Relation of Systemic and Local Muscle Exercise Capacity to Skeletal Muscle Characteristics in Men With Congestive Heart Failure

BARRY M. MASSIE, MD, FACC,*† AMERICO SIMONINI, MD,* PUNEET SAHGAL, MD,* LAUREN WELLS, PT, MS,† GARY A. DUDLEY, PhD‡

San Francisco, California and Athens, Georgia

Objectives. The present study was undertaken to further characterize changes in skeletal muscle morphology and histochemistry in congestive heart failure and to determine the relation of these changes to abnormalities of systemic and local muscle exercise capacity.

Background. Abnormalities of skeletal muscle appear to play a role in the limitation of exercise capacity in congestive heart failure, but information on the changes in muscle morphology and biochemistry and their relation to alterations in muscle function is limited.

Methods. Eighteen men with predominantly mild to moderate congestive heart failure (mean ± SEM New York Heart Association functional class 2.6 ± 0.2, ejection fraction 24 ± 2%) and eight age- and gender-matched sedentary control subjects underwent measurements of peak systemic oxygen consumption (VO₂) during cycle ergometry, resistance to fatigue of the quadriceps femoris muscle group and biopsy of the vastus lateralis muscle.

Results. Peak VO₂ and resistance to fatigue were lower in the patients with heart failure than in control subjects (15.7 ± 1.2 vs. 25.1 ± 1.5 ml/min-kg and 63 ± 2% vs. 85 ± 3%, respectively, both p < 0.001). Patients had a lower proportion of slow twitch, type I fibers than did control subjects (36 ± 3% vs. 46 ± 5%, p = 0.048) and a higher proportion of fast twitch, type IIa fibers (18 ± 3% vs. 7 ± 2%, p = 0.004). Fiber cross-sectional area was smaller, and single-fiber succinate dehydrogenase activity, a mitochondrial oxidative marker, was lower in patients (both p ≤ 0.034). Likewise, the ratio of average fast twitch to slow twitch fiber cross-sectional area was lower in patients (0.780 ± 0.06 vs. 1.05 ± 0.08, p = 0.019). Peak VO₂ was strongly related to integrated succinate dehydrogenase activity in patients (r = 0.896, p = 0.001). Peak VO₂, resistance to fatigue and strength also correlated significantly with several measures of fiber size, especially of fast twitch fibers, in patients. None of the skeletal muscle characteristics examined correlated with exercise capacity in control subjects.

Conclusions. These results indicate that congestive heart failure is associated with changes in the characteristics of skeletal muscle and local as well as systemic exercise performance. There are fewer slow twitch fibers, smaller fast twitch fibers and lower succinate dehydrogenase activity. The latter findings suggest that mitochondrial content of muscle is reduced in heart failure and that impaired aerobic-oxidative capacity may play a role in the limitation of systemic exercise capacity.

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Systemic exercise intolerance and fatigue are common and troublesome symptoms in patients with heart failure, but their severity correlates poorly with indexes of cardiac function (1-3). This discordance appears to be explained in part by reduced blood flow to exercising muscle (4,5), but evidence also suggests an important role for abnormalities of skeletal muscle, including atrophy (6-8), impaired function (9,10) and altered metabolism (11,12).

To determine the basis for these changes, investigators (6,13-16) have evaluated morphologic, histologic and biochemical characteristics of skeletal muscle in patients with congestive heart failure; however, these studies have not yielded a consistent pattern of abnormalities. Muscle biopsy findings have also shown variable, if any, relation with measurements of muscle function, muscle metabolism and exercise capacity. These inconsistencies probably reflect the diversity of functional and biochemical assessments and the heterogeneity of the patient and control groups studied.

The present study was undertaken to determine whether the muscle morphology and histochemistry of patients with heart failure differs from those of age-matched sedentary control subjects and, if so, whether these changes are associated with corresponding abnormalities of muscle function and exercise capacity. To accomplish this, biopsy specimens from the lateral aspect of the quadriceps femoris were analyzed for...
fiber type composition, cross-sectional area, capillarization and single-fiber succinate dehydrogenase activity, a mitochondrial marker. These measurements were then related to quantitative indexes of quadriceps femoris function and systemic exercise capacity.

Methods

Patients. Eighteen men with a history of stable, chronic congestive heart failure of >6 months' duration were recruited from the Department of Veterans Affairs Medical Center in San Francisco. The diagnosis was based on a history of dyspnea on exertion, fatigue or fluid retention, with confirmation of left ventricular dysfunction by a radionuclide ejection fraction of <40% (mean ± SEM 24 ± 2%). Symptoms were classified as New York Heart Association functional class I, II, III and IV in two, seven, seven and two patients, respectively. Ten patients had ischemic cardiomyopathy, and eight were thought to have primary myocardial disease. Patients were excluded if they had had a myocardial infarction within 6 months or if exercise was limited by symptoms other than fatigue or dyspnea. Other criteria for exclusion included alcohol abuse within the previous 12 months; initiation of therapy with an angiotensin-converting enzyme inhibitor, long-acting nitrate, diuretic drug, or digitalis within 3 months; the presence of hemodynamically significant valvular disease; angina pectoris; or exercise limitation by pulmonary, peripheral vascular, arthritic or neurologic disease.

For comparison, eight age-matched sedentary men were recruited from employees or patients followed up in the Dermatology Clinic at the Veterans Affairs Medical Center. These subjects had no history of heart disease and no cardiac, peripheral vascular or musculoskeletal abnormalities on physical examination. To avoid the comparison of patients with highly fit control subjects, these subjects were excluded if their peak systemic oxygen consumption (VO₂) was >1 SD above the mean for their age. The protocol was approved by the Committee on Human Research at the University of California, San Francisco; written informed consent was obtained from all participants.

Physiologic measurements. Systemic exercise capacity. Peak systemic VO₂ was quantified during cycle ergometry according to our previously published techniques (8,10). Briefly, after resting on the cycle for 5 min, subjects performed unloaded exercise for 2 min. Subsequently, the load was increased to 200 kg-m/min for 2 min, and then by 100 kg-m/min every 2 min until exhaustion, as defined by the inability to maintain a pedal frequency of >40 rpm. Standard verbal encouragement was used for all subjects.

Respiratory gas exchange was monitored by utilizing a metabolic cart (Sensormedics, Inc.), with measurements of VO₂ and carbon dioxide production (VCO₂) at 15-s intervals throughout exercise. Peak systemic VO₂, defined as the highest VO₂ achieved, was utilized to quantify exercise capacity, and the respiratory exchange ratio (VO₂/VCO₂) was utilized as an index of the adequacy of exercise. The respiratory exchange ratio exceeded 1.0 in all patients and control subjects.

Knee extensor function. Knee extensor endurance and strength were measured with an isokinetic dynamometer (Cybex 340), as described in detail previously (8,10). Briefly, 15 maximal knee extensions were performed at 90°/s in rapid succession over a period of ~30 s. Verbal encouragement was given in a standardized manner throughout the procedure. Endurance, or the ability to withstand fatigue, was defined as the ratio of the mean peak torque in the last three extensions to the mean peak torque in the first three extensions multiplied times 100. The mean peak torque for the first three extensions was also used as a measure of strength of the quadriceps femoris muscle group.

Muscle biopsies. Two biopsy specimens were taken from two different sites, 16 to 19 cm above the patella, by using the percutaneous needle technique of Bergstrom (17). Specimens were processed for histochemical analysis as described in detail elsewhere (18–21). Briefly, sections were assayed for determination of muscle fiber type (I, IIA, IIB, IIC and IID) (22), capillarity and succinate dehydrogenase activity, an estimate of mitochondrial content.

Area and capillarity of the different fiber types were assessed by using a semiautomated image analysis system and Image software (National Institutes of Health). From these data, fiber type percent, the ratio of fast twitch to slow twitch fiber cross-sectional area, fiber type–specific absolute and relative areas, average fiber area and the number of capillaries surrounding a given fiber were calculated. Regions of a section were excluded from analysis if they contained oblique or histologically abnormal fibers. At least 150 fibers in each section were assessed for area, capillarity and type. Data from the two biopsy sites in each subject were averaged to enhance validity of the results (23). Type IIC fibers were rare, comprising 2 ± 2% overall, and were subsequently excluded from analyses.

Single-fiber succinate dehydrogenase activity was determined by using a quantitative histochemical technique described by Blanco et al. (24), as done previously (21). Fiber type–specific activity was determined by matching fibers assayed for succinate dehydrogenase with those in serial sections assayed for myofibrillar adenosine triphosphatase activity. About 60 fibers/section were thus assayed. Enzyme activities for type IIA and type IIB fibers were averaged and reported as activity for type IIB fibers, because of the low proportion of type IIB fibers in the control subjects. An adequate number of matched fibers was available for performing this fiber-specific analysis in 11 of 18 patients and 7 of 8 control subjects. These data were used with fiber type percent and average fiber cross-sectional area to calculate average integrated succinate dehydrogenase activity irrespective of fiber type, an overall estimate of mitochondrial content.

Statistical analyses. Comparisons between groups were made by using an independent analyses of variance with repeated measures over a given variable or by an independent t test with SuperANOVA software. Percent and ratio data
were arc-sine or log transformed before analyses. Correlations between variables were assessed by using Pearson's product correlations. Data are presented as mean value ± SEM.

Results

Systemic exercise capacity and local muscle function (Table 1). Control subjects and patients with heart failure were comparable in age. The heart failure group had a mean ejection fraction of 24 ± 2% and functional class of 2.6 ± 0.2. Although peak VO₂ in the control group was relatively low, consistent with the age and sedentary status of these subjects, it was significantly higher than that in patients with heart failure (25.1 ± 1.5 vs. 15.7 ± 1.2 ml/kg-min, p < 0.001). The fatigue index of the knee extensors, calculated from the decline in peak torque during the series of 15 knee extensions, was lower in the patients (63 ± 2% vs. 85 ± 3%, p < 0.001). In contrast, knee extensor strength was not significantly reduced in the patients (87 ± 8 vs. 89 ± 7 Newton-meters). Systemic and local exercise capacity, expressed as peak VO₂ and the fatigue index, respectively, were correlated in both patients (r = 0.57, p = 0.026) and control subjects (r = 0.80, p = 0.018).

Muscle biopsy findings (Table 2). The results of the fiber type analyses and morphometry are given in Table 2. Significant intergroup differences were found in fiber type distribution. The percent of type I fibers was less in the patients with heart failure than in the control subjects (36 ± 3% vs. 46 ± 5%, p = 0.048), whereas the percent of type IIa fibers was greater in the patients (18 ± 3% vs. 7 ± 2%, p = 0.004).

In both patients and control subjects, type I and IIa fibers were larger than IIb fibers. Overall fiber cross-sectional area was smaller in patients than in control subjects, reflecting the smaller size of the fast twitch fibers, because the area of type I fibers was similar in the two groups. Therefore, the ratio of average fast twitch to slow twitch fiber area was lower in patients than in control subjects (0.78 ± 0.06 vs. 1.05 ± 0.08, p = 0.019).

The hierarchy of succinate dehydrogenase activity reflected the usual type I > type IIa > type IIb pattern in both groups, but the mean activity per fiber was reduced in the patients (100 ± 5 vs. 119 ± 9 optical density U/min × 10⁻⁴, p = 0.026), reflecting lower values in all fiber types. The number of capillaries around a given fiber followed the hierarchy of type I or type IIa > type IIb or type IIb. However, the number of capillaries per fiber did not differ between groups for any type of fiber.

Relation of exercise capacity to muscle characteristics. The finding that the patients with heart failure and control subjects differed in both distribution and size of fiber types necessitated that skeletal muscle characteristics be weighted

Table 1. Clinical Data and Physiologic Measurements in 26 Men (18 patients with congestive heart failure and 8 control subjects)

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>NYHA Class</th>
<th>EF (%)</th>
<th>Peak VO₂ (ml/kg-min)</th>
<th>Fatigue Index (%)</th>
<th>Strength (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>64 ± 2</td>
<td>2.6 ± 0.2</td>
<td>24 ± 2</td>
<td>15.7 ± 1.2*</td>
<td>63 ± 2*</td>
<td>87 ± 8</td>
</tr>
<tr>
<td>Control subjects</td>
<td>65 ± 3</td>
<td>...</td>
<td>...</td>
<td>25.1 ± 1.5</td>
<td>85 ± 3</td>
<td>89 ± 7</td>
</tr>
</tbody>
</table>

*p < 0.001, patients versus control subjects. Data are presented as mean value ± SEM. EF = ejection fraction; Nm = Newton-meters; NYHA Class = New York Heart Association functional class; VO₂ = oxygen consumption; ... = data not available.

Table 2. Skeletal Muscle Morphology and Histochemical Findings in the 26 Study Subjects

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type IIab</th>
<th>Type IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution (%)†‡</td>
<td>36 ± 34</td>
<td>25 ± 4</td>
<td>18 ± 3*</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Mean cross-sectional area (µm²)§†</td>
<td>6,031 ± 615</td>
<td>5,598 ± 647</td>
<td>4,792 ± 644</td>
<td>3,413 ± 409</td>
</tr>
<tr>
<td>Succinic dehydrogenase activity§† (optical density U/min × 10⁻⁴)</td>
<td>130 ± 7</td>
<td>94 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillaries per fiber†</td>
<td>4.2 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
</tbody>
</table>

*Fiber type by group interaction by analysis of variance (ANOVA), p = 0.022; fiber type by ANOVA, p ≤ 0.043; †p < 0.05, patients versus control subjects; ‡group effect by ANOVA, patients versus control subjects, p = 0.034; §succinic dehydrogenase activity not given for IIab fibers because of small numbers. Data are presented as mean value ± SEM.
Discussion

Major findings of the present study. Several findings in this study provide additional insight into the pathophysiology of skeletal muscle dysfunction and exercise intolerance in patients with heart failure. First, they build on previous reports that have demonstrated a decreased proportion of slow twitch oxidative fibers and reduced aerobic-oxidative enzyme content in such patients (6,13–16). In addition to these findings, our results show that the lower mitochondrial content is not specific to fiber type but is evident for each of the major fiber types of human skeletal muscle, as reflected by single-fiber succinate dehydrogenase activity. Second, the strong relation between average integrated succinate dehydrogenase activity, a cumulative index of mitochondrial aerobic-oxidative enzyme content, and peak Vo2 in patients with heart failure, but not in control subjects, suggests that the impairment of aerobic-oxidative capacity may be physiologically significant and contribute to exercise limitation. Finally, several of our findings in patients with heart failure differ from the characteristic responses to muscle disuse in healthy sedentary individuals (20,25–28).

Skeletal muscle characteristics in heart failure. Several groups have reported a lower aerobic-oxidative enzyme content in homogenates of skeletal muscle from patients with heart failure, irrespective of the indexes measured (6,13–16). In contrast, this study quantified succinate dehydrogenase activity in single-muscle fibers in relation to their contractile material. This technique affords the opportunity to determine mitochondrial content in the three major fiber types of human skeletal muscle and eliminates the potential for extraneous factors, such as extracellular fluid accumulation or fatty infiltration, to affect measures of enzyme activity. As is the case in normal subjects (21,29), aerobic-oxidative enzyme activity in the patients with heart failure showed a hierarchy among fiber types, with type I > type IIa > type IIb. However, irrespective of fiber type, the succinate dehydrogenase activity of patients was lower than that in control subjects. Thus, the reduction in oxidative enzyme activity was not specific to one group of fibers.

The results of this study also extend previous observations concerning differences in fiber type composition between patients with heart failure and control subjects. The previously reported finding (14,15) that such patients have a lower proportion of type I, slow twitch fibers and more type 2h, fast twitch glycolytic fibers than do normal subjects was also evident in this study. In addition, we subclassified fast twitch fibers into three types, because it is now evident that fibers that stain intermediate between types IIa and IIb, and are thus distinct from their counterparts (22). Our results suggest that the higher proportion of type IIb fibers reported previously in patients with heart failure may have been due to a higher proportion of type IIb fibers. These findings suggest transition to skeletal muscle with a lower aerobic-oxidative enzyme content because type IIb or type IIb fibers have lower succinate dehydrogenase activity than do type I or type IIa fibers (21). The mechanisms responsible for the different fiber type composition of skeletal muscle between patients and age-matched sedentary control subjects is not known, but preferential loss of type I fibers through denervation or transformation from slow to fast fibers, as may occur with muscle disuse, may be responsible (30,31).

The majority of previous studies (6,13–16) have reported at least a trend toward smaller type II fibers in patients with heart failure than in control subjects. We found that, irrespective of fiber type, fibers were significantly smaller in patients than in control subjects; in addition, the lower ratio for the cross-
sectional area of fast to slow fibers in patients indicates that atrophy is relatively specific to fast fibers. Findings concerning skeletal muscle capillarization in patients with heart failure have been variable (13–15). We found no difference between groups in the number of capillaries surrounding a given fiber type, but the maintenance of a normal capillary density is of interest because a reduction in proportion to the decrease in mitochondrial content is more typical in normal subjects.

**Functional and metabolic correlates of the muscle biopsy findings.** Data relating histologic characteristics of skeletal muscle to exercise capacity are limited and conflicting. Mancini et al. (13) reported that peak Vo2 and type I fiber percent were positively correlated, whereas peak Vo2 and type IIb fiber percent were inversely related. Lipkin et al. (6) did not find any significant correlations between knee extensor strength and several skeletal muscle characteristics despite abnormalities in both. We found that peak Vo2 correlated with average fast twitch fiber and overall fiber cross-sectional area in patients with heart failure, as did the fatigue index and strength of the knee extensors. No such correlations were found in the control subjects. Whether these findings have mechanistic significance or represent associations with other factors remains uncertain.

Data relating exercise performance or metabolism to the content of oxidative enzymes also have shown mixed results. We found a strong correlation between peak Vo2 and integrated succinate dehydrogenase activity in patients with heart failure (r = 0.90, p < 0.001), but not in control subjects. Although Mancini et al. (13) did not observe a relation between these variables, or between aerobic-oxidative enzyme content and phosphorus-31 magnetic resonance spectroscopy indexes of metabolism, in rats with experimental heart failure, citrate synthase activity and phosphocreatine content during repetitive stimulation of the gastrocnemius muscle were significantly correlated (32). Drexler et al. (15) and Munzel et al. (33) have reported a significant relation between skeletal muscle mitochondrial content and leg oxygen extraction, and parallel increases in these measures during chronic angiotensin-converting enzyme inhibitor therapy.

Thus, our data, in conjunction with previous findings demonstrating that in some patients blood flow to exercising muscle does not limit oxygen uptake, are consistent with the hypothesis that the low mitochondrial content of skeletal muscle in heart failure influences exercise capacity (34,35). This is especially noteworthy for systemic exercise performance, which in healthy subjects is determined primarily by cardiac reserve (36). However, other factors are also likely to be involved in local muscular exercise intolerance in the patients. We found a 40% decrease in force during 30 s of repetitive knee extensions in this and a previous study (10). Patients with heart failure have also shown (37,38) a similar decline in force during isometric ankle flexion or knee extension. Fatigue was rapid and severe whether intermittent blood flow was allowed during knee extensions or blood flow was occluded (10), suggesting that during these local muscle protocols, nonmetabolic factors may also play a role. Such factors may include failure of excitation-contraction coupling (39), inefficiency of chemical to mechanical energy transduction (40) or reflex inhibition of motor unit activation, alone or in combination.

**Exercise intolerance in congestive heart failure and muscle disease.** Since skeletal muscle abnormalities have been recognized in heart failure, a major issue has been whether they occur solely as a secondary consequence of the diminished activity accompanying this condition or whether they are a direct consequence of the syndrome and an additional factor in propagating exercise intolerance (41). Several findings in this study provide circumstantial evidence that a process in addition to muscle disuse is being expressed. First, reduced strength and muscle atrophy are the characteristic responses to inactivity (28), with disuse consistently evoking greater relative decreases in strength than in muscle size (20,25,27,28). In contrast, patients with mild to moderate heart failure exhibit significant atrophy but relatively well preserved strength (8,10, present study). Second, as discussed previously, patients experience rapid and extreme local muscular fatigue (10, present study). In normal subjects, forced inactivity that reduces mitochondrial content by 15% to 20%, the difference noted between patients and control subjects in this study, evokes only a slight decline (−6%) in the ability to withstand fatigue (27). Third, patients with heart failure showed a markedly lower ratio of fast twitch to slow twitch fiber size than did control subjects in this study, suggesting atrophy mainly of fast twitch fibers, as other investigators (6,13–16) have noted. In contrast, this ratio is not altered by muscle disuse in healthy subjects, who exhibit comparable atrophy of all fiber types (20,25,26). Although no definitive conclusions can be drawn from these observations, these findings suggest that as yet undefined factors are responsible for some of the changes in muscle function and protein expression in heart failure.

**Implications of this study.** The results of this study provide additional insight into previous demonstrations of abnormal skeletal muscle function, metabolism and composition in patients with congestive heart failure. They support the concept that reduced aerobic-oxidative capacity and possibly other factors contributing to extreme local muscular fatigue play a role in their exercise intolerance. Although it is not possible to conclude from the present results that these changes are specific for the syndrome of heart failure or to define the role of muscle disuse in their genesis, at least some of the abnormalities appear not to be characteristic of inactivity alone. Additional studies are required to differentiate the contributions of heart failure from those of chronic illness and inactivity in the pathophysiology of skeletal muscle dysfunction in this syndrome.

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