Decreased Proportion of Type I Myofibers in Skeletal Muscle of Dogs With Chronic Heart Failure

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Background. Whether biochemical and histological abnormalities of skeletal muscle (SM) develop in patients with chronic heart failure (HF) remains controversial. In the present study, dogs with chronic HF were used to examine potential alterations of SM fiber type, fiber size, number of capillaries per fiber (C/F), β-adrenergic receptor density (Bₛₛ₅), and fiber ultrastructural integrity.

Methods and Results. HF was produced in 17 dogs by sequential intracoronary microembolizations. Biopsies of the lateral head of the triceps muscle were used in all studies. Type I and type II fibers were differentiated by myofibrillar ATPase (pH 9.4 or 4.2). Bₛₛ₅ was assessed by radioligand binding and SM ultrastructure by transmission electron microscopy. Comparisons were made with biopsies obtained from nine control dogs. The percentage of SM type I fibers was reduced in HF dogs compared with control dogs (19±2% versus 32±5% (p<0.001), whereas the percentage of SM type II fibers was increased (81±2% versus 68±5%) (p<0.001). The change in fiber type composition was not associated with a preferential atrophy or hypertrophy of either fiber type. There was no difference in SM Bₛₛ₅ (198.9±14.3 versus 186.8±17.3 fmol/mg protein) or in C/F (5.37±0.26 versus 5.84±0.21) between HF dogs and control dogs. No ultrastructural abnormalities were present in SM fibers of HF dogs.

Conclusions. In dogs with HF, there is a decrease in the relative composition of the slow-twitch type I SM fibers and an increase in fast-twitch type II fibers. The shift in fiber type composition is not associated with preferential atrophy of either fiber type or with a reduction in C/F, β-adrenergic receptor density, or structural abnormalities of the myofibers. (Circulation 1993;87:1729–1737)

Key Words • heart failure, congestive • muscle, skeletal • ventricular function • receptors, β-adrenergic • microscopy

Exertional fatigue in patients with chronic heart failure has traditionally been attributed to skeletal muscle underperfusion caused by reduced cardiac output, redistribution of blood flow away from the skeletal muscle, and impaired capacity for arteriolar vasodilation.1–4 Recent studies, however, have demonstrated that the reduction of exercise tolerance in this patient population may be associated with skeletal muscle metabolic abnormalities manifested by early reliance on anaerobic metabolism.5–7 Forearm exercise in patients with heart failure was associated with increased phosphocreatine depletion, greater decrease in pH, and increased fatigability compared with normal subjects.5,6 These changes occurred despite normal forearm blood flow, suggesting that skeletal muscle underperfusion was not the mediator of these abnormalities.5–7 These findings provoked speculation that intrinsic abnormalities of skeletal muscle develop in patients with chronic heart failure that impact substrate use and, subsequently, exercise tolerance.7

Several biochemical and histological abnormalities of skeletal muscle have been reported in patients with chronic heart failure. These include downregulation of β-adrenergic receptors,8 endomysial fibrosis, increased lipid deposition and mitochondrial degeneration,9–11 decreased capillary density,7 reduced proportion of type I myofibers,7 and development of fiber atrophy.11

There is no general agreement, however, that these abnormalities are indeed characteristic features of the skeletal muscle in chronic heart failure. A number of studies failed to show downregulation of β-adrenergic receptors in skeletal muscle of patients with heart failure12 or any changes in skeletal muscle fiber capillary density.11 Studies in dogs with heart failure produced by rapid ventricular pacing failed to show any changes in skeletal muscle fiber type composition.13 These conflicting findings may be caused by factors related to the pathogenesis of heart failure, severity of hemodynamic compromise, extent of physical activity or lack thereof, nature of ongoing drug therapy in patients, and possibly to the specific skeletal muscle used for analysis. To
minimize these confounding effects and at the same
time address the question of intrinsic skeletal muscle
abnormalities in chronic heart failure, we examined
skeletal muscle characteristics in a well-described and
standardized canine model of chronic heart failure
produced by multiple sequential intracoronary micro-
embolizations. Triceps muscle specimens were used to
evaluate the potential existence of alterations in 1) 
\( \beta \)-adrenergic receptor density, 2) proportion of type I
and type II myofibers, 3) fiber size, 4) number of
capillaries per fiber (C/F), and 5) myofibrillar and
mitochondrial ultrastructural integrity.

**Methods**

The canine model of chronic heart failure used in the
present study has been previously described and hemo-
dynamically characterized in detail.\(^{14}\) In this prepara-
tion, chronic heart failure is produced by multiple
sequential intracoronary embolizations with polysty-
rene latex microspheres (70–102 \( \mu \)m in diameter) that
lead to loss of viable myocardium.\(^{14}\) The model mani-
fests many of the sequelae of heart failure observed
in patients, including marked and sustained depression
of left ventricular (LV) systolic and diastolic function, LV
hypertrophy and dilation, reduced cardiac output, in-
creased systemic vascular resistance, and enhanced sym-
pathoadrenergic activity evidenced by marked elevation
of plasma norepinephrine concentration.\(^{14}\)

Twenty-six healthy adult mongrel dogs of either sex
(weight, 20–35 kg) were used in the study. Seventeen
underwent a series of cardiac catheterizations and
coronary microembolizations to produce heart failure,
and nine served as normal control dogs. Control dogs
did not undergo cardiac catheterizations. The protocol
was approved by the Henry Ford Hospital Care of
Experimental Animals Committee. All cardiac catheteri-
izations were performed with the chest closed and
under general anesthesia and sterile conditions. Dogs
were anesthetized with an injection of Innovar-Vet (0.1
mg/kg i.v.) (droperidol 2.0 mg/kg and fentanyl citrate
0.04 mg/kg) followed by an injection of sodium pento-
barbital (7.5 mg/kg i.v.). In heart failure dogs, each
animal underwent three to nine embolizations 1–3
weeks apart. Embolizations were discontinued when LV
ejection fraction, determined angiographically, was
\( \pm 35\% \). Heart failure dogs were killed at 3–4 months
after the final coronary embolization.

**Hemodynamic and Angiographic Evaluation**

Hemodynamic and angiographic measurements were
made in heart failure dogs at baseline, before any
microembolizations, and during heart failure (3–4
months after the last embolization). Aortic and LV
pressures were measured with catheter-tip microman-
ometers (Millar Instruments). Peak LV rate of change
of pressure during isovolumic contraction (peak \(+dP/
dt\)) was measured from analog differentiation of the LV
pressure waveform. Mean pulmonary artery wedge
pressure and mean right atrial pressure were measured
with a fluid-filled Swan-Ganz catheter connected to a
P23XI pressure transducer (Spectramed Inc.). Cardiac
output was measured in triplicate by the thermodilution
method. LV stroke work, a measure of LV systolic
function, was calculated as described by Grossman.\(^{15}\)
Systemic vascular resistance was calculated as previ-
ously described.\(^{14}\) Left ventriculograms were performed
with the dog placed on its right side. Ventriculograms
were recorded on 35-mm cine film at 30 frames per
second during the injection of 20 mL of contrast mate-
rial (RENO-M-60, Squibb Diagnostics). Correction for
image magnification was made with a calibrated grid
placed at the level of the LV. Ventricular volumes were
calculated by the area–length method,\(^{16}\) and LV eje-
tion fraction was calculated as previously described.\(^{14}\)
 Plasma norepinephrine concentration in peripheral ve-
nous blood was measured as previously described.\(^{14}\)

**Exercise Tolerance**

Before initiation of coronary microembolization, dogs
in which heart failure was to be induced were trained to
exercise on a treadmill (model Q-65, Quinton Instru-
ments). Eleven of the 17 dogs with heart failure were
successfully trained. A modified Bruce protocol\(^{17}\) was
used to evaluate exercise tolerance at baseline, before
any embolization, and during heart failure. The exercise
protocol was limited on the upper end to stage 5 at a
12\% grade to prevent the animal from slipping on the
treadmill. Once stage 5 was reached, each dog was
allowed to exercise at this stage until it refused to
exercise further. This was always associated with phys-
ical signs of fatigue evidenced by rapid panting, saliva-
ion, and refusal to perform further physical activity.
Maximal exercise duration was based on the average of
the results of two tests performed on consecutive days.
Between the baseline and final exercise tests, dogs were
exercised on the treadmill biweekly for short durations
so as to maintain this ability. All dogs were housed
individually in large pens (36 sq ft) and were allowed
access to exercise runs (200 sq ft) for 1 hour each day.

**Skeletal Muscle Biopsy**

Skeletal muscle specimens were obtained from the
left lateral head of the triceps muscle by use of an open
biopsy procedure. All biopsies were performed under
general anesthesia. In heart failure dogs, tissue speci-
mens were obtained on the final day of the study just
before hemodynamic and angiographic evaluation. In
control dogs, tissue specimens were obtained before
these animals were used for unrelated investigations
that were ongoing in the laboratory. None of these
investigations involved pharmacological interventions.
In all instances, enough skeletal muscle tissue was
obtained for routine histological and histochemical
analysis, ultrastructural evaluation, and determination
of \( \beta \)-adrenergic receptor density. In seven of the 16
heart failure dogs, serial triceps biopsies were obtained
at three time intervals during the course of developing
heart failure, namely, at baseline and at an average of 1
month and 3–4 months after the last embolization. The
baseline and 1-month biopsies were limited to a single
tissue specimen (approximately 100 mg). Tissue from
serial biopsies was used for evaluation of fiber type and
size. In these seven dogs, hemodynamic and angi-
ographic studies were performed at all three time inter-
vals. Exercise testing was performed on five of these
seven dogs.

**Determination of Skeletal Muscle \( \beta \)-Adrenergic
Receptor Density**

Skeletal muscle \( \beta \)-adrenergic receptor density was
assessed in six heart failure dogs and five control dogs.
In all instances, tissue samples (0.5 g) were rapidly removed during the open biopsy procedure, cut into 5-mm³ blocks, flash frozen in liquid nitrogen, and stored at −80°C. Membrane fractions were prepared as previously described.18 Membrane fractions were incubated in a total volume of 5 mL of Tris buffer at 25°C with various concentrations of [³H]dihydroalprenolol for 30 minutes. These conditions permitted the complete equilibration of the radioligand with its receptor. After incubation, samples were filtered under vacuum through Whatman GF/C glass fiber filters, and the filters were washed twice with 5 mL ice-cold Tris buffer using a model M-24R cell harvester (Brandel Instruments). Nonspecific binding was determined by the addition of 1×10⁻⁶ mol/L DL-propranolol to a duplicate set of tubes. Filters were counted by liquid scintillation spectrometry at an efficiency, determined by external standards, of 40–45%. Radioligand binding from saturation isotherms was analyzed by use of the iterative, nonlinear least-squares computer program LIGAND.19 At saturating radioligand concentrations, specific [³H]dihydroalprenolol binding was 68.2±1.3%. The maximum binding (B_max) and dissociation constant (K_d) were determined by Scatchard analysis.20 Protein concentration was determined by the method of Bradford.21

Assessment of Skeletal Muscle Fiber Size and Type

Once removed, skeletal muscle tissue specimens were mounted on platforms, trimmed, and gently manipulated to align the fibers. The block was then covered with a protective embedding medium (Tissue-Tek, O.C.T. Compound, Miles Inc., Elkhart, Ind.), quickly frozen in isopentane cooled to −160°C in liquid nitrogen, and stored at −80°C.22 After cryostat sectioning, skeletal muscle type I and type II myofibers were differentiated by myofibrillar adenosine triphosphatase (ATPase) staining resulting from preincubation at a pH of either 9.4 or 4.2.22 Four fields containing an average of 100 fibers each (total, 404±20 fibers per specimen) were photographed. Photographs were used to count the total number of type I and type II fibers. The proportions of type I and type II fibers for each specimen were calculated as a percent of the total number of fibers in any given section. The ratio of type I to type II fibers was also calculated. The same photographs were used to measure the mean cross-sectional area of type I and type II fibers by computerized planimetry. The cross-sectional area ratio of type I to type II fibers was calculated for each specimen.

Evaluation of Skeletal Muscle Ultrastructural Integrity

The ultrastructural integrity of skeletal muscle fibers was evaluated by transmission electron microscopy (TEM) in five heart failure dogs and six control dogs. TEM specimens were immediately immersed in 0.1 mol/L ice-cold cacodylate-buffered (pH 7.2) 3% glutaraldehyde and cut into 1-mm³ blocks. Tissue blocks were fixed overnight at 4°C and then postfixed for 1 hour in 0.1 mol/L cacodylate-buffered osmium (pH 7.2). After fixation, the specimens were dehydrated in progressive concentrations of ethanol, infiltrated with propylene oxide, oriented longitudinally, and embedded in araldite. After polymerization, thick (1-µm) sections were stained with toluidine blue. Longitudinally oriented sections were photographed and analyzed by use of a computer-based image analysis system.23

Assay of Norepinephrine

Norepinephrine was determined in plasma and calcium arteries using a modification of the fluorometric assay described by Cathala et al.24 in which the tissue was homogenized in a 0.001 N HCl solution containing 1% nonylphenolphosphate, 0.1% 2-mercaptoethanol, and 0.2% E-DTA and extracted with 1:1 chloroform:ethylacetate.25 After extraction, the calcium artery was incubated with 0.002 mol/L sodium nitroprusside, 0.0005 mol/L ascorbic acid, 0.002 mol/L L-arginine, and 0.001 mol/L EF. Nominal [³H]dihydroalprenolol concentrations (2 and 4 nM) were used to determine the steady-state concentrations of [³H]dihydroalprenolol bound to the calcium artery. The effect of calcium ions on the radioligand binding was determined by use of a solution containing 0.003 mol/L EF, 0.0005 mol/L ascorbic acid, 0.002 mol/L L-arginine, 0.001 mol/L EF, and 2 and 4 nM [³H]dihydroalprenolol. The calcium ions were synthesized according to the procedure of Perlman and Scudder.26

TABLE 1. Hemodynamic and Angiographic Findings in 17 Dogs With Chronic Heart Failure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Heart failure</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>58±1</td>
<td>22±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>63±3</td>
<td>96±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>6±1</td>
<td>21±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV +dP/dt (mm Hg/sec)</td>
<td>2,310±90</td>
<td>1,530±60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>3.3±0.2</td>
<td>2.3±0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>6±1</td>
<td>14±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVSW (g · m⁻¹)</td>
<td>51±3</td>
<td>29±3</td>
<td>0.001</td>
</tr>
<tr>
<td>SVR (dyne · sec · cm⁻⁵)</td>
<td>2,266±96</td>
<td>2,837±91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PNE (pmol/L)</td>
<td>337±12</td>
<td>803±141</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

LV, left ventricular; EF, ejection fraction; EDV, end-diastolic volume; EDP, end-diastolic pressure; +dP/dt, peak rate of change of pressure during isovolumic contraction; CO, cardiac output; PAWP, pulmonary artery wedge pressure; LVSW, LV stroke work; SVR, systemic vascular resistance; PNE, plasma norepinephrine concentration.

*p Probability relative to baseline.

TABLE 2. Skeletal Muscle Fiber Type, Size, Capillary Density, and β-Adrenergic Receptor Density in Heart Failure Dogs and Control Dogs

<table>
<thead>
<tr>
<th>Fiber type</th>
<th>Control</th>
<th>Heart failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (%)</td>
<td>32±5</td>
<td>19±2</td>
</tr>
<tr>
<td>Type II (%)</td>
<td>68±5</td>
<td>81±2</td>
</tr>
<tr>
<td>Ratio type I/type II</td>
<td>0.47±0.04</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>Average fiber size</td>
<td>n=9</td>
<td>n=17</td>
</tr>
<tr>
<td>Type I fiber (µm²)</td>
<td>2,996±176</td>
<td>2,707±291</td>
</tr>
<tr>
<td>Type II fiber (µm²)</td>
<td>3,445±240</td>
<td>2,965±149</td>
</tr>
<tr>
<td>Ratio type I/type II</td>
<td>0.88±0.03</td>
<td>0.94±0.12</td>
</tr>
<tr>
<td>Capillary density</td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>Capillaries per fiber</td>
<td>5.84±0.21</td>
<td>5.37±0.26</td>
</tr>
<tr>
<td>β-Adrenergic receptors</td>
<td>n=5</td>
<td>n=6</td>
</tr>
<tr>
<td>B_max (fmol/mg protein)</td>
<td>186.8±17.3</td>
<td>198.9±14.3</td>
</tr>
<tr>
<td>K_d (pmol/L)</td>
<td>379.2±60.4</td>
<td>470.6±71.7</td>
</tr>
</tbody>
</table>

B_max, density; K_d, equilibrium dissociation constant.
FIGURE 2. Panel A: Electron micrograph of type I myofiber from the triceps muscle of a dog with chronic heart failure showing normal myofibrillar content, Z-bands, and normal, matrix-dense, interfibrillar mitochondria with tightly packed cristae. Original magnification, ×19,000. Panel B: Electron micrograph of type II myofiber from the triceps muscle of a dog with chronic heart failure showing normal myofibrillar content, Z-bands, and interfibrillar glycogen with only occasional mitochondria. Original magnification, ×19,000.
sections were selected for ultrathin sectioning. Ultrathin sections were mounted on grids, doubly stained with uranyl acetate as well as lead citrate, and studied in a Philips 201 electron microscope. Multiple micrographs of randomly selected regions were obtained at magnifications of ×3,000 and ×7,000. Myocytes were examined qualitatively for any evidence of degeneration, including myofibrillar loss, disruption of Z-band material, intermyofibrillar lipid content and mitochondrial swelling, matrix clarification, and loss of cristae.

**Assessment of C/F**

The C/F in skeletal muscle was used as a measure of potential microradial alterations. Skeletal muscle C/F was assessed in five heart failure dogs and five control dogs. Capillaries were identified by the *Griffonia simplicifolia* I lectin staining fluorescent tracer technique. The number of capillaries surrounding individual skeletal muscle fibers was determined by the following random sampling method: The number of capillaries surrounding each of four muscle fibers per field was determined. The muscle fibers selected were those within or closest to the four equidistant points of a cross-hair reticle used for photography. Capillaries surrounding the randomly sampled muscle fibers in at least 15 fields were counted.

**Data Analysis**

A flow chart depicting the number of dogs used in the various components of the investigation is shown in Figure 1. Comparisons between control dogs and heart failure dogs were based on a *t* statistic for two means. Among heart failure dogs, paired comparisons of hemodynamic and angiographic parameters between baseline and heart failure were based on Student's *t* test. In the seven heart failure dogs evaluated at three time intervals during the course of developing heart failure, temporal changes of LV function indexes, fiber type, and fiber size were examined by repeated-measures ANOVA with the level of significance set at *α*=0.05. If significance was attained by ANOVA, paired comparisons between baseline and subsequent time points were performed by the Student-Newman-Keuls test. For all these tests, a probability of less than 0.05 was considered significant. All data are reported as the mean±SEM.

**Results**

The hemodynamic and angiographic measures of LV function and plasma norepinephrine concentration obtained at baseline and during heart failure in all 17 dogs are shown in Table 1. Heart failure was associated with a marked reduction of LV ejection fraction, LV +dP/dt, LV stroke work, and cardiac output and a significant elevation of LV end-diastolic volume, LV end-diastolic pressure, and plasma norepinephrine concentration. In 10 dogs in which exercise testing was performed at baseline and during heart failure, the duration of exercise decreased significantly from 39.7±4.5 minutes at baseline to 21.4±3.3 minutes during heart failure (*p*<0.001). There were no differences in any of the LV function parameters between heart failure dogs in which exercise tolerance was determined and those in which it was not.

**β-Adrenergic Receptor Density**

There was no difference in the density (Bmax) of β-adrenergic receptors in heart failure dogs (198.9±14.3 fmol/mg of protein) compared with normal control dogs (186.8±17.3 fmol/mg of protein). Similarly, there was no difference between heart failure dogs and control dogs with respect to the equilibrium dissociation constant (Kd) for [3H]dihydralprenolol (470.6±71.7 versus 379.2±60.4 pmol/L (Table 2). There was no difference in any of the LV function parameters among dogs in which β-adrenergic receptor density was evaluated compared with those in which it was not.

**Number of Capillaries per Fiber**

In heart failure dogs, triceps muscle C/F (5.37±0.26) was not significantly different from control dogs (5.84±0.21) (Table 2). There was no difference in any of the LV function parameters among dogs with heart failure in which C/F was evaluated compared with those in which it was not.

**Triceps Muscle Ultrastructural Integrity**

Qualitative examination of the ultrastructure of mitochondria-rich type I myofibers and glycogen-rich type II fibers in dogs with heart failure showed no abnormalities compared with control dogs. There was no evidence of myofibrillar or Z-band disruption and no evidence of excessive lipid content. Mitochondria were oval and contained electron-dense matrix and orderly tightly packed cristae. None of the mitochondria examined showed evidence of swelling, matrix clarification, or cristae disruption. Typical electron micrographs in a dog with heart failure are shown in Figures 2A and 2B.

**Triceps Muscle Fiber Size**

None of the skeletal muscle sections examined showed any evidence of focal regions of fibrosis. The cross-sectional areas of type I and type II fibers tended to be smaller in dogs with heart failure compared with control dogs (Table 2). This reduction in fiber size, however, was not statistically significant. The ratio of type I to type II average cross-sectional area was similar in heart failure dogs (0.94±0.12) compared with control dogs (0.88±0.03), indicating no preferential atrophy of either fiber type.

**Triceps Muscle Fiber Type Distribution**

The percentage of type I fibers was significantly lower in triceps muscle of heart failure dogs (19±2%) compared with control dogs (32±5%) (*p*<0.001), whereas the percentage of type II fibers was significantly increased in heart failure dogs (81±2%) compared with control dogs (68±5%) (*p*<0.001). The ratio of the number of type I to type II fibers was significantly lower in heart failure dogs than in control dogs (Table 2). Figures 3A and 3B are light micrographs depicting the typical distribution of type I and type II fibers in a control dog and a dog with chronic heart failure.

**Fiber Type Composition During the Evolution of Heart Failure**

The hemodynamic, angiographic, and exercise tolerance findings observed in seven dogs in which serial skeletal muscle biopsies were obtained during the course
FIGURE 3. Panel A: Micrograph of myofibrillar ATPase stain (pH 9.4) from the triceps muscle of a normal control dog. Light fibers are type I, and dark fibers are type II. Panel B: Micrograph of myofibrillar ATPase stain (pH 9.4) from the triceps muscle of a dog with chronic heart failure. Light fibers are type I, and dark fibers are type II. Note the decreased proportion of type I fibers relative to the control dog illustrated in panel A.
of evolving heart failure are shown in Table 3. There was a significant change in indexes of LV function between 1 month and 3–4 months after the last embolization. At 3–4 months, LV ejection fraction, peak +dP/dt, LV stroke work, and cardiac output decreased significantly compared with measures obtained at 1 month (p < 0.05), whereas LV end-diastolic volume and pulmonary artery wedge pressure increased (p < 0.05). The progressive decline in LV function was accompanied by a progressive decrease in the proportion of type I fibers and a progressive increase in the proportion of type II fibers (Table 3). The ratio of the number of type I to type II fibers was 0.51 ± 0.10 at baseline. This ratio decreased to 0.30 ± 0.07 at an average of 1 month after the last embolization and decreased further to 0.21 ± 0.02 at 3–4 months after the last embolization (p < 0.05). The cross-sectional areas of both type I and type II fibers decreased gradually during the course of developing failure (Table 3). More importantly, however, the ratio of type I to type II fiber cross-sectional area was not significantly different between baseline, 1 month, and 3–4 months when examined by repeated-measures ANOVA (Table 3).

Discussion

The present study demonstrates a decrease in the percentage of type I skeletal muscle myofibers and an increase in the percentage of type II myofibers in dogs with chronic heart failure resulting from loss of viable myocardium. Furthermore, these changes in skeletal muscle myofiber composition developed progressively during the course of evolving heart failure. The observed changes of skeletal muscle fiber types are not associated with preferential atrophy of either muscle fiber type and are not accompanied by changes in C/F, β-adrenergic receptor density, or any identifiable myofiber ultrastructural abnormality.

The observed reduction in the percentage of slow-twitch type I fibers and the increase in the percentage of fast-twitch type II fibers in dogs with chronic heart failure are consistent with observations made in patients. Using biopsy specimens from the vastus lateralis muscle, Sullivan and associates showed a 31% reduction in the proportion of type I fibers and a 118% increase in type IIb fibers in patients with chronic heart failure compared with normal subjects. Mancini et al. used biopsies of the gastrocnemius muscle and showed a 13% reduction in the proportion of type I fibers and a 46% increase in the proportion of type IIb fibers in patients with chronic heart failure compared with normal subjects. In contrast with the present observations and those in patients, studies by Wilson and associates in skeletal muscle of dogs with experimental heart failure produced by rapid ventricular pacing failed to show any differences in the composition of skeletal muscle fiber types compared with control animals. This was true for the triceps, gracilis, and gastrocnemius muscles. In the present study, the gracilis and gastrocnemius muscles were not studied so as to avoid potential artifactual findings that may arise as a result of multiple catheterizations of the femoral arteries and veins.

We found no differences in the C/F, β-adrenergic receptor density, or fiber ultrastructural integrity in skeletal muscle of dogs with heart failure compared with control animals. There was a tendency toward smaller type I and type II fibers in dogs with heart failure compared with control dogs and a significant reduction in fiber cross-sectional area of both type I and type II myofibers in dogs in which serial measurements were made. The ratio of type I to type II fiber cross-sectional area, however, was not different in any of the groups, indicating no preferential atrophy of either of the two fiber types. There is no general agreement with respect to skeletal muscle atrophy in patients with heart failure. Lipkin et al. reported atrophy of both type I and type II fibers of the quadriceps muscle of patients with heart failure. Caforio et al. reported selective atrophy of type I fibers of both the right biceps brachii and the right deltid muscle, whereas Mancini et al. reported preferential selective atrophy of type II fibers of the gastrocnemius muscle. In contrast, Martin et al. reported a nearly twofold increase in the cross-sectional area of both type I and type II fibers of the gastrocnemius muscle. The reasons for these wide-ranging differences of skeletal muscle fiber size are not clear. It is possible that variations are caused by differences in the pathogenesis of heart failure, the severity of the disease at the time of biopsy, and the extent of physical activity or the lack thereof. One possible explanation for moderate overall muscle fiber atrophy in the present study may be related to the extent of physical activity. Although every attempt was made to provide the dogs with sufficient space to allow for physical activity (gardening and exercise runs), it is possible that dogs may have become less active as heart failure ensued. It is interesting to note, however, that dogs that were trained to exercise...
on a treadmill manifested the same degree of fiber atrophy as dogs that were not successfully trained. Other potential contributors to overall skeletal muscle atrophy include reduced caloric protein intake and chronic underperfusion of the skeletal muscle. In the present study, all dogs continued to eat throughout the entire observation period.

Skeletal muscle sympathetic neural activation is reportedly increased in patients with chronic heart failure and is thought to have an impact on muscle metabolism and perfusion. Two studies that examined the density of β-adrenergic receptors in skeletal muscle of patients with heart failure reported conflicting results. Drexler et al characterized β-adrenergic receptors by [125I]iodo- 

pindolol radioligand binding in needle biopsies of the vastus lateralis muscle of patients with heart failure. In their study, skeletal muscle β-adrenergic receptor density was increased by nearly 67% in patients with heart failure compared with normal control subjects. In contrast, Martin et al, using autoradiographic techniques in gastrocnemius muscle biopsies, demonstrated a 33% reduction of β-adrenergic receptor density in patients with heart failure compared with control subjects. In the present dog study, the density of β-adrenergic receptors was unchanged in heart failure dogs compared with control dogs. It is noteworthy that the unaltered nature of β-adrenergic receptor density in skeletal muscle is in contrast to the marked downregulation of β-adrenergic receptors in cardiac tissue of this canine model of heart failure. In heart failure, there is a selective downregulation of cardiac β1-adrenergic receptor subtype with little or no change in the β2-receptor population. Recent studies suggest that skeletal muscle contains primarily the β2-adrenergic receptor subtype.

Histological studies of skeletal muscle in patients with idiopathic cardiomyopathy demonstrated increased lipid deposits and fibrosis and often myofibrillar loss, Z-band streaming, and swollen mitochondria, suggesting that a generalized myopathy may be responsible for both cardiac and skeletal muscle abnormalities. Lipkin et al reported increased lipid content in skeletal muscle of patients with long-standing heart failure. Alterations in skeletal muscle mitochondrial morphology, characterized by decreased surface density of cristae, were also described in some patients with chronic heart failure. In patients with dilated or hypertrophic cardiomyopathy, Caforio et al reported extensive mitochondrial abnormalities in type I skeletal muscle myofibers characterized by organellae swelling and matrix clarification attributed to the existence of subclinical myogenic myopathy. In the present study, we found no identifiable fibrosis or ultrastructural abnormalities of skeletal muscle fibers or mitochondria, nor did we observe an increase in lipid deposits compared with normal dogs.

Exertional fatigue in patients with chronic heart failure is associated with early anaerobic metabolism in skeletal muscle. Recent studies by Sullivan et al suggest that reduced aerobic activity in skeletal muscle plays an important role in mediating the early onset of anaerobic metabolism during exercise. It is possible, although by no means established in this study, that the reduction of type I fiber composition and the increase in type II fiber composition is responsible for the reduced aerobic enzyme activity seen in patients with heart failure and the early onset of anaerobic metabolism during exercise. Type I fibers are rich in mitochondria and lipids and can sustain continuous activity, whereas type II fibers are poor in mitochondria content and are unable to sustain prolonged activity. Thus, a downward shift in the ratio of skeletal muscle type I to type II fibers is likely to promote early fatigue during exercise.

The mechanism or mechanisms responsible for the shift in skeletal muscle fiber type composition are not known. In the present study, the observed shift in fiber type composition occurred without changes in C/F, β-adrenergic receptor density, or fiber ultrastructural abnormalities. It is possible that preferential atrophy of a skeletal muscle fiber type can be an impetus for a shift in fiber type. In the present study, we found no selective atrophy of either fiber type, yet a distinct reduction in the percentage of type I fibers was present. A recent study in dogs with heart failure caused by chronic rapid ventricular pacing showed marked atrophy of type I and type II skeletal muscle fibers without any shift in fiber type composition relative to control animals. These observations do not support the notion that fiber atrophy per se has a direct impact on the redistribution of fiber type composition in heart failure. It is also possible that certain adaptations in skeletal muscle innervation may take place in heart failure and can have a role in the determination of fiber type. The classic studies of Buller et al demonstrated that switching a nerve from a slow-twitch type I muscle to innervate a fast-twitch muscle results in transformation of the muscle to a slow phenotype. The converse experiment also transformed a slow muscle to one with a fast phenotype. Subsequent studies have shown that this transformation can be explained, in part, by the differing firing patterns of fast and slow nerves.

Certain limitations of the present study are worth noting. No attempt was made to evaluate the metabolic abnormalities of skeletal muscle at rest or during exercise. Such data would have been useful for correlation with the reduction in the percentage of type I myofibers. Quantification of the density of both β1- and β2-adrenergic receptor subpopulations would have been useful in determining the primary receptor subtype in the triceps muscle of dogs and in determining whether both receptor subtypes remained unchanged in the skeletal muscle of dogs with heart failure compared with control animals. A distinction between the C/F for type I and type II fibers would have also been useful. We also did not distinguish the subtypes of type II myofibers, namely, IIA and IIB. Such a delineation would have been particularly useful in distinguishing which of these fiber subtypes increased in dogs with heart failure. Type IIA fibers have overall cytochemical characteristics intermediate between type I and type IIB and possess both oxidative enzymes and abundant glycogen, whereas type IIB fibers are primarily glycolytic. If the observed increase of type II fibers in the present study was primarily because of an increase in the IIB subtype, such an observation would have provided further support for a role of fiber type composition in the early onset of skeletal muscle anaerobic metabolism reported during exercise in patients with chronic heart failure. On the other hand, if the increase in type II fibers was primarily because of an increase in the IIA subtype, this explana-
tion may be tempered somewhat. Further studies are needed to elucidate the importance of these factors and others so as to ultimately identify the mechanisms responsible for exercise intolerance in heart failure.

In conclusion, the results of this study indicate that in skeletal muscle of dogs with chronic heart failure produced by multiple sequential intracoronary embolizations, there is a decrease in the relative composition of the highly oxidative, slow-twitch type I fibers and an increase in fast-twitch type II fibers. This shift in fiber type composition occurs progressively during the course of developing failure and does not appear to be associated with preferential atrophy of either of the two skeletal muscle fiber types or with a reduction of C/F, β-adrenergic receptor density, or fiber ultrastructural abnormalities.

References

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