Skeletal muscle ouabain binding sites are reduced in rats with chronic heart failure

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THE MOST COMMON SYMPTOM EXPERIENCED by individuals with chronic heart failure (CHF) is a reduced exercise capacity that is associated with the early onset of muscular fatigue (12). Initially, it was thought that early onset of fatigue found in the CHF state was primarily the result of a reduced skeletal muscle blood flow response to exercise (12, 58, 59). However, more recent investigations have shown that abnormalities intrinsic to skeletal muscle may enhance muscle fatigability (3, 17, 30, 34, 36, 52).

Accordingly, a large number of investigators have demonstrated that the oxidative capacity of skeletal muscle is reduced in the CHF state (3, 36, 51). In addition, skeletal muscle atrophy and changes in fiber type composition and/or endurance capacity. Four muscles were chosen that represented slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), and mixed fiber types. Measurements were obtained 8–10 wk postsurgery in 23 myocardial infarcted (MI) and 18 sham-operated control (sham) rats. Eighteen rats had moderate left ventricular (LV) dysfunction [LV end-diastolic pressure (LVEDP) < 20 mmHg], and five had severe LV dysfunction (LVEDP > 20 mmHg). Rats with severe LV dysfunction had significant pulmonary congestion and were likely in a chronic state of compensated congestive failure as indicated by an approximately twofold increase in both lung and right ventricle weight. Run time to fatigue and maximal oxygen uptake (V̇O2 max) were significantly reduced (1, 39 and ↓ 28%, respectively) in the rats with severe LV dysfunction and correlated with the magnitude of LV dysfunction as indicated by LVEDP (run time: r = 0.60, n = 21, P < 0.01 and V̇O2 max: r = 0.93, n = 13, P < 0.01). In addition, run time to fatigue was significantly correlated with V̇O2 max (r = 0.87, n = 15, P < 0.01). The concentration of [3H]ouabain binding sites (Bmax) was significantly reduced (21–28%) in the three muscles comprised primarily of oxidative fibers [soleus: 259 ± 14 vs. 188 ± 17; plantaris: 295 ± 17 vs. 229 ± 18; red portion of gastrocnemius: 326 ± 17 vs. 260 ± 14 pmol/g wet tissue wt]. In addition, Bmax was significantly correlated with V̇O2 max (soleus: r = 0.54, n = 15, P < 0.05; plantaris: r = 0.59, n = 15, P < 0.05; red portion of gastrocnemius: r = 0.65, n = 15, P < 0.01). These results suggest that down-regulation of Na+/K+ pumps that possess a high affinity for ouabain in oxidative skeletal muscle may play an important role in the exercise intolerance that attends severe LV dysfunction in CHF.

Na+/K+ pump; exercise; performance; oxygen uptake; congestive failure

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peated muscular contractions, it has been postulated that the downregulation of these Na\(^{+}\)-K\(^{-}\) pumps can interfere with contractile performance via reductions in membrane excitability (43).

In a recent investigation, Green et al. (16) found that the Na\(^{+}\)-K\(^{-}\)-ATPase concentration in the vastus lateralis muscle of patients with moderate CHF was significantly correlated with the CHF patient's ability to perform exercise as indicated by the individual's peak oxygen uptake (V\(_{\text{O}2}\) peak). This correlation between Na\(^{+}\)-K\(^{-}\) pump activity and V\(_{\text{O}2}\) peak suggests that 1) pump activity or number may be related to the severity of CHF and 2) reductions in pump activity or number may be contribute to the decrements in exercise performance commonly found with this pathological condition. On the basis of the results of Green and colleagues, the present investigation was undertaken to determine whether the reduction in the number of skeletal muscle Na\(^{+}\)-K\(^{-}\) pumps that have a high affinity for ouabain (i.e., the concentration of \([\text{H}]\text{ouabain binding sites}\) found during CHF is related to 1) the severity of the disease state, 2) the fiber type composition of the muscle being investigated, and 3) exercise performance as tested by the duration of an endurance run to the point of fatigue along with the measurement of the animal's maximal oxygen uptake (V\(_{\text{O}2}\) max). We tested the hypothesis that the concentration of \([\text{H}]\text{ouabain binding sites}\) (B\(_{\max}\)) would be reduced in rats with severe left ventricular (LV) dysfunction and CHF but not in rats with moderate LV dysfunction and CHF. We also tested the hypothesis that the reduction in B\(_{\max}\) found in the individual muscles examined in this investigation would be significantly correlated with V\(_{\text{O}2}\) max and/or each animal's endurance capacity as designated by the time to fatigue during a progressive treadmill exercise test. Four muscles were chosen that represented slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), and mixed fiber types.

**METHODS**

Female Wistar rats were obtained from Charles River Laboratories. All rats 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 oxygen mixture. They were intubated, connected to a rodent respirator (model 680, Harvard Apparatus), and maintained on 2% halothane-oxygen mixture. The heart was exposed through a left-sided pericardial sac and opened, and the heart was exteriorized. Rats received either a myocardial infarction (MI) or a sham operation as described previously (42). In rats receiving an MI, the left main coronary artery was ligated between the pulmonary artery and the left atrium. A 6-0 suture was placed around the left main coronary artery and tied. Sham operations were completed by using the same surgical procedure except that the coronary artery was not ligated. After these procedures, the lungs were hyperinflated, and the ribs were approximated with 3-0 gut. The muscles of the thorax were sewn together with 4-0 gut, and the skin incision was closed with 3-0 silk. Analgesic agents were applied to each animal. Anesthesia was withdrawn, and the animals were extubated. Postoperatively, each rat received ampicillin (50 mg/kg sc) for each day for 5 days. All rats were returned to individual cages. Each cage was 6 in. wide and 9 in. long.

**Determination of endurance exercise capacity.** Six weeks after the MI or sham operation, all rats performed a treadmill exercise test to fatigue. Our 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 application of bursts of high-pressure air at 5% hindquarter each. At the end of each exercise test, the end point of fatigue was confirmed by 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 (8). 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 an MI rat with CHF or a noninfarcted sham control rat.

**Determination of V\(_{\text{O}2}\) max.** V\(_{\text{O}2}\) max was determined for 9 sham and 15 MI rats according to previously established methods that have been used extensively in our laboratory (39). This method uses a metabolic chamber (14.5 \(\times\) 43 \(\times\) 7 cm) designed to fit into a stall of a 10-channel rodent treadmill and utilizes the standard techniques described by Brooks and White (6) for determining oxygen uptake (V\(_{\text{O}2}\)) and carbon dioxide production (V\(_{\text{CO}2}\)). V\(_{\text{O}2}\) 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 were increased every 2 min. V\(_{\text{O}2}\) max was defined as the point at which the V\(_{\text{O}2}\) 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\(_{\text{O}2}\) max values in untrained rats (5). However, confirmation that V\(_{\text{O}2}\) 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 maximal test. With the second maximal 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\(_{\text{O}2}\) and V\(_{\text{CO}2}\) were recorded. The treadmill speed was then increased by 3–5 m/min, and V\(_{\text{O}2}\) and V\(_{\text{CO}2}\) were recorded. If the measured V\(_{\text{O}2}\) was similar between the two workloads, the animal was considered to be at V\(_{\text{O}2}\) max, and the exercise test was terminated. If the rat demonstrated an increase in V\(_{\text{O}2}\) during the second maximal exercise test, the test was terminated and the same procedure was repeated after 48 h of recovery. This procedure was repeated until comparable V\(_{\text{O}2}\) values were found between the initial and second (greater) workloads during each subsequent maximal exercise test, thus ensuring an accurate assessment of V\(_{\text{O}2}\) max in each animal (39).
Determination of LV dysfunction. The presence and severity of CHF were evaluated in each rat by using the following structural indexes: changes in LV, right ventricular (RV), and total lung weight normalized to body weight. Hemodynamic indexes included changes in mean arterial pressure and LV end-diastolic pressure (LVEDP). Approximately 8–10 wk after the initial surgery, each rat was anesthetized (pentobarbital sodium, 25 mg/kg ip), and the right carotid artery was cannulated with a 2-Fr catheter-tip pressure manometer (Millar Instruments) for the recording of arterial pressure and heart rate. While the 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 soleus, plantaris, and red and white portions of the gastrocnemius muscles were harvested.

Ouabain binding assay. The number of Na\(^{+}\)-K\(^{+}\) pumps that have a high affinity for ouabain in the soleus, plantaris, and the red and white portions of the gastrocnemius muscles was determined by using a radiolabeled ([\(\text{H}\)]ouabain) binding assay (44, 47). These muscles or muscle parts of the ankle flexors along with a muscle (plantaris) containing a majority of SO (soleus), FOG (red portion of the gastrocnemius), and FG (white portion of the gastrocnemius) types of fibers along with a muscle (plantaris) containing a mixed fiber type composition (2). In addition, these muscles or muscle parts contain a significant range of oxidative capacity as indicated by their citrate synthase (CS) activity (11). Each muscle was cut into 800-μm-thick transverse sections by use of 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 MgSO\(_4\), 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) weighing and digestion, and 5) counting of radiolabel. Stages 1–3 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 unlabeled ouabain (10\(^{-4}\) M). All wells contained 5 nM [\(\text{H}\)]ouabain titrated to the appropriate concentration with unlabeled 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 unlabeled 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 Minaxi Tri-carb scintillation counter (4000 series, Packard). Specific binding was determined from the difference between total and nonspecific binding normalized to grams of wet muscle weight. Quench curves were established for the standard and NaOH solutions to accurately calculate specific ([\(\text{H}\)])ouabain binding sites.

Statistical analysis. Structural and hemodynamic indexes, and indexes of exercise performance (V\(_{\text{O}2}\) max and run time to fatigue) were compared between noninfarcted sham-operated control rats and rats with either moderate (LVEDP < 20 mmHg) or severe (LVEDP > 20 mmHg) LV dysfunction (11) with a one-way ANOVA. When a significant F value was demonstrated by the one-way ANOVA, a Student-Newman-Keuls post hoc test was performed to detect differences between mean values. B\(_{\text{max}}\) and the apparent dissociation constant for ouabain were determined for each rat and each muscle by using Scatchard analysis. These results were also analyzed with a one-way ANOVA. To determine whether a relationship existed between exercise performance parameters (V\(_{\text{O}2}\) max and run time to fatigue) and the degree of LV dysfunction that developed in each animal (LVEDP) and/or the B\(_{\text{max}}\) found in each muscle, the results were examined by use of linear and curvilinear regression analysis. P < 0.05 was considered to be statistically significant. Group data for each variable are expressed as mean ± SE.

RESULTS

MI s were induced in 23 rats, and sham operations were performed in 14 rats. Of the 23 rats that received an MI, 18 were categorized as having moderate LV dysfunction (LVEDP < 20 mmHg) whereas 5 were categorized as having severe LV dysfunction (LVEDP > 20 mmHg). LVEDP in sham rats was significantly lower than either group of MI rats. Body weights were not significantly different between the groups (Table 1).

Structural and hemodynamic indexes indicative of heart failure were prevalent in the MI rats. LV weight normalized to body weight was elevated in rats with

| Table 1. Structural and hemodynamic variables measured in noninfarcted sham-operated control rats and in MI rats with moderate and severe left ventricular dysfunction |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Body Wt, g      | LV Wt Body Wt, mg/g | RV Wt Body Wt, mg/g | Lung Wt Body Wt, mg/g | MAP, mmHg | LVEDP, mmHg |
| Sham           | 359 ± 15        | 2.07 ± 0.4       | 0.49 ± 0.1        | 3.91 ± 0.9       | 100 ± 4     | 4 ± 1         |
| Moderate‡      | 367 ± 11        | 2.37 ± 0.06      | 0.53 ± 0.2        | 3.93 ± 1.0       | 96 ± 4      | 11 ± 1*       |
| Severe§        | 404 ± 19        | 2.48 ± 1.9       | 1.07 ± 19         | 8.12 ± 1.68      | 87 ± 9      | 30 ± 4*       |

Values are means ± SE; n, number of rats. LV, left ventricular; RV, right ventricular; MAP, mean arterial pressure. ‡P < 0.05 vs. Sham; §P < 0.05 vs. Moderate; †LVEDP end-diastolic pressure (LVEDP) was not measured in 2 rats that had small MI.
moderate and severe LV dysfunction. Moreover, these increases in LV weight coincided with increases in LVEDP compared with sham rats, but the rats with severe LV dysfunction also demonstrated significantly greater increases in LVEDP compared with their counterparts with moderate LV dysfunction. Rats with severe LV dysfunction demonstrated increased lung weight normalized to body weight, suggesting that these animals had significant pulmonary congestion. Because increases in RV weight normalized to body weight were also found in these animals, they were likely in a chronic state of compensated congestive heart failure.

Decrement in exercise performance were found in rats with severe LV dysfunction as indicated by reductions in exercise endurance (run time to the point of fatigue) and VO_{2\text{max}} (Fig. 1). Interestingly, these decrements in exercise performance were not found in rats with moderate LV dysfunction. However, if run time to the point of fatigue and VO_{2\text{max}} were plotted as a function of LVEDP measured in MI rats, both indexes of exercise performance were significantly correlated with this index of LV dysfunction (Fig. 2). In addition, the endurance capacity of these MI rats was highly correlated with their VO_{2\text{max}} (Fig. 3).

The concentration of \[^{3}\text{H}\text{]ouabain binding sites (B}_{\text{max}}\) was significantly reduced in the soleus (27%), plantaris (22%), and red portion of the gastrocnemius muscle (20%) of the rats with severe LV dysfunction.

Fig. 1. A: exercise performance as measured by run time to fatigue during a progressive treadmill test for sham (n = 14) and myocardial infarction (MI) rats with moderate (n = 18) and severe (n = 5) left ventricular (LV) dysfunction. B: Similarly, maximal oxygen uptake (VO_{2\text{max}}) was determined for sham (n = 9) and MI rats with moderate (n = 10) and severe (n = 5) LV dysfunction. Values are means ± SE. *P < 0.05 compared with sham. †P < 0.05 compared with moderate group.

Fig. 2. A: relationship between run time to fatigue and LV end-diastolic pressure (LVEDP) measured in MI rats with moderate (n = 16) and severe (n = 5) LV dysfunction. B: relationship between VO_{2\text{max}} and LVEDP in MI rats with moderate (n = 8) and severe (n = 5) LV dysfunction.

Fig. 3. Relationship between run time to fatigue and VO_{2\text{max}} measured in MI rats with moderate (n = 10) and severe (n = 5) LV dysfunction.
Table 2. $[^3]$H]ouabain binding sites and binding affinity in muscles from rats with and without moderate and severe LV dysfunction

<table>
<thead>
<tr>
<th>Group</th>
<th>$B_{\text{max}}$, pmol/g wet wt</th>
<th>$K_d$, nM</th>
<th>$R_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soleus muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>259 ± 14</td>
<td>91 ± 7</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>266 ± 9</td>
<td>91 ± 6</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>Severe</td>
<td>188 ± 17$^*$†</td>
<td>87 ± 6</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td><strong>Plantaris muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>295 ± 17</td>
<td>162 ± 14</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>297 ± 14</td>
<td>158 ± 17</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>Severe</td>
<td>229 ± 18$^*$†</td>
<td>196 ± 33</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td><strong>Red portion of gastrocnemius muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>326 ± 17</td>
<td>129 ± 9</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>314 ± 12</td>
<td>131 ± 15</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Severe</td>
<td>260 ± 14$^*$†</td>
<td>146 ± 19</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td><strong>White portion of gastrocnemius muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>142 ± 15</td>
<td>97 ± 8</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>134 ± 10</td>
<td>98 ± 8</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Severe</td>
<td>101 ± 8</td>
<td>110 ± 8</td>
<td>0.93 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; $B_{\text{max}}$ (maximal binding capacity) and $K_d$ (apparent dissociation constant) represent mean of individual values obtained from Scatchard analysis for each muscle from each rat; $R_s$, average coefficient of correlation for linear regression of individual Scatchard plots. *P < 0.05 vs. Sham; †P < 0.05 vs. Moderate.

Fig. 4. Relationship between $V_O^{\text{max}}$ and number of Na⁺-K⁺ pumps ($B_{\text{max}}$) measured in the soleus (A), plantaris (B), and red (C) and white (D) portions of the gastrocnemius (Gastroc) muscles of chronic heart failure rats with moderate and severe LV dysfunction.
muscles examined. These results suggest that down-regulation of \( \text{Na}^+-\text{K}^+ \) pumps that possess a high affinity for ouabain in oxidative skeletal muscle may play an important role in the exercise intolerance that attends severe LV dysfunction in CHF.

The factors that regulate the concentration of \( \text{Na}^+-\text{K}^+ \) pumps in skeletal muscle remain unclear at this time. Clausen (9) and others have shown that the greatest activation of the pump occurs during exercise and/or muscle contraction, and it has been demonstrated that the number of \( \text{Na}^+-\text{K}^+ \) pumps increases with exercise training and decreases with deconditioning (15, 25, 26, 32, 37, 55). On the basis of these results, one might expect that the reductions in \( \text{Na}^+-\text{K}^+ \) pump number in CHF to be attributed to a decrease in physical activity (i.e., deconditioning). However, our results do not support this conclusion. In this regard, all the rats used in this study were housed individually in cages that were 6 in. wide and 9 in. long. Although physical activity was not measured, we are confident that the size of the cage was sufficient to restrict the amount of activity of each animal such that the amount of deconditioning was similar for all rats (50). Therefore, the reduced number of \( \text{Na}^+-\text{K}^+ \) pumps that have a high affinity for ouabain (i.e., the concentration of \( [\text{H}] \) ouabain binding sites) found in this study is not likely the consequence of physical deconditioning. This conclusion is consistent with previous investigations in which CHF-induced changes in skeletal muscle morphology and biochemistry could not be explained by reductions in animal activity (11, 50), and other factors associated with CHF must therefore be contributing to down-regulation of the \( \text{Na}^+-\text{K}^+ \) pump. Related to this possibility, it has been shown that muscle sympathetic nerve activity is increased in CHF (25). In conjunction with increased levels of plasma catecholamines, skeletal muscle norepinephrine concentrations are elevated in rats with CHF, specifically in muscles that have a high oxidative capacity (41). The possibility exists that chronic stimulation of pump activity via \( \beta \) adrenergic receptors (10) could have resulted in the down-regulation of \( \text{Na}^+-\text{K}^+ \) pump density.

Previous studies have suggested that the number of \( \text{Na}^+-\text{K}^+ \) pumps found in skeletal muscle is related to the oxidative capacity of the muscle (7, 23). Consistent with this observation, we found that the \( [\text{H}] \) ouabain \( B_{\text{max}} \) measured in the present investigation correlates highly with the CS activities measured in the soleus, plantaris, and red and white portions of the gastrocnemius muscles of both sham and MI rats with moderate and severe LV dysfunction from a previous investigation from our laboratory (see Fig. 5; CS activities taken from Ref. 11). Although these results suggest that the down-regulation of \( B_{\text{max}} \) and mitochondrial function coincide with one another in CHF, the precise mechanisms that contribute to each of these phenomena remain unknown, and further research in each of these investigative areas is clearly needed.

Reductions in exercise performance are well documented in CHF (52). In this regard, Weber and colleagues (56) have demonstrated that patients with CHF suffer from decrements in their maximal aerobic work capacity (\( \text{VO}_2 \max \)) compared with normal individuals. Furthermore, these reductions in \( \text{VO}_2 \max \) (or \( \dot{\text{VO}}_2 \text{peak} \)) have been shown recently to correlate with the amount of LV dysfunction that develops in the individual (i.e., decreases in ejection fraction) along with the reduction in skeletal muscle endurance capacity found in CHF patients (33, 38). We have shown previously that a similar phenomenon occurs in rats with CHF (40), and the results from this study confirm that a similar relationship exists between exercise performance and the degree of LV dysfunction in the rat CHF model (see Figs. 2 and 3).

To our knowledge, this investigation is the first to demonstrate that reductions in peak exercise performance (i.e., \( \text{VO}_2 \max \)) are correlated significantly with the reduction in the number of \( \text{Na}^+-\text{K}^+ \) pumps that possess a high affinity for ouabain found in skeletal muscles of rats with CHF. These results are consistent with previous reports that have shown that the number of \( \text{Na}^+-\text{K}^+ \) pumps in skeletal muscle is related to exercise/skeletal muscle performance (13, 26, 27) and also with the proposed hypothesis that electrolyte disturbances found in CHF may lead to an increased skeletal muscle fatigability (31, 43). However, some investigations have not been able to demonstrate this relationship in longitudinal exercise training studies (32, 37), and the possibility exists that the increases in pump number produced by training and their quantitative effect on exercise/skeletal muscle performance may be significantly different from those produced by the down-regulation of pump number in CHF. Because exercise training increases the number of skeletal muscle \( \text{Na}^+-\text{K}^+ \) pumps that possess a high affinity for ouabain, it would be interesting to see whether a program of training would ameliorate the decreases in \( B_{\text{max}} \) found in the present investigation because of...
the development of CHF. It would also be interesting
to determine whether the anticipated training-in-
duced increases in Na\(^+\)-K\(^+\) pump number would
correlate with increases exercise performance in
these individuals.

Green and colleagues (16) found recently that the
number of Na\(^+\)-K\(^+\) pumps measured in the vastus
lateralis muscle of the leg did not change in patients
with moderate CHF compared with normal controls.
However, within the CHF population there was a sig-
ificant correlation between VO\(_2\) peak and the number
of skeletal muscle Na\(^+\)-K\(^+\) pumps measured with the
\([^{3}H]\)ouabain binding assay. The present investigation
 corroborates these findings and extends them to rats
with severe LV dysfunction where significant reduc-
tions in the \([^{3}H]\)ouabain B\(_{\text{max}}\) were found in muscles
that are characterized by a high oxidative capacity
based on their fiber type composition and CS activity
(see Fig. 5). Two important points can be made from
these observations. First, it would appear that the
downregulation of B\(_{\text{max}}\) in skeletal muscle does not
occur in CHF until individuals develop severe LV dys-
fuction and congestive failure. In the present investi-
gation reductions in B\(_{\text{max}}\) did not occur until LVEDP
was >20 mmHg and rats demonstrated signs of
chronic congestive failure as indicated by the increases
in lung weight (pulmonary edema) and RV weight (RV
hypertrophy). It is worth noting that a previous study
(47) demonstrated decreases in B\(_{\text{max}}\) with LVEDPs
<20 mmHg but compensated congestive failure was
evident based on lung and RV weight compared with
sham-operated controls. Second, muscle fiber type com-
position may be important in understanding the skel-
etal muscle adaptations that occur in CHF. Specifi-
cally, the reductions in B\(_{\text{max}}\) produced in CHF were
clearly fiber type dependent in the present investiga-
tion. Because the vastus lateralis muscle contains a
wide range of fiber type composition within and be-
tween humans (49), the possibility exists that a single
biopsy from this muscle may not be representative of
the whole muscle. In comparison, the skeletal muscle
of rats is highly compartmentalized such that a large
number of muscles contain a high proportion of a single
fiber type (2, 11). This model therefore provides the
unique opportunity to determine whether muscles of a
certain fiber type composition are more susceptible to
the adaptations produced in CHF.

Limitations of the study. Relevant to the present
investigation, the Na\(^+\)-K\(^+\) pump (Na\(^+\)-K\(^+\)-ATPase)
have been shown to consist of a catalytic alpha (α) and a
glycosylated beta (β) subunit (29, 54). At least three
isoforms of the subunits have been identified (α\(_1\), α\(_2\), α\(_3\)
and β\(_1\), β\(_2\), β\(_3\)), and, thus far, two isoforms of the α
subunit (α\(_1\) and α\(_2\)) have been shown to be expressed in
rat skeletal muscle (22, 45, 54). Results suggest that
the α\(_1\) subunit plays a major role in maintaining basal
pump activity whereas the regulation and catalytic activity
of the α\(_2\) subunit can be influenced signifi-
cantly by different hormones (4, 14, 21, 24). In addi-
tion, the α\(_2\) subunit of the enzyme has a high affinity
for the Na\(^+\)-K\(^+\) pump inhibitor ouabain whereas the α\(_1\)
subunit does not (54). Because of this difference in
affinity for ouabain, the \([^{3}H]\)ouabain binding assay
used in the present investigation recognizes only the α\(_2\)
subunit of the enzyme, and therefore the reductions in
the skeletal muscle Na\(^+\)-K\(^+\) pump number found in the
present investigation primarily reflect a reduction in
the α\(_2\) isoform. What may have occurred with the
Na\(^+\)-K\(^+\) pumps that contain α\(_1\) isoform remains a
matter of speculation.

Finally, the β subunit is required for the functional
expression of the enzyme (19) and is thought to be
important in preserving the stability of the het-
erodimer complex along with playing a regulatory role
in processing and transporting the mature enzyme
complexes from the intracellular compartment to the
plasma membrane (14, 22, 28). The possibility exists
that modifications in the expression of the β subunit
could alter both Na\(^+\)-K\(^+\) pump activity (28) concomi-
tant with changes in skeletal muscle metabolic func-
tion (24). Consequently, changes in the combined ex-
pression of α and β subunits of the Na\(^+\)-K\(^+\) pump might be contributing factors to the decrements in
skeletal muscle contractile function found in the CHF
state (18).

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