Effect of high-intensity training on exercise-induced gene expression specific to ion homeostasis and metabolism

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Nordsborg, Nikolai, Jens Bangsbo, and Henriette Pilegaard. Effect of high-intensity training on exercise-induced gene expression specific to ion homeostasis and metabolism. J Appl Physiol 95: 1201–1206, 2003. First published June 6, 2003; 10.1152/japplphysiol.00257.2003.—Changes in gene expression during recovery from high-intensity, intermittent, one-legged exercise were studied before and after 5.5 wk of training. Genes related to metabolism, as well as Na+, K+, and pH homeostasis, were selected for analyses. After the same work was performed before and after the training period, several muscle biopsies were obtained from vastus lateralis muscle. In the untrained state, the Na+-K+-ATPase α1-subunit mRNA level was approximately threefold higher (P < 0.01) at 0, 1, and 3 h after exercise, relative to the preexercise resting level. After 3–5 h of recovery in the untrained state, pyruvate dehydrogenase kinase 4 and hexokinase II mRNA levels were elevated 13-fold (P < 0.001) and 6-fold (P < 0.01), respectively. However, after the training period, only pyruvate dehydrogenase kinase 4 mRNA levels were elevated (P < 0.05) during the recovery period. No changes in resting mRNA levels were observed as a result of training. In conclusion, cellular adaptations to high-intensity exercise training may, in part, be induced by transcriptional regulation. After training, the transcriptional response to an exercise bout at a given workload is diminished.

sodium-potassium-adenosine 5′-triphosphatase; lactate/hydrogen ion transport; messenger ribonucleic acid; regulation of substrate utilization; glycolysis

TRAINING REDUCES THE RISE in arterial and venous plasma K+ during high-intensity exercise (23). This is most likely a consequence of reduced K+ accumulation in the muscle interstitium, which probably leads to improved high-intensity exercise performance (34). The reduction of K+ release from the exercising muscles to the blood might be caused by the increased amount of Na+-K+-ATPases typically found after training (23). The increase in Na+-K+-ATPase amount may be a result of an increased transcription, because Na+-K+-ATPase mRNA has been shown to be elevated in a subunit- and fiber-type-specific manner immediately after 1 h of exercise in rats (37). However, the importance of exercise-induced transcriptional regulation in relation to Na+-K+-ATPase expression in human skeletal muscle is unknown. An enhanced high-intensity exercise performance after high-intensity training could also be related to an improved capacity for lactate and H+ transport (11, 16, 28). An increased amount of monocarboxylate transporters 1 and 4 (MCT1, MCT4), and Na+/H+ exchanger (NHE1) protein is likely to be one reason for the increased lactate and H+ transport capacity (16, 17, 28). In rats, mRNA and protein expression has been reported to correlate for MCT1, but not MCT4, during postnatal development (12). Nevertheless, long-term stimulation of red and white rat skeletal muscle did not show any clear correlation between MCT1 mRNA and protein expression and did not affect MCT4 mRNA and protein expression (6). Thus the molecular basis for adaptations in MCT1, MCT4, and NHE1 induced by high-intensity exercise training in humans is still unknown.

High-intensity exercise demands a high-glycolytic flux, and a typical adaptation to high-intensity training is increased activity of various glycolytic enzymes (32). After high-intensity exercise training, phosphofructokinase (PFK) activity is increased (9, 14, 31, 33, 40), whereas endurance training reduces PFK activity (10, 39). These findings underline the general concept of training specificity. It has been shown that endurance exercise leads to a transient increase in hexokinas II (HKII) transcription and mRNA levels in human skeletal muscle (29, 30), but it is unknown whether high-intensity intermittent exercise has the same impact on transcriptional regulation of the HKII gene. This does seem possible based on the results from endurance exercise studies and the fact that HKII activity normally increases after high-intensity training (21, 22). A possible effect of increased HKII activity during recovery from high-intensity exercise may be an increased rate of glycogen replenishment, because blood glucose is the major precursor for glycogen replenishment after high-intensity exercise (4). Another effect of endurance exercise, related to regulation of substrate choice, is an increase in pyruvate dehydrogenase kinase 4 (PDK4) mRNA levels and transcription (30). An increased amount of PDK4 during recovery from exercise is probably a mechanism to ensure muscle glycogen replenishment after exercise via inac-

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tivation of the PDH complex. Because of the rapid glycogen breakdown during high-intensity exercise (3), PDK4 expression may also increase after high-intensity exercise to ensure glycogen replenishment.

Accumulation of mRNA as a result of repeated training sessions has been suggested to be a general model for cellular adaptations to exercise-training (25, 41). It is unclear, however, whether such an adaptation occurs with regular training and whether a trained muscle responds in the same manner as an untrained muscle to a given work performed.

Thus the present study was designed to test the hypotheses that 1) an acute bout of high-intensity exercise induces a transient increase in transcription of genes related to regulation of metabolism and ion homeostasis; 2) several weeks of high-intensity exercise training lead to elevated level of mRNA at rest; and 3) transient increases in mRNA after a high-intensity exercise bout are reduced when the exercise is performed at the same absolute workload after a training period due to the reduced stress on the muscle cells.

METHODS

Six healthy habitually active male subjects participated in the study. Age, height, and weight were, on average, 25.3 ± 1.2 yr, 185.0 ± 1.6 cm, and 82.8 ± 4.8 kg (means ± SE), respectively. Before training, maximal oxygen uptake determined by an incremental bicycle test was 50.2 ± 0.5 ml O2·min⁻¹·kg⁻¹. The mass of the quadriceps femoris muscle was estimated based on Simpson’s rule, as described by Jones and Pearson (15), and corrected based on a comparison between the anthropometric measurements and computed axial tomography scan determinations (ratio 1.0:80). The mean knee-extensor mass of the experimental leg was 2.47 ± 0.06 kg before training, with no difference between the right and left leg. The subjects were informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate. The study conforms to the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

Design. High-intensity intermittent training was performed with one leg for 5.5 wk. Before and after the training period, one training session was carried out at the same absolute workload. Changes in mRNA levels were followed in the recovery phase from these two training sessions.

Training. The leg to be trained was selected randomly. The subjects performed one-legged knee-extensor exercise in the supine position, permitting the exercise to be confined to the quadriceps muscle (1). The subjects were familiarized with the one-legged knee-extensor model at least three times before the training started. The training intensity corresponded to ~150% of the leg maximal oxygen uptake. The training intensity was adjusted every second week to maintain the same relative workload throughout the training period. The training period lasted 5.6 ± 0.5 wk and consisted of 23.2 ± 1.5 training sessions. Each training session consisted of a 5-min warm-up at 10 W, 5-min rest, and 15 exercise intervals composed of kicking for 1 min at the pre-determined workload followed by 3-min rest. Training was conducted three times per week in weeks 1–2, four times per week in weeks 3–4, and five times per week for the remainder of the period. After the training period, an incremental test was performed with the control and the trained leg separately. The power output was initially 40 W, and increased 10 W every second minute until exhaustion. Time to exhaustion was 10.6 ± 0.7 min for the trained leg and 8.2 ± 0.7 min for the untrained leg (P < 0.05).

Experimental days. On the experimental days, the exercise protocol was the same as used for the training sessions, and the workload was the same before and after the training period. Muscle samples were obtained from vastus lateralis muscle under local anesthesia with the use of the percutaneously needle biopsy technique with suction (5). Muscle biopsies were obtained at rest, immediately after exercise, and 3 h into the recovery period. Additional biopsies were obtained after 1, 5, and 24 h of recovery in the untrained state.

RNA isolation. RNA was isolated from ~25-mg wet wt muscle tissue by a modified guanidinium thiocyanate (GT)-phenol-chloroform extraction method adapted from Chomczynski and Sacchi (8), as described previously (30). Briefly, the samples were homogenized in the GT solution. Extraction was performed by centrifugation after adding NaOAc, pH 4.0, diethyl pyrocarbonate-saturated-phenol, and chloroform-isooamyl-oxygen-hydrogen. RNA was precipitated by centrifugation after addition of isopropanol. Yeast RNA was added before precipitation to facilitate localization of the pellet. To solubilize DNA, the pellet was incubated in 4 M lithium chloride. The resulting pellet was resuspended in GT solution. A second isopropanol precipitation was carried out, and the pellet was then rinsed with 75% EtOH and resuspended in 2 μl of 0.1 mM EDTA containing nuclelease-free water per milligram original tissue.

Reverse transcription. The reverse transcription reaction was performed by using the Superscript II RNase H⁻ system and oligo(dT) primers, as described by the manufacturer (Invitrogen, Carlsbad, CA). A volume of 11 μl of RNA was reverse transcribed, and the RT product was diluted in nuclease-free water to a total volume of 150 μl.

Real-time PCR. Specific primers and probes were designed for each of the mRNA sequences of interest (Table 1). The cDNA sequences were obtained from the National Center for Biotechnology Information and The Sanger Centre databases. Primers and probes were designed by use of Primer Express version 2.0 (Applied Biosystems). Primers were labeled with 6-carboxy-fluorescein at the 5' end and 6-carboxy-11-mercaptotetramethylrhodamine at the 3' end. Specificity of the obtained product sequence was confirmed by a search in the National Center for Biotechnology Information Blast database. It was verified that amplification of RNA samples not subjected to reverse transcription did not result in a detectable PCR product within the cycle number used for analysis of mRNA expression. GAPDH mRNA content was determined by using commercially available primers and probes (Applied Biosystems). Primer and probe optimization and validation of amplification efficiency were carried out. Validation of the different PCR product sizes was performed by electrophoresis (ethidium bromide containing 2.5% agarose gel). The ABI 7700 real-time PCR system was used for relative quantification. Each reaction was composed of 2.5 μl of the diluted cDNA and 12.5 μl of 2× TaqMan Universal MasterMix (AppliedTaq Gold DNA Polymerase, AmpErase uracil N-glycosylase, dNTPs with dUTP, buffer components, ROX as passive reference; Applied Biosystems). Primers, probe, and water were added to give a final reaction volume of 25 μl. Triple analyses were performed for each sample. Before PCR cycling, incubation at 50°C for 2 min followed by 95°C for 10 min was performed to activate the uracil N-glycosylase enzyme and AmpliTag Gold enzyme, respectively. PCR cycling was performed by heating to 95°C for 15 s followed by 60°C for 60 s. A total of 40 cycles were completed.

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Table 1. Primers and probes used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺(^{-})K⁺-ATPase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α₁</td>
<td>GCCAATGGTGGCGGAAGG</td>
<td>CATTGCTTGGCGCGTA</td>
<td>TTTAGCTCAGGCTCGCTGCG</td>
</tr>
<tr>
<td>α₂</td>
<td>GAATGAAAGCTTACGATCG</td>
<td>CAAAGTGTGGAAGAGGCCG</td>
<td>CCAACTACGCTCTGCGCTAC</td>
</tr>
<tr>
<td>β₁</td>
<td>TCCAGTATTTTAAAACCCCATATCA</td>
<td>CTTCCTGATCTGAGGAATCTGTT</td>
<td>TTTGGTGTCAGGCTCGCTGAC</td>
</tr>
<tr>
<td>K(_{ATP})</td>
<td>CAGCGTACAGCTTCTGCTGCTGCT</td>
<td>CAGACGACGCGCACGCGACG</td>
<td>TTTGGTGTCAGGCTCGCTGAC</td>
</tr>
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<td>MCT1</td>
<td>CAGCGGCTGACGACGACGACG</td>
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<td>TTTGGTGTCAGGCTCGCTGAC</td>
</tr>
<tr>
<td>MCT4</td>
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<td>TTTGGTGTCAGGCTCGCTGAC</td>
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<tr>
<td>NHE1</td>
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<td>TGGCGGCAACTACGCTGATC</td>
</tr>
<tr>
<td>PDK4</td>
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<td>TTTGGTGTCAGGCTCGCTGAC</td>
<td>TTTGGTGTCAGGCTCGCTGAC</td>
</tr>
<tr>
<td>HKII</td>
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<td>TGGCGGCAACTACGCTGATC</td>
<td>TGGCGGCAACTACGCTGATC</td>
</tr>
<tr>
<td>PFK</td>
<td>TGGCGGCAACTACGCTGATC</td>
<td>TGGCGGCAACTACGCTGATC</td>
<td>TGGCGGCAACTACGCTGATC</td>
</tr>
</tbody>
</table>

α₁, α₂, and β₁, Na⁺/K⁺-ATPase subunits; K\(_{ATP}\), ATP-sensitive potassium channel; MCT1 and MCT4, monocarboxylate transporters 1 and 4; NHE1, Na⁺/H⁺ exchanger; PDK4, pyruvate dehydrogenase kinase 4; HKII, hexokinase II; PFK, phosphofructokinase.

**Data analysis.** All samples from each subject were analyzed simultaneously. The cycle at which the fluorescence signal rises above a manually chosen threshold level is defined as the threshold cycle. The average standard deviation in determination of threshold cycle within triplicates was 0.14 (0.01–0.20). All samples were analyzed for the content of the different target mRNA sequences and a reference mRNA sequence (GAPDH). An arbitrary concentration of each gene was calculated from external standard curves. The ratio of the target gene content to the content of the reference gene was calculated.

**Statistics.** Before the statistical analysis was performed, all targets to reference gene ratios were logarithmic transformed to obtain normal distributed data. Changes in mRNA levels after one acute exercise bout were tested by a one-way ANOVA for repeated measurements. When the untrained state was tested, samples obtained before and 0, 1, 3, 5, and 24 h after exercise were included. Samples obtained before, immediately after, and 3 h after exercise were included in the test of the trained state. A two-way ANOVA for repeated measurements was used to test if any interaction between training state and sample time was present and to test whether any difference in the mRNA response existed between the untrained and trained state. The level of significance was set to P < 0.05. Because of the logarithmic transformation for the statistical analyses, the data reported in the text and shown in Figs. 1 and 2 are the antilogarithmic values (geometric mean) of the target gene to reference gene mRNA ratio, normalized to the ratio of the sample obtained in the untrained state. The 95% confidence intervals are reported and depicted on the graphs because of the antilogarithmic transformation.

**RESULTS**

**Na⁺\(^{-}\)K⁺-ATPase subunits and the ATP-sensitive K⁺ channel.** In the untrained state, high-intensity intermittent exercise elevated (P < 0.01) Na⁺\(^{-}\)K⁺-ATPase α₁-subunit mRNA approximately threefold after 0, 1, and 3 h of recovery, whereas no increases were observed in the Na⁺\(^{-}\)K⁺-ATPase α₂- and β₁-subunit mRNA content (Fig. 1). After the training period, no changes in Na⁺\(^{-}\)K⁺-ATPase α₁-, α₂-, and β₁-subunit mRNA, as an effect of exercise, could be detected. No change in ATP-sensitive K⁺ channel mRNA content was observed in either the untrained or the trained state (Tables 2 and 3).

**MCT1, MCT4, and NHE1.** In neither the untrained nor the trained state did high-intensity intermittent exercise induce significant changes in MCT1, MCT4, or NHE1 mRNA levels during recovery (Tables 2 and 3).

**DISCUSSION**

The major findings of the present study were as follows: 1) high-intensity exercise elevated Na⁺\(^{-}\)K⁺-ATPase α₁-subunit, HKII, and PDK4 mRNA content in untrained human skeletal muscle; 2) no changes could be detected in mRNA resting levels of the investigated genes as a result of training; and 3) of the investigated transcripts, only PDK4 mRNA was elevated after exercise in the trained state.

**Effect of intense, repeated exercise on transcription.** Our finding of a high-intensity, exercise-induced increase in the Na⁺\(^{-}\)K⁺-ATPase α₁-subunit mRNA level indicates, for the first time, that expression of this catalytic subunit may be regulated at the transcriptional level in human skeletal muscle. Our results are supported by results from a study on rats that have been exercised for 1 h on a treadmill, which led to an increase in Na⁺\(^{-}\)K⁺-ATPase α₁-subunit mRNA, but not α₂- and β₁-subunit mRNA (37). Thus exercise apparently does not induce increases in Na⁺\(^{-}\)K⁺-ATPase α₂ and β₁ mRNA levels. Nevertheless, the expression of these subunits probably is regulated at the transcriptional level in rats, the thyroid state affects Na⁺\(^{-}\)K⁺-ATPase α₂ and β₁ mRNA content, but not α₁ mRNA content (2, 13). Furthermore, glucocorticoids induce increased transcription of Na⁺\(^{-}\)K⁺-ATPase α₂- and β₁-but not α₁-subunits in rat skeletal muscle (36). Thus different stimuli and pathways apparently regulate the expression of the various subunits of the Na⁺\(^{-}\)K⁺-ATPase. It cannot be excluded that exercise for a
longer duration, with a larger muscle mass can lead to an increase in Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\) and \(\beta_1\) mRNA as well, because this type of exercise results in an elevated plasma cortisol level (19). The mechanism caus-

Table 2. Effect of one acute high-intensity exercise bout on mRNA levels during recovery

<table>
<thead>
<tr>
<th>Sample</th>
<th>(K_{ATP})</th>
<th>NHE1</th>
<th>MCT1</th>
<th>MCT4</th>
<th>PPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTPre</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>UT 0 h</td>
<td>2.1</td>
<td>1.6</td>
<td>1.5</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>(1.0–4.5)</td>
<td>(0.7–3.8)</td>
<td>(0.6–3.7)</td>
<td>(0.4–7.5)</td>
<td>(0.3–9.7)</td>
</tr>
<tr>
<td>UT 1 h</td>
<td>1.8</td>
<td>1.8</td>
<td>1.1</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(0.4–8.2)</td>
<td>(0.4–8.0)</td>
<td>(0.3–3.9)</td>
<td>(0.1–48.1)</td>
<td>(0.5–4.0)</td>
</tr>
<tr>
<td>UT 3 h</td>
<td>1.4</td>
<td>1.1</td>
<td>1.2</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(0.3–5.7)</td>
<td>(0.5–2.7)</td>
<td>(0.6–2.5)</td>
<td>(0.7–3.3)</td>
<td>(0.3–3.8)</td>
</tr>
<tr>
<td>UT 5 h</td>
<td>1.4</td>
<td>1.1</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
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<tr>
<td></td>
<td>(0.6–2.9)</td>
<td>(0.6–1.9)</td>
<td>(0.9–2.4)</td>
<td>(0.8–5.1)</td>
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</tr>
<tr>
<td>UT 24 h</td>
<td>0.9</td>
<td>0.6</td>
<td>0.9</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>(0.2–3.7)</td>
<td>(0.2–1.4)</td>
<td>(0.4–2.1)</td>
<td>(0.5–3.4)</td>
<td>(0.3–2.6)</td>
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<tr>
<td>TPre</td>
<td>1.4</td>
<td>1.1</td>
<td>1.1</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>(0.4–4.5)</td>
<td>(0.5–2.8)</td>
<td>(0.6–1.9)</td>
<td>(0.6–5.7)</td>
<td>(0.7–3.1)</td>
</tr>
<tr>
<td>T 0 h</td>
<td>1.7</td>
<td>1.3</td>
<td>1.0</td>
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<td>(0.6–4.9)</td>
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<tr>
<td>T 3 h</td>
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<td>0.8</td>
<td>1.0</td>
<td>1.3</td>
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Values are geometric means and 95% confidence intervals (in parentheses) of the nonsignificant mRNA fold changes observed after high-intensity exercise, but not depicted in Figs 1 and 2. Muscle biopsies were obtained before (Pre) exercise and after 0 and 3 h of recovery in the untrained (UT) and trained (T) state. In UT, additional biopsies were obtained after 1, 5, and 24 h of recovery.

Another enzyme that is key to glucose metabolism is HKII (38). We found an increase in HKII mRNA after high-intensity exercise training in the untrained state, which is in agreement with previous studies with the use of various exercise regimes (20, 29, 30). Previously, it has been shown that increases in the HKII mRNA 

Fig. 1. Na\(^+\)-K\(^+\)-ATPase subunit mRNA changes after a high-intensity intermittent exercise bout carried out at the same workload before and after 5.5 wk of high-intensity exercise training. Shown are ratios of Na\(^+\)-K\(^+\)-ATPase \(\alpha_1\) (A), \(\alpha_2\) (B), and \(\beta_1\) (C) mRNA to GAPDH mRNA. Solid bars, untrained state; open bars, trained state. UTPre, presample obtained in the untrained state. Values are geometric means and 95% confidence intervals. Significantly different from *the sample taken before exercise in the untrained state and *0 h in the untrained state: \(P < 0.05\).
levels correlate well with an increase in HKII protein content and activity (20, 26). Thus the increased HKII mRNA level may reflect the need for an increased glucose phosphorylation, both during and after exercise, thus ensuring substrate availability for glycolysis during exercise and for glycogen synthesis in the recovery period.

After exercise in both the trained and untrained state, PFK mRNA was unaltered. Previous studies on PFK activity after high-intensity sprint training have repeatedly found increases in PFK activity (9, 14, 31, 33, 40). Higher PFK activity is, therefore, expected after training in the present study, leading to the suggestion that changes in PFK activity after a training period are independent of changes in PFK mRNA. This is in agreement with the finding of equal amounts of PFK mRNA in control subjects and endurance athletes but lower PFK activity and protein content in endurance athletes (39).

Effect of training. Although transient increases in Na\(^+\)/K\(^+\)-ATPase \(\alpha_1\)-subunit, HKII, and PDK4 mRNA levels were apparent after exercise in the untrained state, training did not result in increased mRNA resting levels for the transcripts investigated in the present study. However, nearly all of the investigated mRNA levels were, on average, nonsignificantly higher (Figs. 1 and 2, Table 1) after the training period (range: 1.1- to 2.1-fold). Thus we cannot exclude that a general increase existed but was too modest to be detected. It is possible that a higher training frequency, or longer training duration, could have lead to detectable increases in, at least some, mRNA resting levels. Although no changes could be detected in PDK4 mRNA resting levels after the training period, a reduction existed in five of the six subjects. This finding underlines that regulation of gene expression by exercise is gene specific.

As a result of the training period, no changes were observed in Na\(^+\)/K\(^+\)-ATPase \(\alpha_1\)-subunit mRNA after exercise at the same work intensity. After high-intensity exercise in the trained state, a reduced increase was also seen for HKII and PDK4 mRNA (Fig. 2). The reduced induction of HKII and PDK4 mRNA, and the reduction of PDK4 mRNA resting levels in five subjects, may have been caused by higher levels of muscle glycogen (29) as a result of the training period. It is possible that the mRNA changes during recovery from exercise would have been the same in the trained and untrained state, if exercise had been carried out at the same relative instead of the same absolute workload. Further studies are needed to clarify the role of exercise intensity for induction of gene expression after a period of training.

In summary, the present study shows that Na\(^+\)/K\(^+\)-ATPase \(\alpha_1\)-subunit, PDK4, and HKII mRNA are elevated after high-intensity exercise, implying that transcriptional regulation of selected genes is important in relation to adaptation to high-intensity training. Furthermore, a reduction or abolishment of the increase in mRNA was found when subjects exercised at the same absolute workload after a high-intensity training period. No change in resting mRNA content as a conse-
quence of intense exercise training was found for the investigated transcripts.

REFERENCES