased mRNA level for the investigated transcripts. Previously, it has been demonstrated that electrical stimulation of rat muscle reduces the α₁-, α₂-, and β₂-mRNA expression in EDL (37). Moreover, extremely well-trained athletes have been reported to have reduced α₁- and α₂-mRNA expression compared with recreational active controls (28). Thus it appears that a high volume of strong contractile stimuli is able to induce reductions of mRNA expression instead of the hypothesized increases.

Day 3 protein expression was not significantly different between the control and training groups. However, higher expression levels than observed in the groups that exercised on day 1 were apparent for α₁ in soleus and EDL, as well as for α₂ in soleus. These observations show that protein de novo synthesis occurred with exercise from day 1 to day 3. Moreover, the clearest reductions of mRNA expression from day 1 to day 3 coincide with the most pronounced increases of protein expression from day 1 to day 3. It should be noted that the observed differences in protein expression between day 3 and day 1 do not necessarily reflect an increased maximal activity of the Na⁺-K⁺ pump. However, similar increases of Na⁺-K⁺ pump protein expression and maximal activity have been observed in human muscle after a dexamethasone-induced increase of expression (35). Moreover, there appears to be an association between increased Na⁺-K⁺ pump expression and training-induced improvements of extracellular K⁺ regulation during exercise (33). Thus it may be expected that observed differences in expression also reflect a difference in maximal enzymatic activity.

A pronounced change of protein expression occurred in EDL β₂ after both endurance and sprint exercise. At the same time, the β₂-mRNA level was observed to be reduced on day 3 compared with day 1 after sprint exercise, but was otherwise unaffected. This observation clearly demonstrates that β₂ expression is not exclusively determined by the prevailing mRNA expression, but possibly by the mRNA levels during previous days. Likewise, no changes were observed in FXYD1 mRNA expression, whereas soleus FXYD1 protein expression was increased after sprint exercise, compared with expression on day 1. Moreover, FXYD1 protein expression and soleus α₁-protein expression changed in a similar pattern, indicating a possible link between regulations of these protein expressions.

Our present findings confirm previous results, showing that Na⁺-K⁺ protein expression can be altered by 3 days of training in humans (15) and thus confirms one of the study hypotheses. However, our detailed analyses of mRNA and protein expression revealed that protein expression is increased at the same time as the corresponding mRNA levels are reduced, which is in contrast to the proposed hypothesis that increased constitutively mRNA expression would be associated with increased protein expression. In contrast, the finding indicates that elevated mRNA levels preceded the increased protein expressions, and that the increased protein expres-
sion by an unknown mechanism suppressed mRNA expression. After 3 days of training, the most pronounced regulations were observed after sprint exercise, and only FXYD1 showed muscle-type differences.

\[ \text{Na}^+\text{-K}^+ \text{ Pump, FXYD1, and NHE1 Expression After 3 wk of Training} \]

Before the study, it was hypothesized that one bout of exercise subsequent to 3 wk of training would result in increased mRNA expression of the studied proteins and a corresponding increase in protein levels of the investigated targets. Surprisingly, the expression of the investigated proteins was not increased after the training period. Numerous other studies have reported increased expression of Na\(^{+}\)-K\(^{+}\) pumps after training (5). An important aspect in interpretation of the present results is that the exercise frequency and intensity were held constant during the 3-wk training period. This was done to investigate if a standardized and constant training stimulus would result in a new “steady-state” protein expression, despite the gradual reduction of relative exercise intensity. However, no increases of protein expression were evident after 3 wk of training, leading to the conclusion that adaptation to exercise training is only maintained as long as the training regime causes a certain degree of stimuli.

However, the mRNA expression of several targets was affected when investigated after exercise subsequent to the 3-wk training. For example, the \( \alpha_1 \)-mRNA expression was reduced in EDL after sprint exercise, whereas the mRNA level of \( \alpha_2 \) was increased by sprint exercise in soleus and by endurance exercise in EDL. However, the unchanged protein expression shows that these changes of mRNA expression were too modest to cause protein de novo synthesis. Interestingly, the expression of \( \beta_2 \)-protein after endurance exercise was reduced, whereas the corresponding mRNA level was increased. Possibly, the reduced protein expression facilitated the exercise-induced increase of mRNA expression, corresponding to the reduced mRNA expression associated with increased protein levels observed after sprint training on day 3.

It may be noted that the training intensities used in the present study are higher than intensities that have previously been observed to cause increased Na\(^{+}\)-K\(^{+}\) pump protein expression (32) and similar to intensities causing increased glycogen content and GLUT-4 expression at rest after a period of exercise training (26). According to a previous report on the intensity of rat running, the applied running speed would correspond to an oxygen uptake well above 60% of the maximal (19).

Perspectives and Significance

In contrast to the research hypothesis, changes with exercise training in expression of Na\(^{+}\)-K\(^{+}\) pump subunits, FXYD1, and NHE1 do not appear to be a simple effect of altered constitutive mRNA expression, as evidenced by the observed response to exercise after 3 days of training. It was observed for \( \alpha_1 \), \( \alpha_2 \), and \( \beta_1 \) at day 3 that exercise caused a reduction in mRNA and increased protein expression, relative to day 1, suggesting that a negative feedback mechanism exists or that exercise regulates protein expression by regulating protein degradation rate. Importantly, the applied exercise regime did not increase the expression of the investigated proteins after 3 wk of training, despite the observed changes on day 3 relative to day 1. The reason is likely to be the constant exercise training workload. Thus a certain stimuli, e.g., perturbation in ion homeostasis and not just the mechanic activation of the muscle resulting from the contraction, appears to be necessary to achieve adaptation. This observation has important implications for exercise training regimes in both a health and performance perspective. Generally stated, exercise training needs to contain high-intensity sessions performed at a high relative workload, not only to induce adaptation at the onset of training, but also to preserve the changes gained at the onset of a training period. However, it is worth noticing that the experimental setup only gives a “snap shot” of the dynamic changes in mRNA and protein expression, and further investigations need to deal with the time dependencies of exercise-induced changes in mRNA and its relation to protein expression.

DISCLOSURES

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

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