Contraction-induced increases in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA levels in human skeletal muscle are not amplified by activation of additional muscle mass

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Submitted 11 November 2004; accepted in final form 8 February 2005

Nordsborg, Nikolai, Martin Thomassen, Carsten Lundby, Henriette Pilegaard, and Jens Bangsbo. Contraction-induced increases in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA levels in human skeletal muscle are not amplified by activation of additional muscle mass. Am J Physiol Regul Integr Comp Physiol 289: R84–R91, 2005.—The present study tested the hypothesis that exercise with a large compared with a small active muscle mass results in a higher contraction-induced increase in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA expression due to greater hormonal responses. Furthermore, the relative abundance of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit α1, α2, α3, αλ, β1, β2, and β3 mRNA in human skeletal muscle was investigated. On two occasions, eight subjects performed one-legged knee extension exercise (L) or combined one-legged knee extension and bilateral arm cranking (AL) for 5.00, 4.25, 3.50, 2.75, and 2.00 min separated by 3 min of rest. Leg exercise power output was the same in AL and L, but heart rate at the end of each exercise interval was higher in AL compared with L. One minute after exercise, arm venous blood lactate was higher in AL than in L. A higher level of blood epinephrine and norepinephrine was evident 3 min after exercise in AL compared with L. Nevertheless, none of the exercise-induced increases in α1, α2, β1, and β3 mRNA expression levels were higher in AL compared with L. The most abundant Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit at the mRNA level was β1, which was expressed 3.4 times than α2. Expression of α1, β2, and β3 was less than 5% of the α2 expression, and no reliable detection of α3 and α4 was possible. In conclusion, activation of additional muscle mass does not result in a higher exercise-induced increase in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit-specific mRNA.

The Na\textsuperscript{+}-K\textsuperscript{+}-ATPase plays an important role in the regulation of potassium in contracting muscles, and thereby systemic levels, during exercise. Therefore, the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content in exercise-trained muscles (8, 28, 32, 42) is likely to cause the observed training-induced reduction of plasma (18) and interstitial K\textsuperscript{+} accumulation during exercise and the concomitant enhanced exercise performance (32). Nevertheless, not until recently has the subunit-specific adaptation of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase to exercise and a period of training been studied at the protein (9, 15, 32) and mRNA level (33). In a previous study of high-intensity exercise, we found an increase in α1 mRNA but did not detect increases in α2 and β1 mRNA (33). In that study, expression of α1 and α2 protein increased, whereas β1 was unchanged after a period of training (32). One study has investigated α3, β2, and β3 mRNA after intense exercise in humans (29), and increases in mRNA for these subunits as well as for α1, α2, and β1 were found, whereas no change in protein expression was detected. Resistance training for 6 wk has been shown to increase α1, α2, and β1 protein expression (9), and 6 days of prolonged cycle training have resulted in a higher α2 and β1 but not α1 protein expression (15). Thus the effect of exercise on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit expression at the protein and mRNA level varies between studies, possibly dependent on the active muscle mass as well as the intensity and duration of the exercise investigated. In addition, methodological variation is seemingly larger in studies of mRNA changes (29, 33, 37) than the more traditional approach using [3H]ouabain to quantify non-subunit-specific changes in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase protein expression (8). One mRNA transcript consistently reported to increase with exercise of various types is pyruvate dehydrogenase kinase 4 (PDK4) (35). Thus analysis of PDK4 mRNA is a sensible positive control for ascertaining that an exercise-induced mRNA change is detected if present.

Hormones, such as catecholamines and insulin, are important regulators of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity (8). Furthermore, several hormones are potent regulators of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase expression (12). It can be hypothesized that during high-intensity exercise, the size of the active muscle mass and the concomitant hormonal response will affect the exercise-induced upregulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase expression. The major endocrine regulator of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase basal expression seems to be thyroid hormone (8), but cortisol and possibly growth hormone (GH) also affect Na\textsuperscript{+}-K\textsuperscript{+}-ATPase expression (8, 11, 12, 45). Furthermore, hormonal induction of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit expression may be subunit-specific, as evidenced by the selective upregulation of α2 and β1 mRNA and protein expression shown by analysis of α1, α2, β1, and β2 expression in rat skeletal muscle after treatment with the artificial glucocorticoid dexamethasone (46). Furthermore, treatment with dexamethasone has been shown to result in a dose-dependent increase in [3H]ouabain binding sites in rat skeletal muscle (10, 39), confirming that changes at the mRNA level are likely to cause changes in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase protein expression. Plasma levels of thyroid hormone do not change with exercise (13, 43), but both cortisol (5, 47, 48) and GH (14) increase during and after exercise. The increases in cortisol and GH seem to be largest with intense exercise of a long duration (5, 14, 47, 48). Therefore, the largest effect of an exercise-associated hormone-induced upregulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase expression may be expected when performing high-intensity exercise engaging a large muscle mass for a substantial duration. However, it has been argued that hormones are not
important for the exercise-induced upregulation of Na⁺-K⁺-ATPase content because adaptation after a period of exercise training only occurs in the exercise-trained and not in the nontrained muscles (8, 25, 44). On the other hand, this argument does not take into account that blood flow increases up to ~20-fold in the active musculature during intense exercise (2), resulting in a substantially increased availability of circulating hormones to the exercising muscles compared with the inactive muscles, and therefore a muscle may have to be activated to adapt. Thus there is a need to examine the effect of the active muscle mass on Na⁺-K⁺-ATPase subunit adaptation to exercise.

In homogenates of human skeletal muscle, expression of Na⁺-K⁺-ATPase isoforms 1, 2, 3, 4, and mRNA has been reported (29) as well as expression of α4 mRNA (34). Nevertheless, other studies have not been able to detect α3 and α4 mRNA (20, 41), and the β2 protein has not been observed in membrane fractions of muscle cells (19, 22), possibly because of a general low expression level or because of low protein recovery during fractionation (17). When investigating and interpreting the physiological importance of exercise-induced relative changes of the Na⁺-K⁺-ATPase subunit mRNA levels, it is important to know the basal subunit-specific abundance. Previously, only fold changes have been reported (29, 33). Data on the relative abundance of Na⁺-K⁺-ATPase subunit mRNA in human skeletal muscle do not exist, but from rat muscle it is known that α2 and unspecified β mRNA are the most abundant, followed by α1 mRNA (34). At the protein level, β1 is the most abundant subunit, followed by α2 and then α1 (26). A similar difference in the abundance of Na⁺-K⁺-ATPase subunits can be expected in human skeletal muscle. Thus information about basal mRNA expression levels can help to clarify the uncertainties regarding the expression of Na⁺-K⁺-ATPase isoforms in human skeletal muscle and provide important knowledge about how to interpret the physiological importance of fold changes in Na⁺-K⁺-ATPase mRNA levels after exercise.

Therefore, the purpose of the present study was to evaluate the hypothesis that intense intermittent exercise with a large active muscle mass induces a greater change in Na⁺-K⁺-ATPase mRNA expression in an active muscle than when exercise is carried out with a smaller total active muscle mass. A second aim was to quantify the relative amount of the various Na⁺-K⁺-ATPase subunit mRNAs in resting human skeletal muscle.

METHODS

Eight male recreationally active subjects [24 ± 2 (mean ± SD) yr, 76.5 ± 9.0 kg] participated in the study after giving their informed consent. 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 (KF 01-183/02).

Exercise Protocols

Each subject completed at least three pretrials separated by 8 ± 3 days, to get acquainted with the protocol and exercise model. In one of two experimental sessions, subjects performed intermittent, one-legged knee extensor exercise (1) at a rate of 60 contractions/min in an upright sitting position (L). Subjects exercised for 5, 4.25, 3.50, 2.75, and 2 min separated by 3 min of rest. The leg power output (56 ± 5 W) was determined during pretrials to result in fatigue after 6–7 min of exercise. In the other experimental session, subjects performed the L protocol as well as concomitant bilateral arm cranking (82 ± 18 W) at 60 rpm on a modified Monark ergometer (AL). The workload for the arm exercise was determined in pretrials to result in the highest possible heart rate after 6–7 min (maximal heart rate) of combined arm and leg exercise. A standardized meal was given to the subjects on the evening and morning before the experiments. All experiments were initiated in the morning (9:28 AM ± 1 h). The order of the two experimental sessions was randomized, and the experiments were completed at least 2 wk apart (mean: 24 ± 9 days). The first experimental day was a minimum of 4 days after (mean: 18 ± 17 days) the last pretrial.

A muscle biopsy was obtained from vastus lateralis muscle at rest and at 0, 1, 3, and 5 h after the last exercise interval. Biopsies were obtained from the same leg after L and AL exercise. Blood was drawn from an antecubital vein at rest and 1 min after each exercise period as well as 3 and 16 min after the final exercise period. The muscle biopsies were analyzed for mRNA expression (n = 8), and the blood samples were analyzed for lactate (n = 8) and catecholamines (n = 4). Heart rate was measured continuously during exercise with a Polar heart rate monitor. The kicking frequency was recorded by a computer.

Analysis

RNA isolation. From each biopsy, two ~25-mg wet weight muscle tissue samples were used for duplicate RNA extraction by a modified guanidinium thiocyanate-phenol-chloroform extraction method (7) as described previously (36). Briefly, the samples were homogenized in the guanidinium thiocyanate solution. Extraction was performed by centrifugation after adding sodium acetate, pH 4.0, diethyl pyrocatecholate-saturated phenol, and chloroform-isooamyl alcohol. RNA was precipitated by centrifugation after addition of isopropanol. The pellet was then rinsed with 75% ethanol and resuspended in 50 μl of nuclease-free water containing 0.1 mM EDTA. The quality of the extracted RNA was ensured by detection of 18S and 28S bands using ethidium bromide (formaldehyde- and ethidium bromide-containing 2.5% agarose gel). Furthermore, the ratio of absorbance at 260 and 280 nm (260/280) was >1.7.

Reverse transcription. The reverse transcription reaction was performed 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 reverse transcription 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 and information on exon/intron boundaries were obtained from the National Center for Biotechnology Information (NCBI) and Sanger Centre databases. Primers and probes were designed using Primer Express v. 2.0 (Applied Biosystems). Probes were labeled with 6-carboxyfluorescein at the 5' end and 6-carboxy-N,N,N',N'-tetramethylrhodamine at the 3' end. Specificity of the obtained product sequence was confirmed by a search in the NCBI 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 numbers used for analysis of mRNA expression. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA content was determined using commercially available primers and probes (Applied Biosystems). Primer and probe optimization and validation of amplification efficiency (Table 1) were carried out. Validation of the different PCR product sizes was performed by electrophoresis (ethidium bromide-containing 2.5% agarose gel). The ABI 7900 real-time PCR system was used for relative quantification. Each reaction was composed of 1 μl of the diluted cDNA and 5 μl of 2X TaqMan Universal MasterMix [AmpliTaq Gold DNA polymerase,
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>
<th>Efficiency</th>
<th>Exon</th>
</tr>
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<tbody>
<tr>
<td>Na⁺-K⁺-ATPase subunit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α₁</td>
<td>AAAAGCTGTGCTTCCATTAGGAA</td>
<td>CGACACTTCCCTGCGGATT</td>
<td>CCGGCGGAGAGAGTGATGAGTAAAT</td>
<td>1.98</td>
<td>4/5</td>
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<tr>
<td>α₂</td>
<td>GAATGAGGCTTCCATTAGGAGT</td>
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<td>CAGGTGAGGAGGATGAGTAAAT</td>
<td>1.90</td>
<td>18/19</td>
</tr>
<tr>
<td>α₃</td>
<td>ATGGCTTCCCTGCGGATTCCAG</td>
<td>CGCCGCGAGAGAGTGATGAGTAAAT</td>
<td>CCGGCGGAGAGAGTGATGAGTAAAT</td>
<td>1.91</td>
<td>12/13</td>
</tr>
<tr>
<td>α₄</td>
<td>GTGTCCTGAGGCTTCCATTAGGAGT</td>
<td>CGCCGCGAGAGAGTGATGAGTAAAT</td>
<td>CCGGCGGAGAGAGTGATGAGTAAAT</td>
<td>1.91</td>
<td>12/13</td>
</tr>
<tr>
<td>β₁</td>
<td>TCAGTCGTTTCTAGAGGAGGATTCCAG</td>
<td>CTTCTGCTGAGGAGAAGGAG</td>
<td>CAGGTGAGGAGGATGAGTAAAT</td>
<td>1.85</td>
<td>2/3</td>
</tr>
<tr>
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<td>CGCCGCTTCTGGAGGAGGAGGATTCCAG</td>
<td>CCGGCGGAGAGAGTGATGAGTAAAT</td>
<td>CCGGCGGAGAGAGTGATGAGTAAAT</td>
<td>1.85</td>
<td>2/3</td>
</tr>
<tr>
<td>β₃</td>
<td>GTGGCGGCTGAGGAGGAGGATTCCAG</td>
<td>CTTCTGCTGAGGAGAAGGAG</td>
<td>CAGGTGAGGAGGATGAGTAAAT</td>
<td>1.74</td>
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<td>TCCAGCTGACAGGAGCCCTTTGGCTG</td>
<td>TGGCAAGCCGTAACCAAAT</td>
<td>ATRAATCCCGGAAATGCTTTTGGCTG</td>
<td>1.95</td>
<td>9/10</td>
</tr>
</tbody>
</table>

PDK4, pyruvate dehydrogenase kinase 4.
were not significant (*the differences in epinephrine (72%) and norepinephrine (72%) existed in AL than in L (Fig. 2), whereas 16 min after exercise, exercise-induced changes in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA levels

An exercise-induced increase \((P < 0.001)\) in \(\alpha_1\) mRNA expression was evident 1, 3, and 5 h into recovery in both AL and L with no differences between AL and L (Fig. 3). In addition, exercise induced an increase \((P < 0.001)\) in \(\alpha_2\) mRNA expression. In AL, an increase in \(\alpha_2\) mRNA was apparent after 1 and 3 h of recovery, whereas an increase was observed at 1, 3, and 5 h of recovery in L (Fig. 3), with no differences between AL and L.

The level of \(\beta_1\) mRNA increased \((P < 0.001)\) after exercise, being evident after 1 and 3 h of recovery in AL and after 1, 3, and 5 h of recovery in L (Fig. 4). At 5 h, the level of \(\beta_1\) was higher \((P = 0.020)\) in L compared with AL (1.5- vs. 2.4-fold). No exercise-induced increase in \(\beta_2\) mRNA expression could be determined at any time point after exercise (Fig. 4), whereas an increase \((P < 0.001)\) in \(\beta_3\) mRNA after exercise was detected at all time points after both AL and L exercise (Fig. 4). No difference in the exercise-induced increase in \(\beta_3\) existed between AL and L.

Exercise-Induced Changes in PDK4 and GAPDH mRNA Expression

GAPDH mRNA tended to be elevated \((P = 0.080)\) after exercise, with no differences between AL and L (Fig. 5). Exercise induced an increase \((P < 0.001)\) in PDK4 mRNA (Fig. 5), with no differences between AL and L. At the end of L exercise, PDK4 mRNA was ~5-fold \((P = 0.028)\) higher than before exercise, and it was ~21-fold higher \((P < 0.001)\) 5 h into the recovery period. For AL exercise, PDK4 mRNA was elevated ~8-fold \((P = 0.002)\) after 5 h of recovery.

DISCUSSION

The findings in the present study could not support the hypothesis that exercising with an additional large muscle mass induces a higher increase in the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit mRNA expression of an exercising muscle. We also found that the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunits \(\alpha_2\) and \(\beta_1\) are the most highly expressed at the mRNA level in human skeletal muscle, followed by \(\alpha_1\), \(\beta_2\), and \(\beta_3\). The levels of \(\alpha_3\) and \(\alpha_4\) did not allow for a reliable detection.

Exercise with a large compared with a small muscle mass did not induce any difference in the mRNA expression level during recovery for any of the investigated subunits, except at 5 h after exercise, when the expression of \(\beta_1\) mRNA was lower for AL compared with L. In preliminary studies, we observed the intra- and interindividual coefficient of variation to be ~30\% for \(\alpha_1\), \(\alpha_2\), and \(\beta_1\) mRNA expression in human skeletal muscle. The substantial, but typical, variation in mRNA expression data (29, 30, 33, 37) underlines the need for caution when interpreting data where no differences are found. In the present study, we used duplicate RNA isolations from each biopsy specimen and normalization of target mRNA amount to the amount of RNA: cDNA hybrids in the sample used for real-time PCR. This was done to increase the power of the applied analysis and to circumvent the problem of using...
endogenous controls that possibly can be affected by exercise (6, 30). Thus differences of 0.7-fold in mRNA expression between AL and L exercise would have been detected as revealed by a power analysis. The lack of a higher Na\(^+\)-K\(^+\)-ATPase subunit mRNA expression after AL compared with L exercise is not likely to have been caused by an insufficient difference in the physiological response between AL and L. The epinephrine and norepinephrine concentrations 3 min after exercise were 141 and 380% higher for AL than for L. The exercise intensity was 90% of maximal oxygen consumption (V\(\dot{O}_2\)max) for AL and 60% of V\(\dot{O}_2\)max for L, as estimated from the heart rate response during exercise. Previously, plasma cortisol has been shown to increase from a resting level of 135–275 nM and reach 550 nM after intermittent high-intensity cycling of duration and intensity comparable to that of AL exercise (16, 27), whereas plasma cortisol has been reported to increase from a resting level of 135–275 nM after intermittent exercise at an intensity <70% of V\(\dot{O}_2\)max (27). Furthermore, exercise at intensities >60% of V\(\dot{O}_2\)max is generally suggested to be required to elicit an exercise-induced increase in GH (14), and as little as 1.5 min of exhaustive running has been shown to increase GH 35% (24). Therefore, it seems valid to assume that both cortisol and GH levels were higher for AL than for L both during and after exercise. From these results, it appears reasonable to conclude that higher hormonal responses brought about by activation of additional muscle mass do not amplify mRNA expression of Na\(^+\)-K\(^+\)-ATPase subunits after intense exercise.
In addition to the systemic effects, it is likely that differences in intracellular ion homeostasis of the active muscle cells existed between AL and L exercise. In a previous study in which intense knee extensor exercise was performed after a period of intense intermittent arm exercise, it was observed that lactate release from the leg muscle was reduced, K⁺ release was elevated, and muscle pH in the quadriceps muscle was lowered (3). A lower pH may have affected Na⁺-K⁺-ATPase subunit gene expression by inducing changes in intracellular Na⁺ concentration (31) by reducing the open probability of Na⁺ channels (40) or increasing Na⁺/H⁺ exchange (21). In addition, changes in intracellular Ca²⁺ homeostasis endorsed by changes in intracellular Na⁺ and, subsequently, Na⁺/Ca²⁺ exchange rate could be of importance for Na⁺-K⁺-ATPase mRNA expression (38). It may be that these intracellular differences brought about by the addition of arm exercise are responsible for the reduced β₁ expression 5 h after AL exercise compared with L exercise. Nevertheless, none of the possible intracellular differences found between AL and L exercise were of a magnitude that significantly changed the exercise-induced increase in mRNA expression of the other Na⁺-K⁺-ATPase subunits.

Even though the active muscle mass seems to be of little importance for changes in Na⁺-K⁺-ATPase mRNA expression, differences in training status, exercise intensity, and exercise duration have the potential to affect the increase in Na⁺-K⁺-ATPase mRNA after exercise. Previously, our group reported a reduced increase in α₁ mRNA after exercise when subjects performed the same exercise after a period of training (33), suggesting that relative workload is of importance for the exercise-induced increase in at least some of the Na⁺-K⁺-ATPase subunits. Furthermore, findings of 16, 9, and 39% increases in α₁, α₂, and β₁ protein expression after 6 days of 2-h cycling (15) compared with 37, 22, and 33% after resistance training for 30 min three times per week for 6 wk (9) suggest that either the higher intensity or longer duration of the training period leads to the larger changes in α₁ and α₂ expression after resistance training compared with cycling. Nevertheless, the importance of exercise duration, intensity, and fitness level for changes in Na⁺-K⁺-ATPase mRNA needs further investigation.

This is the first study to describe the relative mRNA expression level of the Na⁺-K⁺-ATPase subunits present in human skeletal muscle. Expression of α₂ and β₁ mRNA was the most abundant, followed by α₁, β₃, and β₂. No reliable detection of α₃ and α₄ was possible. The current finding of α₂ mRNA being most expressed is in agreement with data from rat skeletal muscle (34). It should be noted that expression of the Na⁺-K⁺-ATPase α₃ and α₄ mRNA in the present study corresponded to <1 molecule/μl in the sample subjected to PCR analysis. In the majority of samples, Na⁺-K⁺-ATPase α₄ expression was undetectable, even though the use of synthetic oligonucleotides corresponding to the expected target sequences showed that any expression would have been detected. Furthermore, α₄ expression in commercially obtained RNA from human testes was detected as previously reported (41). A recent study did find expression of α₄ mRNA in human skeletal muscle (23). Because the primers used in the present study span exons 2 and 3 of the ATP1A4 gene that was shown to be expressed in muscle (23), no obvious explanation for the controversy exists. Nevertheless, α₄ seems to be of little quantitative importance compared with the high level of α₂ and β₁ mRNA expression. Furthermore, it should be noted that low abundant expression detected in muscle biopsies could stem from other tissues in the biopsy, such as adipose, nerve, vascular, or connective tissue cells.

Although no differences were found between AL and L exercise, except for β₁ mRNA expression, significant increases in Na⁺-K⁺-ATPase subunit mRNA were observed for α₁, α₃, α₄, β₂, and β₃ after the high-intensity intermittent exercise protocol. Likewise, an increase in PDK4 expression was apparent (35). GAPDH mRNA tended to increase after exercise, and when the changes observed at 0, 1, 3, and 5 h were averaged, a calculation termed average postexercise response in a previous study of mRNA expression (29), GAPDH mRNA expression was higher (P = 0.005) after exercise. The analysis of the relative amount of Na⁺-K⁺-ATPase subunit mRNA in resting human skeletal muscle allows us to conclude that the changes in α₂ and β₁ mRNA are quantitatively the most important, even though the fold change for α₁ was the highest. However, this does not necessarily mean that changes in α₂ and β₁ mRNA expression are physiologically the most relevant, exemplified by the finding that both α₁ and β₁ protein expression in rat
alveolar epithelial cells can be increased by dexamethasone when only $\beta_1$ and not $\alpha_1$ mRNA abundance is increased (4). The increase in $\beta_1$ protein expression has previously been suggested to cause increased $\alpha_1$ stability (46), suggesting posttranslational regulation of expression.

The non-subunit-specific Na$^+$-K$^+$-ATPase expression determined by $[^{3}H]$ouabain binding is consistently reported to be ~300 pmol/g wet weight in human skeletal muscle (see Ref. 8), and after various forms of exercise training, the Na$^+$-K$^+$-ATPase content increases around 14–22% (8). In contrast, changes in Na$^+$-K$^+$-ATPase mRNA expression of various subunits were between two- and fivefold in the present and other studies (29, 33) and are less consistent than changes in protein data. Thus a substantially larger change in mRNA expression seems to precede more modest protein adaptations. Furthermore, repeated increases in mRNA levels may be necessary before an increase in protein expression can be expected, because elevated Na$^+$-K$^+$-ATPase-specific mRNA levels after one exercise bout do not result in increased protein expression (29). The finding of an increase in $\alpha_1$ mRNA is in agreement with a previous finding by our group (33), whereas changes in $\alpha_2$ and $\beta_1$ mRNA were not previously observed (33). When average postexercise response was calculated in the previous study (33), only one subject did not show an increase in $\alpha_2$ mRNA ($P = 0.100$) and all subjects had an increase in $\beta_1$ mRNA ($P = 0.044$), making it likely that an undetected increase did exist. Furthermore, the present finding of an increase in Na$^+$-K$^+$-ATPase subunit $\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_3$ mRNA after AL and L exercise is in agreement with data from a recent study (29). The lack of an exercise-induced increase in $\beta_3$ mRNA expression in the current study is in contrast to the twofold increase in $\beta_2$ mRNA average postexercise response previously found (29). In the present study, $\beta_2$ mRNA average postexercise response was 1.3-fold, and a power analysis revealed a 2-fold increase to be necessary for significant detection. Nevertheless, the low expression level of $\beta_2$ mRNA found in the present study indicates that changes in $\beta_2$ mRNA are of little quantitative importance.

In summary, the present study has shown that the engagement of a large compared with a small muscle mass during intense intermittent exercise does not result in a higher exercise-induced increase of muscle Na$^+$-K$^+$-ATPase subunit-specific mRNA. Furthermore, it was demonstrated that of the Na$^+$-K$^+$-ATPase subunits, $\beta_1$ and $\alpha_2$ mRNA are the most abundantly expressed in resting human skeletal muscle. Thus the typical finding of a two- to fivefold increase in mRNA expression of all Na$^+$-K$^+$-ATPase subunits after exercise leads to the conclusion that increases in $\alpha_2$ and $\beta_1$ mRNA after exercise are the quantitatively most important.

Perspectives

The results from this study suggest that intracellular events are responsible for the exercise-induced upregulation of Na$^+$-K$^+$-ATPase content in human skeletal muscle, although the mechanism remains uncertain. Further research is needed to clarify potential signaling cascades involved in the regulation of Na$^+$-K$^+$-ATPase subunit gene expression in relation to exercise.

ACKNOWLEDGMENTS

We thank Merete Vamby for skilled technical assistance.

GRANTS

This study was supported by grants from Team Danmark and Copenhagen Muscle Research Centre (504-14 Danish National Research Foundation).

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