Training-induced changes in skeletal muscle Na\(^{+}\)-K\(^{+}\) pump number and isoform expression in rats with chronic heart failure

Bryan Helwig,1 Katherine M. Schreurs,1 Joslyn Hansen,2 K. Sue Hageman,1 Michael G. Zbreski,2 Richard M. McAllister,1,2 Kathy E. Mitchell,1,2,* and Timothy I. Musch1,2,*

Departments of 1Anatomy and Physiology and 2Kinesiology, Kansas State University, Manhattan, Kansas 66506-5802

Submitted 30 March 2002; accepted in final form 24 January 2003

CHRONIC HEART FAILURE (CHF) produces a reduction in exercise capacity that is commonly associated with the early onset of muscular fatigue (19). Initially, it was proposed that the exercise deficits associated with CHF were primarily the result of the skeletal muscle blood flow abnormalities produced in this disease state (19, 68, 69). However, more recent studies have suggested that abnormalities intrinsic to skeletal muscle may be associated with the early onset of fatigue in CHF, including muscle atrophy, reductions in oxidative enzyme capacity, along with changes in fiber type composition, myosin heavy chain expression, and excitation-contraction coupling (4, 18, 25, 39, 43, 52, 59, 61–63).

In addition, sarcoplasmic reticulum (SR) Ca\(^{2+}\)-release and reuptake are perturbed in CHF such that muscle twitch and tetanic force generation are reduced (29, 52, 67). Moreover, these perturbations coincide with a downregulation in sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) gene expression, and it has been postulated that the downregulation of the SERCA gene may be related to the contractile dysfunction found in the CHF state (53). Along with these changes in SR function, it has been suggested that perturbations in Na\(^{+}\) and K\(^{+}\) balance across the sarcolemmal membrane could also be contributing factors to the decrements in muscle performance found in CHF (41). Consistent with this hypothesis, a number of studies have shown that skeletal muscle Na\(^{+}\)-K\(^{+}\) pump number is reduced in CHF (49, 51, 56). These pumps are essential in restoring the sarcolemmal transmembrane potential during repeated muscular contractions, and a reduction in the number of these pumps could interfere with contractile performance via reductions in membrane excitability (50). However, contrary to this hypothesis, recent studies on humans (24) and rats (40) have shown that skeletal muscle Na\(^{+}\)-K\(^{+}\) pump number is maintained in the CHF condition. Therefore, the premise that CHF results in a reduction

ouabain; exercise; performance; oxygen uptake; congestive heart failure

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org 8750-7587/03 $5.00 Copyright © 2003 the American Physiological Society 2225

in the number of sarcolemma Na\(^{+}\)-K\(^{+}\) pumps remains controversial.

The Na\(^{+}\)-K\(^{+}\) pump consists of a catalytic alpha (\(\alpha\)) subunit and a glycosylated accessory beta (\(\beta\)) subunit (33, 64). Thus far, two isoforms of the \(\alpha\) subunit (\(\alpha_1\) and \(\alpha_2\)) have been found to be expressed in rat skeletal muscle, with the \(\alpha_2\)-isoform of the enzyme being more abundant than the \(\alpha_1\)-isoform (64). Both of the \(\alpha\)-isoforms are expressed at greater levels in individual muscles that possess a high oxidative capacity (i.e., soleus, red portion of the gastrocnemius (gastrocnemius\(_{\text{red}}\) muscle) compared with their low-oxidative highly glycolytic (i.e., white portion of the gastrocnemius (gastrocnemius\(_{\text{white}}\) muscle) counterparts (31). Moreover, the \(\alpha_2\)-subunit of the enzyme has a much greater affinity for ouabain than the \(\alpha_1\)-subunit (64), and there is evidence suggesting that functional differences may exist between the two isoforms (17).

The exact role that the \(\beta\)-subunit has in defining sarcolemmal Na\(^{+}\)-K\(^{+}\) pump activity is not known. However, pump stability, correct folding, and transport of the pump to the plasma membrane are dependent on the interaction of the \(\alpha\) and \(\beta\)-subunits (11, 14, 44). Presently, three \(\beta\)-isoforms have been identified (\(\beta_1\), \(\beta_2\), and \(\beta_3\)) and are known to exist in rat skeletal muscle (5, 37). The \(\beta_1\)-isoform is highly expressed in slow-twitch oxidative fibers (SO; i.e., soleus), whereas the \(\beta_2\)-isoform is found predominantly in fast-twitch glycolytic fibers (FG; i.e., gastrocnemius\(_{\text{white}}\) muscle) (32). In comparison, both \(\beta_1\)- and \(\beta_2\)-isoforms are expressed in muscle fibers that are fast-twitch, highly oxidative, and glycolytic in nature (FOG; i.e., gastrocnemius\(_{\text{red}}\) muscle) (32). Studies in both human and rats show that each of the \(\beta\)-subunits imparts unique pharmacological and transport properties to the pump (12, 17).

Recently, our laboratory demonstrated that the number of ouabain binding sites was reduced in the soleus, plantaris, and the gastrocnemius\(_{\text{red}}\) muscle of rats with CHF (49). Because these reductions in the number of ouabain binding sites coincided with decrements in maximal oxygen uptake (\(V_O_2\max\)) and endurance capacity, it suggested that a reduction in the number of Na\(^{+}\)-K\(^{+}\) pumps (Na\(^{+}\)-K\(^{+}\)-ATPase) produced in CHF may be a contributing factor to the reductions in exercise performance found in this disease state. The \(^{[3]H}\)ouabain binding assay used in our previous investigations primarily reflects changes in the expression of the \(\alpha_2\)-subunit of the enzyme (22, 49, 56, 64). Therefore, the possibility exists that a compensatory increase in the \(\alpha_1\)-subunit could have occurred such that Na\(^{+}\)-K\(^{+}\)-ATPase activity was maintained in the CHF animals. Moreover, changes in \(\beta\)-isoform expression could significantly impact the number of functional pumps found at the sarcolemma, thereby influencing total enzyme activity via \(\alpha\)-\(\beta\) interaction (9, 10, 26).

The present investigation was undertaken to determine whether the expression of the \(\alpha\) and \(\beta\)-subunits of the Na\(^{+}\)-K\(^{+}\) pump is modified in the skeletal muscle of rats with CHF. On the basis of results from our laboratory (49, 56), we tested the hypothesis that the expression of the \(\alpha_2\)-subunit would be reduced in muscle that possessed a high oxidative capacity (i.e., the gastrocnemius\(_{\text{red}}\)). In addition, we expected that \(\alpha_1\)-subunit expression would not be significantly changed in either the gastrocnemius\(_{\text{red}}\) or the gastrocnemius\(_{\text{white}}\) muscle. In regard to \(\beta\)-isoform expression, we tested the hypothesis that \(\beta\)-subunit expression would remain stable in the CHF condition. This hypothesis is based on the recent findings of Lunde and colleagues (40) where these investigators demonstrated that the expression of the \(\alpha\) and \(\beta\)-subunit of the Na\(^{+}\)-K\(^{+}\) pump did not change in the muscles of rats with CHF compared with non-CHF controls. Finally, we tested the hypothesis that exercise training would increase the number of ouabain binding sites along with \(\alpha\) and \(\beta\)-subunit expression in the muscle of rats with CHF. This hypothesis is based on the fact that endurance exercise training has been shown to increase Na\(^{+}\)-K\(^{+}\) pump number (16, 21, 23, 36) along with the finding that acute exercise has been shown to increase \(\alpha\)- and \(\beta\)-subunit expression in the skeletal muscle of normal rats (66).

### METHODS

Female Wistar rats were obtained from Charles River Laboratories. All rats were housed in 6 \(\times\) 9-in. cages, received rat chow and water ad libitum, and were maintained on a 12:12-h light-dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

**Surgically induced myocardial infarction.** Rats were anesthetized initially with a 5% halothane-95% oxygen mixture. Rats were intubated, connected to a rodent respirator (model 680, Harvard Apparatus), and maintained on 2% halothane-68% oxygen mixture. The heart was exposed through a left-sided thoracotomy between the pulmonary artery and the left atrium. A 6-0 suture was approximated with 3-0 silk. Analgesic agents were applied to each animal. Anesthesia was withdrawn, and the animals were extubated. All rats were returned to individual cages. Postoperatively, each rat received ampicillin (50 mg/kg sc) for each day for 5 days.

**Determination of endurance exercise capacity.** Six weeks after the MI or sham operation, all rats performed a treadmill exercise test to fatigue. Our laboratory’s use of this exercise test has previously demonstrated that rats with CHF suffer from reduced exercise capacity as indicated by the early onset of fatigue compared with noninfarcted sham-operated control animals (1). The exercise protocol consisted of a graded running test in which each rat initially ran up a 5% grade at a speed of 25 m/min for 15 min. Thereafter, the treadmill speed was increased 5 m/min every 15 min until each animal reached the point of fatigue. The criterion for fatigue was the rat’s inability to keep pace with the treadmill,
even though the animal was encouraged to run by applying bursts of high-pressure air at the hindquarters. At the end of each exercise test, the end point of fatigue was confirmed by the loss of the animal’s righting reflex. Time from the beginning of the exercise to the removal of the rat from the treadmill was measured and recorded to the nearest half minute.

All exercise tests were initiated between 9 and 10 AM to prevent the confounding effects from the diurnal variation in tissue glycogen (15). The exercise test was administered by an observer blinded to the animal’s condition. Therefore, the observer did not know whether the animal being tested was a MI rat with CHF or a noninfarcted sham control rat.

**Determination of \( V_{O_2 \text{max}} \)**. \( V_{O_2 \text{max}} \) was determined for all rats according to previously established methods that have been used extensively in our laboratory (46). This method uses a metabolic chamber (14.5 × 43 × 7 cm) designed to fit into a stall of a 10-channel rodent treadmill and utilizes the standard techniques described by Brooks and White (13) for determining oxygen uptake (\( V_{O_2} \)) and carbon dioxide production (\( V_{CO_2} \)).

\( V_{O_2 \text{max}} \) was determined by having each rat perform a maximal exercise test. This test consisted of a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min, respectively. The treadmill speed and/or grade was increased every 2 min. \( V_{O_2 \text{max}} \) was defined as the point at which the \( V_{O_2} \) did not increase with further increases in workload or when the rat was unable to or unwilling to continue running. These criteria have been shown to produce similar \( V_{O_2 \text{max}} \) values in untrained rats (8). However, confirmation that \( V_{O_2 \text{max}} \) was truly attained in each animal was demonstrated by having each rat perform a subsequent maximal exercise test after 48 h of recovery from the initial test. With the second test, each rat was given a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min. The treadmill grade and speed were then increased to the highest workload each animal was able to sustain during the initial maximal test. \( V_{O_2} \) and \( V_{CO_2} \) were recorded. The treadmill speed was then increased by 3–5 m/min, and \( V_{O_2} \) and \( V_{CO_2} \) were recorded. If the measured \( V_{O_2} \) was similar between the two workloads, the animal was considered to be at \( V_{O_2 \text{max}} \), and the exercise test was terminated. If the rat demonstrated an increase in \( V_{O_2} \) during the second exercise test, the test was terminated, and the same procedure was repeated after another 48 h of recovery. This procedure was repeated until comparable \( V_{O_2} \) values were found between the initial and second (greater) workloads during each subsequent maximal exercise test, thus ensuring an accurate assessment of \( V_{O_2 \text{max}} \) in each animal (46).

**Exercise training protocol.** After the endurance capacity and \( V_{O_2 \text{max}} \) had been determined for each animal, the MI rats were separated randomly into exercise training and sedentary control groups. The rats in the exercise training (MI-T) group were subjected to an exercise training protocol that consisted of having each animal run on a motor-driven treadmill at a speed of 27 m/min up a 10% grade for 60 min/day (47). The MI-T rats ran 5 days/wk and trained for 6 wk. Familiarity with treadmill running was maintained in both the sedentary control groups of MI and sham rats by having each animal run on the treadmill for 5 min/day at a treadmill speed of 20 m/min up a 10% grade. At the conclusion of the 6-wk training period or sedentary control period, all rats had their endurance capacity and \( V_{O_2 \text{max}} \) redetermined using the same protocols as described above. During this time, the rats in the MI-T group continued training until all measurements of exercise capacity were complete.

**Determination of left ventricular dysfunction.** Left ventricular (LV) function was determined for each rat after post-training or postsedentary control measurements of endurance capacity and \( V_{O_2 \text{max}} \) had been completed (6–8 wk after training and 12–14 wk after the initial sham or MI surgery had been performed).

Twenty-four hours after the last exercise bout, each rat was anesthetized (pentobarbital sodium, 35 mg/kg ip), and the right carotid artery was cannulated with a 2-Fr catheter-tip pressure transducer (Millar Instruments) for the recording of arterial pressure and heart rate. Each rat was breathing spontaneously, the micromanometer was advanced into the LV in a retrograde fashion for measuring ventricular systolic and diastolic pressures. Immediately after measurement of ventricular pressures, the micromanometer was removed from the animal, and the right and left soleus muscle and the right plantaris muscle were harvested for the determination of \( Na^{+}-K^{+} \)-pump number and affinity using a \(^{3}H\)-ouabain binding assay. The left plantaris muscle was also removed and frozen immediately in liquid nitrogen for the determination of citrate synthase (CS) activity. The gastrocnemiusred and gastrocnemiuswhite portions of both the right and left muscles were removed and frozen in liquid nitrogen for the determination of \( Na^{+}-K^{+} \)-ATPase isoform concentrations along with the determination of \( Na^{+}-K^{+} \)-ATPase activity measured via an enzyme-coupled assay.

After the removal of the muscles, each rat was killed with an overdose of anesthetic (pentobarbital sodium, 100 mg/kg ip). The lungs were excised and weighed. The heart was then removed, the right ventricle (RV) was surgically separated from the LV and septum, and both tissues were weighed. The LV was examined for scar tissue on the LV free wall for documentation that a large myocardial infarction had been produced in the MI and MI-T animals. Rats were considered to have a significant degree of LV dysfunction when the LV end-diastolic pressure (LVEDP) and LV change in pressure over time (dP/dt) were significantly increased and decreased compared with sham rats (49, 54). In addition, rats were considered to have chronic CHF when RV weight-to-body weight and lung weight-to-body weight ratios were increased compared with their sham counterparts (49).

\(^{3}H\)-ouabain binding. The number and affinity of \( Na^{+}-K^{+} \) pumps in the soleus and plantaris muscles were determined by using a radiolabeled (\(^{3}H\)ouabain) binding assay (51, 56). These muscles of the ankle extensor group of the rat’s hindlimb were selected because they are recruited significantly during exercise (48). In addition, the soleus muscle contains nearly 100% SO fibers, whereas the plantaris muscle contains a mixed fiber type composition (SO, FG, and FOG) (3). Moreover, previous results from our laboratory have demonstrated that the number of \( Na^{+}-K^{+} \) pumps that have a high affinity for ouabain found in these muscles was significantly reduced in MI rats with CHF (49).

Each muscle was cut into 800-μm-thick transverse sections using a McIlwain tissue chopper (Brinkman Instruments). Each muscle slice was placed in a separate well of a tissue culture plate. Each well was filled with 2 ml of a standard solution (in mM): 10 Tris-HCl, 3 MgSO₄, 1 sodium vanadate, and 250 sucrose (pH 7.3). The binding assay was accomplished in five stages: 1) preincubation wash, 2) incubation with radiolabel, 3) wash out of unbound radiolabel, 4) washing and digestion, and 5) counting of radiolabel. In this study, 1–3 washes were performed in the standard solution with appropriate concentration of ouabain at constant temperature (37°C). All measurements were performed in triplicate.

**Assay protocol.** The preincubation wash in the standard solution alone was performed on ice for 20 min to remove...
extracellular K⁺. Slices were incubated in fresh standard solution with radiolabel and gently shaken at 37°C for 3 h in a Dubnoff metabolic incubator. Total binding was obtained over the concentration range of 5–1,000 nM ouabain. Non-specific binding was determined by using an excess of unlabelled ouabain (10⁻⁴ M). All wells contained 5 nM [³H]ouabain titrated to the appropriate concentration with unlabelled ouabain. Free ouabain in the incubation solution was determined at the end of the incubation by removing 250 μl of the incubation medium from the wells with the most dilute concentration of ouabain (5 nM).

Slices were washed on ice for a total of 20 min (2 washes × 10 min). Slices from the total binding wells were washed in standard solution; slices from nonspecific binding wells were washed with an excess of unlabelled ouabain (10⁻⁴ M) during the assay. Individual slices were blotted, weighed, placed in liquid scintillation vials, and digested overnight (at least 12 h) in 250 μl of 1 M NaOH.

Three milliliters of liquid scintillation cocktail (Ecolite+; ICN Biomedicals) were added to the scintillation vials, and counting was performed in a Tri-Carb liquid scintillation analyser. Parallel control experiments were performed for each assay, and binding was determined to the spectrophotometric method of Srere (60). All assays were probed to ensure complete removal of antibodies and typically ranged from 4 to 6 mg/ml.

Homogenization was performed in a Polytron at a medium setting for 3 × 30 s with a 1-min interval on ice between periods of homogenization. The homogenate was then centrifuged at 11,000 g for 14 min. Tissue pellets were resuspended in isolation buffer and homogenized a second time, followed by centrifugation at 100,000 g for 90 min. Tissue pellets were again resuspended in a final ice-cold isolation buffer with a protease inhibitor and stored at −70°C. Protein concentration was determined by microBCA assay (Pierce Chemical) and typically ranged from 4 to 6 mg/ml.

Immunoblotting and densitometry. Skeletal muscle proteins (30 μg) were incubated in Laemmli sample buffer and heated at 37°C for 30 min. Proteins were run on a 4–20% acrylamide gel (Bio-Rad). On completion of the run, proteins were transferred to a nitrocellulose membrane by electrophoretic transfer in a tank system with plate electrodes. Membranes were incubated at room temperature with a primary polyclonal antibody (1:2,000 vol/vol) in 5% nonfat milk and Tris-buffered saline (TBS: 100 mM Tris and 0.9% NaCl, pH 7.5) containing 0.1% Tween 20. After incubation, membranes were washed four times with 0.1% TBS Tween. All antibodies used in this investigation (α1, α2, β1, β2, β3, and dihydropyridine receptor (DHPR)) were obtained from Upstate. The membrane was incubated at room temperature in a horseradish peroxidase-labeled secondary antibody was diluted (1:25,000) in TBS with 0.1% Tween. After four washes with TBS Tween, blots were visualized with chemiluminescence (West Femto, Pierce Chemical) and recorded on radiographic film. After visualization, membranes were stripped of all antibodies (Restore Western blot stripping buffer, Pierce Chemical) via incubation at 37°C for 1 h. Membranes were probed to ensure complete removal of antibodies and then probed with a second primary antibody as described above. Densitometry was performed by using AlphaEase Image software. Densitometry was compared within groups and assigned arbitrary units. Linear range of protein expression was established by loading 0–80 μg of membrane protein on gels followed by subsequent exposure of the blots to the radiographic film at timed intervals to ensure that the signals were in the linear range. All analyses shown are of the 30 μg of membrane protein, which was shown to be within the linear range of detection. In addition, all immunoblots were run with prepared brain tissue as a positive control.

Enzyme-coupled assay of ouabain-sensitive Na⁺-K⁺-ATPase activity. NADH was measured to assess ouabain sensitive activity as described by Schwinger et al. (58). Samples (with and without ouabain) were treated with either 0.5% saponin or assay buffer and incubated on ice for 1 h before spectrophotometry. The assay was started by adding 50 μl of NADH (1.28 μM) to a cuvette containing 900 μl of assay buffer (150 mM NaCl, 10 mM KCl, 100 mM NaHCl, 5 mM MgCl₂, 5 mM ATP, 2.5 mM phosphoenolpyruvate, and 150 mM imidazole HCl, pH 7.25). In addition 50 μl of phosphoenolpyruvate (58 units/mg protein) were added to the assay buffer. A 50-μl protein sample was added to the cuvette, and the decrease in absorbance was measured every 10 s for 1 min at a wavelength of 340 nm on a spectrophotometer (Shimadzu UV-120). The difference between maximal activity and ouabain-sensitive activity was used to determine Na⁺-K⁺-ATPase activity.

Determination of CS activity. CS activity, an index of oxidative capacity, was determined for the plantaris muscle of each rat. Tissue samples were homogenized at 0°C in a volume of 100 mM KPO₄ buffer such that a 1:20 (wt/vol) homogenate was obtained. CS activity was measured according to the spectrophotometric method of Serrè (60). All assays were linear with respect to time and dilution, and each sample was analyzed in duplicate.

Statistical analysis. Structural and hemodynamic indexes, plantaris CS activity, the maximal number of specific ouabain binding cites (Bmax), the apparent dissociation constant (Kd), densitometry results from the α- and β-subunit protein expression and the Na⁺-K⁺-ATPase activity measured with the enzyme-coupled assay were compared between sham, MI, and MI-T rats with a one-way ANOVA. When a significant F value was demonstrated by the ANOVA, a Student-Newman-Kuels post hoc test was performed to detect differences between mean values. P < 0.05 was considered to be statistically significant.

RESULTS

Structural and hemodynamic indexes indicative of LV dysfunction and CHF were prevalent in both MI and MI-T rats. Accordingly, the MI and MI-T group of rats demonstrated elevations in LVEDP compared with sham rats along with reductions in mean arterial pressure and LV dp/dt (Table 1). LV weight normalized to body weight was elevated in both the MI and MI-T groups of rats. Moreover, these increases in LV weight coincided with increases in lung weight-to-body weight.
for the MI-T group of rats increased after 6 wk of sedentary control conditions. In addition, run time to fatigue level as the sham group of rats after 6 wk of endurance training and actually exceeded those found for their counterparts (Table 2). On the other hand, Bmax was increased (20%) in the soleus in the MI-T group of rats compared with their sedentary MI counterparts. Moreover, Bmax was increased (45%) in the plantaris of the MI-T group of rats compared with their sedentary MI counterparts and actually exceeded those found for the sedentary sham rats. In comparison, the affinity (Kd) of the single population of [3H]ouabain binding sites found for all muscles examined was similar across the different groups of animals.

Both α- and β-subunits of the Na\(^+\)-K\(^+\)-ATPase were found to be expressed in both the gastrocnemius\(_{\text{white}}\) and gastrocnemius\(_{\text{red}}\) muscles of the sham, MI, and MI-T group of rats (Fig. 3). DHPR expression was used as a positive control and to ensure that our results were not a result of differences in protein loading of the gels because DHPR expression in skeletal muscle remains unchanged in the CHF condition (40). DHPR expression was similar for all three groups of rats in both the gastrocnemius\(_{\text{white}}\) and gastrocnemius\(_{\text{red}}\) muscle.

![Figure 1](image)

**Fig. 1.** Maximal oxygen uptake (VO\(_{2\text{max}}\)) was determined for sham sedentary control rats (sham; n = 10), myocardial infarction sedentary control rats (MI; n = 16), and myocardial infarction endurance-trained rats (MI-T; n = 16) both before (solid bars) and after 6–8 wk of endurance training or sedentary control conditions (hatched bars) (A). Similarly, run time to fatigue was determined for the same animals (B). Values are means ± SE. *P < 0.05 vs. sham. †P < 0.05 compared with pretraining value.

Table 1. Structural and hemodynamic variables measured in sham, sedentary, and trained myocardial infarction rats with CHF

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Weight, g</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>LV dP/dt/mmHg/sec</th>
<th>LV Weight/Body Weight, mg/g</th>
<th>RV Weight/Body Weight, mg/g</th>
<th>Lung Weight/Body Weight, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10</td>
<td>274 ± 6</td>
<td>354 ± 13</td>
<td>106 ± 3</td>
<td>8 ± 1</td>
<td>6,650 ± 536</td>
<td>2.59 ± 0.03</td>
<td>0.58 ± 0.03</td>
<td>4.99 ± 0.12</td>
</tr>
<tr>
<td>MI</td>
<td>16</td>
<td>279 ± 5</td>
<td>325 ± 13</td>
<td>89 ± 2*</td>
<td>23 ± 3*</td>
<td>4,586 ± 201*</td>
<td>2.89 ± 0.06</td>
<td>0.85 ± 0.09*</td>
<td>7.54 ± 1.07*</td>
</tr>
<tr>
<td>MI-T</td>
<td>16</td>
<td>275 ± 3</td>
<td>323 ± 9</td>
<td>95 ± 2*</td>
<td>28 ± 2*</td>
<td>4,107 ± 190*</td>
<td>2.95 ± 0.05</td>
<td>1.06 ± 0.11*</td>
<td>8.57 ± 1.00*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats; CHF, chronic heart failure; sham, sham-operated rats; MI, myocardial infarction sedentary control rats; MI-T, myocardial infarction endurance-trained rats; LV, left ventricular; RV, right ventricular; HR, heart rate; MAP, mean arterial pressure; LVEDP, LV end-diastolic pressure; LV dP/dt; LV change in pressure over time. *P < 0.05 vs. sham.
In the gastrocnemius<sub>white</sub> muscle, α-isofrom expression along with β-isoform expression was similar for the sham, MI, and MI-T groups of rats (Fig. 4). In contrast, α- and β-isoform expression in the gastrocnemius<sub>red</sub> muscle was significantly different between these groups (Fig. 5). In the gastrocnemius<sub>red</sub> muscle, α<sub>1</sub>-isofrom expression was similar for the sham, MI, and MI-T groups of rats (Fig. 5A). However, α<sub>2</sub>-isofrom expression was reduced in the MI group of rats compared with the sham, but at the same time, α<sub>2</sub>-isofrom expression found in the MI-T group of rats was not significantly different from those found in the MI or the sham group of rats (Fig. 5A). Similar to that found in the gastrocnemius<sub>white</sub>, β<sub>1</sub>- and β<sub>2</sub>-isoform expression in the gastrocnemius<sub>red</sub> muscle was similar between the sham, MI, and MI-T groups of rats (Fig. 5B). In addition, β<sub>2</sub>-isofrom expression was similar between the sham and MI group of rats. However, β<sub>2</sub>-isofrom expression was significantly greater in the MI-T group of rats compared with the sham, and there was a trend for the β<sub>2</sub>-isofrom expression to be greater (P = 0.09) in the MI-T group of rats compared with their sedentary MI counterparts (Fig. 5B).

Table 2. [<sup>3</sup>H]ouabain binding sites and binding affinity in soleus and plantaris muscles from sham, MI, and MI-T rats with CHF

<table>
<thead>
<tr>
<th></th>
<th>B&lt;sub&gt;max&lt;/sub&gt;, pmol/g wet wt</th>
<th>K&lt;sub&gt;d&lt;/sub&gt;, nM</th>
<th>R&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soleus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>312 ± 22</td>
<td>97 ± 11</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>MI</td>
<td>274 ± 7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>77 ± 6</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>MI-T</td>
<td>330 ± 12&lt;sup&gt;†&lt;/sup&gt;</td>
<td>88 ± 9</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td><strong>Plantaris</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>323 ± 13</td>
<td>115 ± 20</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>MI</td>
<td>282 ± 7&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>102 ± 8</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>MI-T</td>
<td>410 ± 15&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>125 ± 7</td>
<td>0.96 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; B<sub>max</sub> (maximal binding capacity) and K<sub>d</sub> (apparent dissociation constant) represent mean of individual values obtained from Scatchard analysis for each muscle from each rat; R<sub>a</sub>, average coefficient of linear correlation for regression of individual Scatchard plots. *P < 0.05 vs. sham. †P < 0.05 vs. MI.

DISCUSSION

The present investigation is the first to demonstrate that CHF alters the α<sub>2</sub>-isofrom expression of the Na<sup>+</sup>-K<sup>+</sup>-ATPase pump within rat skeletal muscle and that endurance exercise training can partially reverse these alterations. Specifically, in CHF rats, we found a down-regulation of the α<sub>2</sub>-subunit (without a compensatory...
upregulation of the α1-subunit) within high-oxidative muscle (gastrocnemius<sub>red</sub>) that was associated with a reduced exercise capacity (i.e., VO₂<sub>max</sub> and exercise endurance). These changes were not found in the low-oxidative highly glycolytic gastrocnemius<sub>white</sub> muscle. Training-induced normalization of α<sub>2</sub>-subunit expression and increased β<sub>2</sub>-subunit expression occurred in CHF rats concomitant with an elevation of ouabain binding sites to control levels in soleus and above control levels in plantaris muscles in conjunction with an augmented exercise capacity. Within the muscles examined, results suggest that exercise training is an effective stimulus for reversing CHF-induced Na<sup>+</sup>-K<sup>-</sup>-ATPase activity impairment and also that maintenance of Na<sup>+</sup>-K<sup>-</sup>-pump activity is associated with exercise capacity.

Effects of CHF on the Na<sup>+</sup>-K<sup>-</sup>-pump. Previous results from our laboratory have shown that the number of ouabain binding sites is reduced in the soleus, plantaris, and gastrocnemius<sub>red</sub> but maintained in the gastrocnemius<sub>white</sub> muscle of rats with severe LV dysfunction and CHF (49). The present investigation confirmed these observations because we found the number of ouabain binding sites to be reduced in the soleus and plantaris muscles of the MI group of rats compared with their sham counterparts. Moreover, the amount of α<sub>2</sub>-isoform that was expressed in the gastrocnemius<sub>red</sub> muscle was significantly reduced in these animals (Figs. 3 and 5) and is consistent with a reduction in Na<sup>+</sup>-K<sup>-</sup>-ATPase activity that was measured in the same muscle samples (Fig. 6). In contrast, α<sub>2</sub>-subunit expression was maintained in the gastrocnemius<sub>white</sub> muscle of the MI rats compared with their sham counterparts. This maintenance of the α<sub>2</sub>-subunit of the pump is consistent with previous findings from our laboratory where the number of ouabain binding sites was maintained in the gastrocnemius<sub>white</sub> muscle of rats with a similar degree of LV dysfunction and CHF (49). The reductions in ouabain binding sites found in the soleus and plantaris muscles in the present investigation are similar to those our laboratory found previously for rats with severe CHF (49, 56). However, these results disagree with those of Green et al. (24) where the number of ouabain binding sites was found to be maintained in patients with CHF. Specifically, Green

![Fig. 4. Densitometry results of the immunoblot analysis of the α (A)- and β-isoforms (B) measured in the gastroc<sub>white</sub> muscle of sham, MI, and MI-T rats. Values are means ± SE. Number of observations in each group of sham, MI, and MI-T rats are the following: α₁, n = 6; α₂, n = 8; β₁, n = 6; β₂, n = 8; β₃, n = 5.](#)

![Fig. 5. Densitometry results of the immunoblot analysis of the α (A)- and β-isoforms (B) measured in the gastroc<sub>red</sub> muscle of sham, MI, and MI-T rats. Values are means ± SE. Number of observations in each group of sham, MI, and MI-T rats are the following: α₁, n = 7; α₂, n = 8; β₁, n = 6; β₂, n = 5; β₃ n = 4. *P < 0.05 vs. sham group of rats. §MI-T and sham group of rats are not significantly different, P > 0.05.](#)
et al. demonstrated that the numbers of ouabain binding sites measured in the vastus lateralis muscle of patients with moderate CHF were similar to those measured in age-matched healthy controls. The reason for these differences may be related to the degree of LV dysfunction and the duration of CHF that had developed in the individual or animal. For example, Green et al. studied patients that were classified as having moderate CHF. These patients were on stable medical regimens for 3 mo before their study, and it appears that none of these individuals was suffering from severe congestive heart failure as categorized by the American Heart Association. Therefore, the possibility exists that the degree of LV dysfunction and the duration of congestive CHF may not have been severe or long enough to produce a reduction the number of ouabain binding sites found in the vastus lateralis muscle of these individuals. Consistent with this hypothesis, our laboratory has shown previously that perturbations in skeletal muscle morphology and biochemistry (including the decreases in the number of ouabain binding sites) do not occur in MI rats until they have reached a stage of congestive CHF (18, 49, 55). The criteria we used to demonstrate that severe chronic congestive heart failure had been produced in our MI rats included large increases in 1) LVEDP to demonstrate severe LV dysfunction, 2) lung weight normalized to body weight to demonstrate pulmonary congestion, and 3) RV weight normalized to body weight to demonstrate that the rats were in a chronic congestive state. Because a vast majority of CHF patients normally receive prolonged medical therapy to minimize their symptoms before study, what may be perceived initially as disparate findings between studies may be related to subtle differences in the degree of LV dysfunction and the duration of CHF found to exist between patient and animal model populations.

The reduction in the expression of the α2-isoform of the pump found in the gastrocnemiusred muscle of MI rats with severe LV dysfunction and stable congestive CHF is a new finding. However, this finding contrasts those of Lunde et al. (40), who did not find any changes in the expression of either the α- or β-subunit of the pump in the flexor digitorum brevis (FDB) muscle of MI rats compared with sham-operated controls. The reason for this difference remains obscure, but again, it may be related to the degree of LV dysfunction and the duration of severe CHF that had developed along with the specific muscle being examined. Inasmuch as the MI rats in the study by Lunde et al. showed clinical signs of severe heart failure (LVEDP > 20 mmHg, pulmonary congestion, tachypnea, and pleural effusions), it would appear that the degree of LV dysfunction produced in the MI rats from both studies were similar to one another. However, two important questions arise concerning the study by Lunde and colleagues. The first deals with the length of time that these animals were in congestive failure. Because Lunde and colleagues did not provide RV weights in their investigation, it remains unclear whether the congestive failure found in these animals was of an acute or chronic nature. The second question deals with the fiber type composition of the FDB muscle. Because the FDB muscle contains almost exclusively fast-twitch fibers, the probability exists that the FDB muscle may be very similar to the gastrocnemiuswhite muscle in its fiber type composition and its response to CHF. If this is true, one would not expect to find any changes in the expression of the α- or β-subunits of the Na\(^+\)-K\(^+\) pump in the FDB muscle based on the results of the present investigation. Consistent with this conclusion, we have shown that skeletal muscle blood flow abnormalities (48) along with reductions in oxidative enzyme capacity (18, 55) and the number of ouabain binding sites (49) produced in MI rats with CHF are located predominantly in muscles containing a majority of SO and FOG types of fibers. In contrast, these perturbations are nearly absent in muscles containing a majority of FG types of fibers. Altogether, the results are consistent with the hypothesis that not all muscles of the rat are affected by CHF in a homogeneous manner. They also suggest that the fiber type composition of the muscle being examined in CHF may be contributing to the disparity of results found between investigations.

The factors that regulate Na\(^+\)-K\(^+\)-ATPase activity in skeletal muscle remain relatively complicated and unclear at this time. However, it is known that the greatest activation of the pump occurs during exercise and/or muscle contraction (16), and it has been demonstrated that the number of Na\(^+\)-K\(^+\) pumps increase with exercise training and decrease with deconditioning (23, 34, 35, 42, 45, 66). On the basis of previous studies, one may postulate that the reductions in Na\(^+\)-K\(^+\) pump number (as indicated by the reduction in ouabain binding sites) along with the changes in α-subunit expression found in the MI group of rats could be attributed to a decrease in physical activity (i.e., deconditioning). However, previous studies do not support this hypothesis (59), and great care was taken...
in the present investigation to ensure that the amount of physical activity that each animal was exposed to on a daily basis was not significantly different between the sedentary sham and MI groups of rats. Accordingly, rats were housed in small individual cages (6 in. wide and 9 in. long) to restrict their aerobic activity. In addition, both sedentary sham and MI rats were subjected to treadmill exercise for a period of 5 min/day to maintain acclimation to running on the treadmill, but the treadmill speed and the exercise duration were minimized to ensure that a training effect would not be produced in the animal (20). On the basis of these experimental procedures, we believe that the reductions in the number of ouabain binding sites and the associated changes in α-isof orm expression found in the MI group of rats cannot be attributed to differences in physical activity compared with their sham counterparts.

Previous studies have shown that the α1-subunit of the enzyme plays a major role in maintaining basal pump activity, whereas the regulation and catalytic activity of the α2-subunit can be influenced significantly by hypokalemia and/or different hormones (6, 7, 22, 30, 33, 65). In our investigation, we found that expression of the α1-subunit of the pump was maintained in the MI group of rats compared with their noninfarcted sham sedentary controls. These results are consistent with the idea that the α1-subunit plays an important role in maintaining basal pump activity in CHF. On the other hand, because we found that the expression of the α2-subunit was decreased in the gastrocnemiusred muscle of the MI group of rats, we believe that these results suggest that the regulation of the α2-isoform of the pump may be associated with the neuroendocrine and/or hormonal changes produced in the CHF state (57).

An intriguing question that remains to be answered is whether the Na+/K+ pump activity of the different muscles examined in the present investigation may be modified by changes in β-subunit expression. In this capacity, studies have shown that the β1-isoform is expressed predominantly in muscles that have a high oxidative capacity, whereas the β2-isoform is expressed to a greater degree in muscles that are primarily fast-twitch in their characteristics (32). The expression of the β3-isoform has also been shown to occur in rat skeletal muscle (5), but its abundance relative to the β1- and β2-isoforms in muscles of a specific fiber type remains unclear. The β-subunit is required for the functional expression of the enzyme (28) and is thought to be important in preserving the stability of the heterodimer complex along with playing a regulatory role in processing and transporting the mature enzyme complexes from the intracellular compartment to the plasma membrane (9, 10, 22, 26, 37). The possibility exists that modifications in the expression of the β-subunit could alter both Na+/K+ pump activity (37) concomitant with changes in skeletal muscle metabolic function (33). Consequently, changes in β-subunit expression could be contributing factors to the decrement in skeletal muscle contractile function found in the CHF state (27).

In the present investigation, we found that all three isoforms of the β-subunit were expressed in both the gastrocnemiuswhite and gastrocnemiusred muscle. However, contrary to the idea that Na+/K+ pump activity may be modified by changes in β-subunit expression in the CHF state, we discovered that the expression of the β1-, β2-, and β3-isoforms for both the gastrocnemiuswhite and gastrocnemiusred muscle of the MI group of rats was similar to that found for their sedentary sham counterparts (Figs. 4B and 5B). These results are consistent with our initial hypothesis that skeletal muscle β-isof orm expression remains stable in the CHF condition. Moreover, they suggest that any changes in skeletal muscle Na+/K+ pump activity produced in CHF are mediated primarily through changes in the α2-isoform of the enzyme.

Effects of endurance exercise training on the Na+/K+ pump. Studies have shown that acute exercise and/or muscle contractions will significantly increase skeletal muscle Na+/K+ pump activity (16) along with inducing the expression of both α1- and α2-subunits of the enzyme (66). Similarly, chronic exercise or endurance exercise training has been shown to increase the number of Na+/K+ pumps as indicated by the number of ouabain binding sites found in skeletal muscle (23, 35, 42, 45). Based on these studies, one would expect the expression of the α1-subunit to increase with chronic treadmill exercise training. However, the question as to whether chronic endurance exercise training would significantly increase the expression of the α1-subunit as suggested by the effects of acute exercise was central to the design of the present investigation. As expected, endurance exercise training (chronic treadmill running) increased the number of ouabain binding sites found in the soleus and plantaris muscles (Table 2) along with the expression of the α2-isoform in the gastrocnemiusred muscle (Fig. 5). However, contrary to our expectations endurance exercise training did not increase the expression of the α2-isoform in the gastrocnemiuswhite muscle (Fig. 4), nor did this type of training significantly increase the expression of the α1-subunit in either of these muscles (Figs. 4 and 5).

Why exercise training selectively increased the expression of the α2-isoform in the gastrocnemiusred muscle but not in the gastrocnemiuswhite muscle remains unclear. Our first impression would be to ascribe the differential expression due to differences in muscle recruitment during exercise. That is, one may argue that the muscles containing a majority of SO and FOG types of fibers were recruited to a greater degree than the muscles containing a majority of FG types of fibers with the exercise (treadmill speed of 27 m/min up a 10% grade) regimen used in this investigation (2). However, closer examination of this hypothesis fails because Tsakiridis et al. (66) have shown that both α1- and α2-isoforms of the enzyme are expressed to a greater degree in both predominantly red (SO and FOG fiber types) and white (FOG and FG fiber types) muscles after an acute bout of exercise that was performed.

J Appl Physiol • VOL 94 • JUNE 2003 • www.jap.org
at a significantly lower level (treadmill speed of 20 m/min up a 10% grade). Therefore, the differential increased expression of the α₂-isoform found in the gastrocnemius<sub>red</sub> muscle of the MI-T group of rats does not appear to be related to differences in muscle recruitment per se.

Endurance exercise training also selectively increased the expression of the β₂-isoform of the gastrocnemius<sub>red</sub> muscle compared with the gastrocnemius<sub>white</sub>. What affect this increase in β-isoform expression may have on the Na<sup>+</sup>-K<sup>+</sup> pump remains ambiguous at this time, but on the basis of the regulatory role that these proteins have on pump assembly, transport, and activity, one may postulate that they may be important in contributing to the increases in Na<sup>+</sup>-K<sup>+</sup>-ATPase activity found commonly after endurance exercise training. Interestingly, this training-induced adaptation occurred in the gastrocnemius<sub>red</sub> muscle, whereas it was absent in the gastrocnemius<sub>white</sub>. This result suggests that the long-term regulation of the Na<sup>+</sup>-K<sup>+</sup> pump induced by exercise training may be specifically targeted toward muscle containing a majority of oxidative fibers. This result also supports the hypothesis that the effects of CHF and exercise training are not homogeneous among muscles of varying fiber type characteristics.

Model considerations or limitations. Relevant to the present investigation, a number of limitations in regard to data interpretation should be acknowledged. First, only limited amounts of gastrocnemius<sub>red</sub> and gastrocnemius<sub>white</sub> muscle were obtained from the animals used in these experiments. To ensure that the tissues harvested from the gastrocnemius muscle were truly representative of the red and white regions, we attempted to minimize the opportunity for any fiber type contamination from adjacent regions of the muscle. Because of limited amounts of tissue, Na<sup>+</sup>-K<sup>+</sup>-ATPase activity (using a coupled-enzyme assay) was measured on the same gastrocnemius<sub>red</sub> muscle samples that were subjected to immunoblot analysis from only a very limited number of animals (n = 3) found in each experimental group. Thus the Na<sup>+</sup>-K<sup>+</sup>-ATPase activities expressed in the present investigation (Fig. 6) should be construed as preliminary in nature.

A second limitation of the present investigation deals with the fact that exercise training was not imposed on a sham group of rats. In this regard, having a sham group of rats exposed to the same training regimen as that used with the rats with CHF could have been beneficial from the standpoint of data interpretation. However, our primary intention was to determine whether exercise training would ameliorate or reverse the reductions in the number of ouabain binding sites found in skeletal muscle of rats with CHF. In addition, we wanted to determine whether directional changes in the number of binding sites would coincide with similar directional changes in the catalytic α₁- and α₂-isoform expression of the pump. Indeed, the increase in plantaris CS activity (Fig. 2) produced in the MI-T group of rats clearly established that a training effect had been produced in these animals. Furthermore, the increase in the number of ouabain binding sites found in the soleus and plantaris muscles, the normalization of the expression of the α₂-subunit of the enzyme, along with the increase in β₂-isoform expression in the gastrocnemius<sub>red</sub> muscle of the MI-T group of rats demonstrated that exercise training produced what may be interpreted as beneficial effects in the muscle of rats with severe congestive CHF. Because previous studies have shown that exercise training will increase the number of ouabain binding sites in skeletal muscles of normal individuals and rats (23, 35, 42, 45), we chose not to include a trained sham group of rats in our experimental paradigm. In doing so, we minimized the number of animals needed for the completion of our study.

The studies were supported by research funds from National Institutes of Health (NIH) Grant AG-19228 and the American Heart Association, Kansas Affiliate (to T. I. Musch); from NIH Grant P20-RR-15563 Kansas State University Provost University Sponsored Research Grant (to K. E. Mitchell); and from the Kansas State University Cancer Research Institute (to B. Helwig).

REFERENCES


