Changes in skeletal muscle biochemistry and histology relative to fiber type in rats with heart failure

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Delp, Michael D., Changping Duan, John P. Mattson, and Timothy I. Musch. Changes in skeletal muscle biochemistry and histology relative to fiber type in rats with heart failure. J. Appl. Physiol. 83(4): 1291-1299, 1997.—One of the primary consequences of left ventricular dysfunction (LVD) after myocardial infarction is a decrement in exercise capacity. Several factors have been hypothesized to account for this decrement, including alterations in skeletal muscle metabolism and aerobic capacity. The purpose of this study was to determine whether LVD-induced alterations in skeletal muscle enzyme activities, fiber composition, and fiber size are 1) generalized in muscles or specific to muscles composed primarily of a given fiber type and 2) related to the severity of the LVD. Female Wistar rats were divided into three groups: sham-operated controls (n = 13) and rats with moderate (n = 10) and severe (n = 7) LVD. LVD was surgically induced by ligating the left main coronary artery and resulted in elevations (P < 0.05) in left ventricular end-diastolic pressure (sham, 5 ± 1 mmHg; moderate LVD, 11 ± 1 mmHg; severe LVD, 25 ± 1 mmHg). Moderate LVD decreased the activities of phosphofructokinase (PFK) and citrate synthase in one muscle composed of type IIB fibers but did not modify fiber composition or size of any muscle studied. However, severe LVD diminished the activity of enzymes involved in terminal and β-oxidation in muscles composed primarily of type I fibers, type IIA fibers, and type IIB fibers. In addition, severe LVD induced a reduction in the activity of PFK in type IIB muscle, a 10% reduction in the percentage of type IId/X fibers, and a corresponding increase in the proportion of type IIb fibers. Atrophy of type I fibers, type IIA fibers, and/or type IIB fibers occurred in soleus and plantaris muscles of rats with severe LVD. These data indicate that rats with severe LVD after myocardial infarction exhibit 1) decrements in mitochondrial enzyme activities independent of muscle fiber composition; 2) a reduction in PFK activity in type IIB muscle; 3) transformation of type IId/X to type IIB fibers, and 4) atrophy of type I, IIA, and IIB fibers.

3-hydroxyacyl-CoA dehydrogenase; lactate dehydrogenase; malate dehydrogenase

ONE OF THE PRIMARY CONSEQUENCES of heart failure is a decrease in work capacity. Several peripheral mechanisms have been proposed to account for this loss, including reductions in skeletal muscle perfusion and abnormalities in muscle metabolism (17, 18). In regard to the alteration in metabolism, several studies have shown that decrements in muscle oxidative potential, fiber transformation, and fiber atrophy occur in humans with heart failure (8, 12, 13, 15, 21, 27, 28) and in laboratory rats (2, 3) in which left ventricular (LV) dysfunction after myocardial infarction (MI) was experimentally induced. For example, Arnolda et al. (2) and Brunotte et al. (3) have shown that LV dysfunction in rats lowers the activity of oxidative enzymes from gastrocnemius-plantaris-soleus muscle homogenate without influencing glycolytic enzyme activities. Furthermore, the reduction of oxidative enzyme activities was related to the severity of the LV dysfunction.

What is still unclear is whether LV dysfunction-induced reductions in mitochondrial enzyme activities predominantly occur in muscles composed of a given fiber type. For example, if heart failure primarily affects muscle composed of a specific fiber type, then it is possible that measurements of enzyme activities from calf muscle homogenates during moderate LV dysfunction could mask subtle fiber-specific changes within a given muscle. Correspondingly, a change in muscle oxidative capacity with severe LV dysfunction may not be generalized to all muscle types but may occur predominantly in a muscle of a given fiber type, such as slow-twitch soleus muscle. Therefore, the purpose of this study was threefold: first, to determine whether LV dysfunction after MI induces changes in the activity of enzymes involved in glycolysis (phosphofructokinase (PFK) and lactate dehydrogenase (LDH)), terminal oxidation [citrate synthase (CS) and malate dehydrogenase (MDH)], and β-oxidation of fatty acids [3-hydroxyacyl-CoA dehydrogenase (HADH)] in muscles composed of different fiber types; second, to determine whether LV dysfunction induces fiber transformation or atrophy in muscles with diverse fiber types; and, third, to determine whether the extent of muscular alterations is related to the severity of the LV dysfunction. The results demonstrate that moderate LV dysfunction produces modest alterations in muscle enzyme activities, whereas severe LV dysfunction induces 1) decrements in mitochondrial enzyme activities (CS, MDH, and HADH) independent of muscle fiber composition; 2) a reduction in PFK activity in muscle composed predominantly of type IIB fibers; 3) transformation of type IId/X to type IIB fibers, and 4) atrophy of type I, IIA, and IIB fibers. In addition, changes in muscle oxidative enzyme capacity, type IIB fiber composition, and atrophy of type I and IIB fibers are related to the severity of the LV dysfunction.

METHODS

The methods employed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The investigation conforms with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).
Animals and surgical procedure. Female Wistar rats (Charles River Laboratories), weighing ~315 g, were individually housed in 6 × 9-in. cages, maintained on a 12:12-h light-dark cycle, and fed rat chow and given water ad libitum. After an habituation period of at least 1 wk, the animals were assigned to one of two groups: sham-operated control rats and rats receiving a MI. The rats were subsequently anesthetized with 3% halothane, intubated, placed on a rodent respirator (model 680, Harvard), and maintained on a 2% halothane-oxygen mixture. Rats received either a sham operation or MI as previously described (16). Briefly, the heart was exposed through a left thoracotomy between the fifth and sixth ribs, and the pericardium was opened. For animals in which a moderate-sized MI was produced, a 6-0 Cardiopoint suture was passed under the left main coronary artery at a point ~1–2 mm distal to the edge of the left atrium, and the artery was ligated; a large-sized MI was induced in rats with a ligature placed around the segment of the coronary artery that was closer to the aortic root. For animals in which a sham operation was performed, the suture around the coronary artery was not ligated. After the surgery, the animals were returned to their cages and closely monitored for the next 24 h. The survival rate for the rats receiving the sham operation was 100%, whereas the survival rate for the rats receiving a MI was ~60%.

Hemodynamic measures. Eight weeks after the initial surgery, all rats were anesthetized with 1 ml/kg of a 1:1 volumetric solution of ketamine hydrochloride (100 mg/ml) and xylazine (20 mg/ml) injected intramuscularly. After induction of anesthesia, the right carotid artery was cannulated with a 2-Fr catheter-tip pressure manometer (Millar Instruments) for recording arterial pressure and heart rate. With the rat breathing spontaneously, the carotid artery catheter was advanced into the LV in a retrograde fashion for measuring ventricular systolic and end-diastolic pressures and the rate of rise and fall of LV pressure (dP/dt1 and −dP/dt, respectively). Immediately after ventricular pressure was measured, the catheter-tip pressure manometer was removed and the animal was given an overdose of pentobarbital sodium (100 mg/kg body wt ip).

Tissue samples. Immediately after the rats were euthanized, the heart, lungs, and soleus, plantaris, and gastrocnemius muscles were quickly excised; the gastrocnemius muscle was further separated into deep red, middle, and superficial white portions (6, 17). The portions of gastrocnemius muscle were frozen in liquid nitrogen and stored at −70°C for determination of enzyme activities. The soleus and plantaris muscles were cut in half through the midbelly region. One-half of each muscle was frozen in liquid nitrogen and stored at −70°C for subsequent determination of enzyme activities, and the other one-half was frozen in melting isopentane (−159°C) and stored at −70°C for subsequent histochemical determination of fiber composition and fiber cross-sectional area (CSA).

Enzyme assays. The frozen muscle samples were pulverized under liquid nitrogen, and total cellular enzymes were extracted by homogenizing the muscle powder in a cold extraction buffer (6, 23). The assay mixtures for PFK, LDH, CS, MDH, and HADH were the same as previously described (4, 23). Enzyme activities, expressed as micromoles per minute per gram of wet weight, were measured spectrophotometrically in 1-ml assay mixtures at 30°C.

Histochemical analysis. Previous analysis of single muscle fibers has demonstrated that specific myosin heavy chains I, IIA, IID/X, and IIB correspond to the histochemically defined fiber types I, IIA, IID/X, and IIB, respectively (29). Therefore, these four fiber types were delineated by myosin adenosine-triphosphatase (ATPase) histochemistry as previously described (7, 9). Three serial transverse cross sections (8 µm thick) near the middle portion of soleus and plantaris muscles were cut in a microtome cryostat at −24°C, mounted on glass coverslips, and air dried. Type I fibers stained dark after preincubations at pH 4.3 and 4.45 and very light after a formaldehyde pretreatment and preincubation at pH 10.4; the reverse was true for IIA fibers. Type IID/X and IIB fibers were both stained very light at preincubation pH 4.3 and medium at preincubation pH 4.45, but IID/X fibers stained medium after formaldehyde-alkaline pretreatment whereas IIB fibers stained light.

In addition to the four "pure" fiber types (types I, IIA, IID/X, and IIB), intermediate hybrid fibers (types IC, IIC, IIAAD, and IIDD) were also evident in plantaris muscle and to a much lesser extent in soleus muscle (see Refs. 7 and 9 for staining pattern). These hybrid fibers coexpress several myosin heavy chains (19). Type IC and IIC fibers, the only hybrid fibers observed in soleus muscle, contain both heavy chains I and IIA. Type IIAAD fibers contain heavy chains IIA and IID/X, and hybrid IIDDB fibers contain heavy chains IID/X and IIB (19). In all cases, these hybrid fibers made up only a small portion (~5%) of the total number of fibers in both soleus and plantaris muscles. When these fibers were categorized into one of the four fiber types, type IC fibers were designated type I because this hybrid fiber contains more heavy chain I than IIA (19). Conversely, hybrid IIC fibers were counted as type IIA because they contain more heavy chain IIA than I. One-half of the hybrid IIDAB fibers were designated as type IIA and the other one-half as type IID/X. Similarly, one-half the hybrid IIDDB fibers were counted as type IID/X and one-half as type IIB. Fiber CSA of hybrid fibers was not measured in this study, but has been reported in a previous study (7).

Determination of muscle fiber composition and CSA. Serial cross sections of muscles stained for myosin ATPase were analyzed. All the fibers contained in each muscle cross section were typed to determine the relative population of each fiber type. Muscle cross sections were then divided into five evenly spaced regions. Representative fascicles with fibers cut perpendicular to their long axes were chosen from each of the regions for measurement of fiber areas. Fiber CSA was measured from an image obtained by use of a Matrox Image 874 card and the software package of an Olympus image processing and analysis system (6). A minimum of five fibers of each type were measured in each of the five regions of the muscle. Therefore, in every muscle, fiber area for each of the four fiber types was measured in 25–40 fibers. Similar sampling techniques have been previously used to determine fiber population and area (6, 7).

Determination of LV infarct size. The heart and lungs were cleaned, blotted, and weighed after excision. The right ventricle (RV) of the heart was separated from the LV and septum and weighed. The LV (with the septum intact) was weighed and placed in 10% Formalin for a minimum of 72 h. The LV was then cut into four transverse sections from the base to the apex in parallel with the atrioventricular groove. The four sections of the LV were subsequently dehydrated in alcohol and xylene and embedded in paraffin. Transverse sections (7 µm thick) were cut, mounted, and stained with Masson's trichrome stain from which hematoxylin was omitted to provide maximal discrimination between fibrous areas of infarct and viable muscle. These sections were magnified and projected, and the size of the infarcted areas was determined by planimetry with a Digital Image Analyzer (Carl Zeiss MOPS 3) according to the technique described by Pfeffer and co-workers (20).

Data analysis. Rats with MI were separated into two groups based on the measurements of LV end-diastolic pres-
Table 1. Body, tissue, and hemodynamic characteristics of sham-operated control rats and rats with moderate and severe left ventricular dysfunction

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Moderate LVD</th>
<th>Severe LVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Myocardial infarct size, %</td>
<td>0 ± 0</td>
<td>45 ± 2*</td>
<td>59 ± 4†</td>
</tr>
<tr>
<td>Initial body wt, g</td>
<td>318 ± 6</td>
<td>307 ± 11</td>
<td>328 ± 11</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>334 ± 6</td>
<td>340 ± 3</td>
<td>334 ± 9</td>
</tr>
<tr>
<td>Right ventricular wt, mg</td>
<td>176 ± 6</td>
<td>240 ± 8*</td>
<td>319 ± 44†</td>
</tr>
<tr>
<td>Right ventricular/body wt ratio, mg/g</td>
<td>0.53 ± 0.02</td>
<td>0.71 ± 0.02*</td>
<td>0.96 ± 0.13†</td>
</tr>
<tr>
<td>Left ventricular wt, mg</td>
<td>687 ± 17</td>
<td>797 ± 11*</td>
<td>771 ± 34*</td>
</tr>
<tr>
<td>Left ventricular/body wt ratio, mg/g</td>
<td>2.06 ± 0.05</td>
<td>2.35 ± 0.04*</td>
<td>2.31 ± 0.05*</td>
</tr>
<tr>
<td>Lung wt, mg</td>
<td>155 ± 7</td>
<td>178 ± 10</td>
<td>237 ± 19†</td>
</tr>
<tr>
<td>Lung/body wt ratio, mg/kg</td>
<td>463 ± 18</td>
<td>523 ± 28</td>
<td>712 ± 59†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>294 ± 8</td>
<td>294 ± 19</td>
<td>282 ± 10</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>104 ± 5</td>
<td>98 ± 5</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>Left ventricular systolic pressure, mmHg</td>
<td>127 ± 7</td>
<td>119 ± 5</td>
<td>105 ± 7</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure, mmHg</td>
<td>5 ± 1</td>
<td>11 ± 1*</td>
<td>25 ± 1†</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>8,421 ± 603</td>
<td>5,970 ± 420*</td>
<td>4,685 ± 437*</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−7,188 ± 519</td>
<td>−4,245 ± 302*</td>
<td>−3,346 ± 285*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. LVD, left ventricular dysfunction. +dP/dt, rate of rise of left ventricular pressure; −dP/dt, rate of fall of left ventricular pressure. *Mean is different from mean of sham-operated rats, P < 0.05. †Mean is different from mean of rats with moderate LVD, P < 0.05.

RESULTS

Infarct size. The infarct size for rats in the moderate LV dysfunction group (n = 10) was 45 ± 2% of the LV endocardial circumference (Table 1). Rats in the severe LV dysfunction group (n = 7) had infarcts that were 59 ± 4% of the LV endocardial circumference. Infarct size in rats classified as having severe LV dysfunction was significantly larger than that in rats classified as having moderate LV dysfunction. Both of these groups had infarcts significantly larger than those of control animals (n = 13), which demonstrated no discernable damage to the LV from the sham operation.

Body, LV, RV, and lung weights. Neither the initial nor the final body weights were different among groups (Table 1). RV weight, RV-to-body weight ratio, LV weight, and LV-to-body weight ratio were greater in rats with moderate and severe LV dysfunction compared with controls (Table 1). Additionally, RV weight and RV-to-body weight ratio were greater in rats with severe LV dysfunction than in rats with moderate LV dysfunction. Lung weight and lung-to-body weight ratio were not different between control rats and rats with moderate LV dysfunction (Table 1). However, both lung weight and lung-to-body weight ratio in rats with severe LV dysfunction were greater than in control animals and animals with moderate LV dysfunction.

Hemodynamic variables. Heart rate, mean arterial pressure, and LVSP were not significantly different among groups (Table 1). However, LVEDP was higher in rats with moderate LV dysfunction than in controls, and LVEDP was greater in rats with severe LV dysfunction than in control animals and animals with moderate LV dysfunction (Table 1). The rise in LVEDP was significantly related to the size of the LV infarct (Fig. 1; r = 0.831). Both +dP/dt and −dP/dt were less in the two LV dysfunction groups than in sham-operated controls; there was no difference in these variables between the two LV dysfunction groups.

Enzyme activities. There were no differences in the activity of LDH among groups in any of the muscles studied (Table 2). However, the activity of PFK decreased in the white portion of gastrocnemius muscle from rats with moderate and severe LV dysfunction. The activity of mitochondrial enzyme CS was lower in
soleus muscle of rats with severe LV dysfunction than in soleus muscles of control animals and rats with moderate LV dysfunction. In the red portion of gastrocnemius muscle, CS was lower in rats with severe LV dysfunction than in controls, and in the white portion of gastrocnemius muscle, CS was lower in rats with moderate and severe LV dysfunction compared with sham-operated controls. The activity of mitochondrial enzyme MDH was lower in the red and white portions of gastrocnemius muscle from rats with severe LV dysfunction than in control rats. The activity of HADH was lower in the red portion of gastrocnemius and plantaris muscles of rats with severe LV dysfunction than in control animals and animals with moderate LV dysfunction.

Regression analyses indicated there was a significant linear relationship between LVEDP and the percent increase in type IIB fibers in plantaris muscle (Fig. 5; r = 0.475). CSA of fibers in soleus muscle was not different between control rats and rats with moderate LV dysfunction (Table 4). In soleus muscle of rats with severe LV dysfunction, CSA of type I and IIA fibers was less than that in sham rats and rats with moderate LV dysfunction. In plantaris muscle, type I fibers were smaller in rats with moderate and severe LV dysfunction than in control animals, but there was no size difference in type I fibers between the two LV dysfunction groups.

Table 2. Activity of phosphofructokinase, lactate dehydrogenase, citrate synthase, malate dehydrogenase, and 3-hydroxyacyl-CoA dehydrogenase in muscles from sham-operated rats and rats with left ventricular dysfunction

<table>
<thead>
<tr>
<th>Muscle</th>
<th>PFK</th>
<th>LDH</th>
<th>CS</th>
<th>MDH</th>
<th>HADH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8 ± 1</td>
<td>157 ± 10</td>
<td>24 ± 1</td>
<td>695 ± 21</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>9 ± 1</td>
<td>160 ± 18</td>
<td>22 ± 1</td>
<td>670 ± 33</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>8 ± 1</td>
<td>152 ± 9</td>
<td>18 ± 1</td>
<td>685 ± 43</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>40 ± 4</td>
<td>743 ± 34</td>
<td>22 ± 1</td>
<td>465 ± 20</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>44 ± 3</td>
<td>772 ± 31</td>
<td>18 ± 2</td>
<td>487 ± 20</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>38 ± 6</td>
<td>790 ± 74</td>
<td>20 ± 2</td>
<td>457 ± 37</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Gastrocnemius, red</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>37 ± 3</td>
<td>541 ± 26</td>
<td>37 ± 2</td>
<td>779 ± 27</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>34 ± 6</td>
<td>574 ± 32</td>
<td>34 ± 1</td>
<td>727 ± 24</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>35 ± 4</td>
<td>466 ± 58</td>
<td>31 ± 1</td>
<td>656 ± 37</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Gastrocnemius, mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>45 ± 4</td>
<td>727 ± 33</td>
<td>23 ± 2</td>
<td>437 ± 34</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>44 ± 4</td>
<td>628 ± 74</td>
<td>21 ± 2</td>
<td>340 ± 36</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>37 ± 3</td>
<td>746 ± 25</td>
<td>20 ± 2</td>
<td>388 ± 35</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Gastrocnemius, white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>68 ± 4</td>
<td>866 ± 34</td>
<td>8 ± 1</td>
<td>210 ± 9</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>56 ± 3*</td>
<td>872 ± 22</td>
<td>6 ± 1*</td>
<td>188 ± 8</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>56 ± 3*</td>
<td>840 ± 39</td>
<td>6 ± 1*</td>
<td>171 ± 4*</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE given in µmol·min⁻¹·g wet wt⁻¹; n = 11–13 for sham, n = 9–10 for moderate LVD, and n = 6–7 for severe LVD. PFK, phosphofructokinase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; HADH, 3-hydroxyacyl-CoA dehydrogenase. *Mean is different from mean of sham-operated rats, P < 0.05; †mean is different from mean of rats with moderate LVD, P < 0.05.
type IIB fibers in plantaris muscle from rats with severe LV dysfunction was less than in control animals and rats with moderate LV dysfunction. There was also a tendency ($P = 0.07$) for CSA of type IIA fibers in plantaris muscle of rats with severe LV dysfunction to be less than that of type IIA fibers in control rats. There was a significant linear relationship between LVEDP and the decrease in type I fiber CSA (Fig. 6; $r = -0.645$).

**DISCUSSION**

Numerous studies in the literature have shown that heart failure can induce skeletal muscle abnormalities and dysfunction. The present study demonstrates that moderate LV dysfunction in rats has little impact on skeletal muscle; i.e., there were modest changes in glycolytic and oxidative enzyme activities in one muscle section but no alterations in fiber composition or fiber CSA. However, severe LV dysfunction resulted in 1) reductions in the activity of enzymes involved in terminal oxidation and $\beta$-oxidation of fatty acids independent of muscle fiber composition; 2) reductions in the activity of PFK in muscle composed predominantly of type IIB fibers; 3) alterations in muscle fiber composition; and 4) atrophy of type I, IIA, and IIB fibers. In addition, the present study is the first to demonstrate a linear relationship between the severity of LV dysfunction and the decreases in mitochondrial enzyme activi-

**Table 3.** Percent distribution of type I, IIA, IID/X, and IIB fibers in soleus and plantaris muscles of sham-operated control rats and rats with left ventricular dysfunction

<table>
<thead>
<tr>
<th>Muscle</th>
<th>I</th>
<th>IIA</th>
<th>IID/X</th>
<th>IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>Sham</td>
<td>92±2</td>
<td>8±2</td>
<td>1±0</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>95±2</td>
<td>4±2</td>
<td>1±1</td>
<td>0±0</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>94±2</td>
<td>6±2</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>Plantaris</td>
<td>Sham</td>
<td>11±2</td>
<td>14±2</td>
<td>28±3</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>11±2</td>
<td>15±2</td>
<td>25±3</td>
<td>49±3</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>7±2</td>
<td>15±2</td>
<td>18±2</td>
<td>59±1†</td>
</tr>
</tbody>
</table>

Values are means ± SE given in %; $n = 7$ for sham, $n = 6$ for moderate LVD, and $n = 6$ for severe LVD. *Mean is different from mean of sham-operated rats, $P < 0.05$. †Mean is different from mean of rats with moderate LVD, $P < 0.05$. 

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**Fig. 3.** Scattergrams showing linear relationship between LV end-diastolic pressure and malate dehydrogenase (MDH) activity in red (GR; A; $n = 26$) and white (GW; B; $n = 28$) portions of gastrocnemius muscle. A: $r = -0.685$, $y = 824.3 - 7.489x$. $P < 0.01$. B: $r = -0.553$, $y = 215.5 - 1.861x$. $P < 0.01$.

**Fig. 4.** Scattergrams showing linear relationship between LV end-diastolic pressure and $3$-hydroxyacyl-CoA dehydrogenase (HADH) activity in GR (A; $n = 27$) and plantaris (B; $n = 29$) muscles. A: $r = -0.560$, $y = 18.4 - 0.25x$. $P < 0.01$. B: $r = -0.331$, $y = 12.4 - 0.104x$. $P < 0.1$. 

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...
ties (Figs. 2–4), increases in the percentage of type IIB fibers (Fig. 5), and atrophy of type I fibers (Fig. 6). LV dysfunction was induced in two groups of rats through MI. The degree of LV dysfunction, defined as an increase in LVEDP, correlated with the size of the MI (Fig. 1). In both of the infarcted groups, the animals were in a state of partially decompensated heart failure, based on the RV hypertrophy and increase in LVEDP (Table 1). Although there was a continuum in the severity of the heart failure among the infarcted rats, the separation of the LV dysfunction animals into two groups produced clear differences between moderate and severe heart failure. In the group of animals we refer to as having moderate LV dysfunction (infarct size $\leq 45\%$ of the LV), RV-to-body weight ratio and LVEDP were higher than that in sham-operated controls. Rats we considered to have severe LV dysfunction had significantly larger MIs ($59\%$ of the LV) than did animals in the moderate LV dysfunction group, and this was associated with greater RV hypertrophy, a higher LVEDP, and an elevation in the lung-to-body weight ratio, indicating congestive heart failure was induced in this group. Others (20) have similarly grouped rats according to MI size and shown that ventricular function is directly related to the extent of infarction.

The present study builds on previous observations that severe, but not moderate, LV dysfunction reduces mitochondrial enzyme activities of calf muscle homogenates (2, 3). The present study was in part performed to determine whether LV dysfunction-induced alterations in muscle are specific to muscles composed of a given fiber type or are generalized in muscles regardless of fiber composition. The data demonstrate that the decrease in oxidative capacity, as indicated by decreases in mitochondrial enzyme activities (CS, MDH, and HADH), is not specific to a particular fiber type but occurs in muscles composed primarily of type I fibers (soleus muscle), type IIA fibers (red portion of gastrocnemius muscle), and type IIB fibers (white portion of gastrocnemius muscle). Thus severe congestive heart failure diminishes the aerobic capacity of all types of fibers in both deep and superficial limb muscles. These findings are in close agreement with a recent report demonstrating that the succinate dehydrogenase activity of type I, IIA, and IIB fibers was lower in human muscle biopsy samples from patients with chronic congestive heart failure (15).

Table 4. Cross-sectional area of type I, IIA, IID/X, and IIB fibers in soleus and plantaris muscles of sham-operated control rats and rats with left ventricular dysfunction

<table>
<thead>
<tr>
<th>Muscle</th>
<th>I</th>
<th>IIA</th>
<th>IID/X</th>
<th>IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>4,587±155</td>
<td>3,708±234</td>
<td>3,570±454</td>
<td></td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>3,946±200</td>
<td>3,993±327</td>
<td>3,229±640</td>
<td>3,229±640</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>3,481±294*</td>
<td>2,874±211†</td>
<td>3,565±1,039</td>
<td></td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2,113±128</td>
<td>2,030±169</td>
<td>3,098±180</td>
<td>4,511±238</td>
</tr>
<tr>
<td>Moderate LVD</td>
<td>1,542±197*</td>
<td>1,708±146</td>
<td>3,046±134</td>
<td>4,612±238</td>
</tr>
<tr>
<td>Severe LVD</td>
<td>1,258±48*</td>
<td>1,599±43*</td>
<td>2,815±135</td>
<td>3,873±107†</td>
</tr>
</tbody>
</table>

Values are means ± SE given in µm²; n = 6 for each group. * Mean is different from mean of sham-operated rats, P < 0.05. † Mean is different from mean of rats with moderate LVD, P < 0.05. ‡ Mean is different from mean of sham-operated rats, P = 0.07.
Although the changes in oxidative enzyme activities induced by moderate and severe LV dysfunction in the present study are similar to those previously reported in rats (2, 3), disparities exist with regard to glycolytic enzyme activities (2). Specifically, we observed a decrease in PFK activity in the white portion of gastrocnemius muscle from rats with moderate and severe LV dysfunction. From calf muscle homogenates, Arnolada et al. (2) observed no change in PFK activity. In addition, PFK activity in muscle biopsy samples from heart failure patients was not different from that from control subjects (13, 27). The reason for the disparity between the present study and others (2, 13, 27) is unknown but may be related to the fiber “purity” of the muscle samples. For example, the white portion of gastrocnemius muscle, the only muscle section showing a decrease in PFK activity, consists of 92% type IIB fibers (6). Neither the muscle homogenate (2) nor the biopsy samples (13, 27) had this degree of fiber homogeneity. Thus the decrease in PFK activity observed in both groups of infarcted animals in the present study may only be present or detectable in homogenous muscle composed predominantly of type IIB fibers.

We observed that severe congestive heart failure induces a shift in fiber composition, with a decrement in the portion of type IID/X fibers and an increase in percentage of IIB fibers in plantaris muscle (Table 3). In addition, there was atrophy of type I, IIA, and IIB fibers (Table 4). It was surprising that an increase in the activity of glycolytic enzymes did not accompany the increase in the percentage of type IIB in plantaris muscle. Perhaps increases in the proportion of type IIB fibers are offset by decreases in the activity of glycolytic enzymes in type IIB fibers, similar to the decrease in PFK activity occurring in the white portion of gastrocnemius muscle. Alternatively, the lack of a change in glycolytic enzyme activities may be related to the fiber atrophy occurring in the muscle.

The increase in the percentage of type IIB fibers and the fiber atrophy observed in the present study differs from that reported by Brunotte et al. (3). These investigators found no change in fiber composition or fiber size with heart failure in rats, although they did report a reduction in calf muscle weight. There are several possibilities that may account for the disparity between the two studies, such as differences in the muscles studied or differences in the techniques used to determine muscle fiber composition. For example, the histological fiber typing method employed in the present study enabled discrimination between type IID/X and type IIB fibers, whereas the method used by Brunotte and co-workers would classify IID/X fibers as IIB (see Ref. 6 for details). Thus a change in fiber composition from type IID/X to type IIB, which was observed in the present study, could not be detected.

Changes in muscle fiber composition and size have also been reported in patients suffering from chronic heart failure. For example, several studies have reported decreases in the percentage of type I fibers (8, 15, 27) and increases in the percentage of type IIB fibers (8, 13). In the present study, there was not a significant change in the percentage of type I fibers (Table 3), although in the plantaris muscle there were 36% fewer type I fibers in animals with severe LV dysfunction than in control rats or animals with moderate LV dysfunction. It is possible that longer periods of infarction may be required for the type I-to-type II fiber conversion to become evident in the rat. In addition to changes in fiber composition, atrophy of type I (12), type IIA (13), and type IIB (13, 27) fibers has also been reported in heart failure patients.

The underlying mechanism(s) inducing decreases in aerobic capacity, fiber transformation, and fiber atrophy in skeletal muscle remains uncertain. One possibility is that these muscle abnormalities are secondary to reductions in physical activity. Support for this notion comes from observations that exercise training ameliorates muscle metabolic alterations from developing in rats with heart failure (3). However, to our knowledge only one study has directly assessed physical activity as a factor in the muscle alterations induced by heart failure (26). Simonini and colleagues (26) measured cage activity in rats with and without MIs and found that there was no difference in the activity levels of infarcted rats and control animals. However, despite the lack of a difference in activity between the groups, the LV dysfunction rats still demonstrated decreases in muscle citrate synthase activity, reductions in the portion of type I fibers, and reductions in mRNA for β-myosin heavy chains and cytochrome-c oxidase. These data suggest that inactivity is not obligatory for the development of skeletal muscle abnormalities with heart failure.

Data from the present study also suggest inactivity alone is not responsible for muscle alterations that develop during heart failure. For example, there were reductions in the activities of oxidative enzymes in muscle composed predominantly of type IIB fibers (i.e., the white portion of gastrocnemius muscle). Muscles composed of IIB fibers are only recruited during high-intensity exercise (1, 6) and thus would remain inactive during normal cage activity. The reduction of mitochondrial enzyme activities in quiescent muscle suggests the involvement of other factors not directly related to physical activity.

A second possibility is that alterations in skeletal muscle perfusion may impact the size and metabolic characteristics of muscle fibers. Several studies have demonstrated that muscle blood flow is diminished by heart failure (cf. Ref. 18). For example, in rats with MIs, hindlimb muscle blood flow is reduced at rest and during exercise (17). However, the reduction in muscle blood flow is related to the degree of LV dysfunction. In animals with moderate LV dysfunction, muscle blood flow is not altered at rest but is lower than in sham-operated controls during exercise. In rats with more severe LV dysfunction, muscle perfusion is lower at rest and during exercise than it is in control and moderate LV dysfunction rats. Thus moderate LV dysfunction does not alter resting muscle blood flow (17), and this is associated with little or no change in the size and metabolic characteristics of the muscle fibers (Ref. 2;
results in RV hypertrophy and increases in lung wet
LV dysfunction produces only modest alterations in
ability of muscle to sustain high power outputs for
anaerobic metabolism undoubtedly contributes to
anaerobic metabolism. This shift to greater dependence
more oxidative and fatigue-resistant fibers to type IIB
the reliance on anaerobic metabolism to support the
composition found in the study.

One of the hallmark features of congestive heart
failure is an intolerance to exercise. It appears evident
that the decreased exercise capacity of individuals
with heart failure is due, at least in part, to alterations in
the characteristics of skeletal muscle. Reductions in
mitochondrial enzyme activities with heart failure diminish
the potential of muscle to utilize O2 and thus increase
the percentage of type IIB fibers, and the atrophy of type I fibers in skeletal muscle.

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