Effect of heart failure on the regulation of skeletal muscle protein synthesis, breakdown, and apoptosis

Rebecca Persinger, Yvonne Janssen-Heininger, Simon S. Wing, Dwight E. Matthews, Martin M. LeWinter, and Michael J. Toth

Departments of Medicine, Chemistry, and Pathology, University of Vermont, Burlington, Vermont 05405; and Department of Medicine, McGill University, Montreal, Quebec, Canada H3A 2B2

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PERSINGER, Rebecca, Yvonne JANSSEN-HEININGER, Simon S. WING, Dwight E. MATTHEWS, Martin M. LEWINTER, and Michael J. TOTH. Effect of heart failure on the regulation of skeletal muscle protein synthesis, breakdown, and apoptosis. Am J Physiol Endocrinol Metab 284:E1001–E1008, 2003.—Heart failure is often characterized by skeletal muscle atrophy. The mechanisms underlying muscle wasting, however, are not fully understood. We studied 30 Dahl salt-sensitive rats (10 male, 20 female) fed either a high-salt (HS; n = 15) or a low-salt (LS; n = 15) diet. This strain develops cardiac hypertrophy and failure when fed a HS diet. LS controls were matched to HS rats for gender and duration of diet. Body mass, food intake, and muscle mass and composition were measured. Skeletal muscle protein synthesis was measured by isotope dilution. An additional group of 27 rats (HS, n = 16; LS; n = 11) were assessed for expression of genes regulating protein breakdown and apoptosis. Gastrocnemius and plantaris muscles weighed less (16 and 22%, respectively) in HS than in LS rats (P < 0.01). No differences in soleus or tibialis anterior weights were found. Differences in muscle mass were abolished after data were expressed relative to body size, because HS rats tended (P = 0.094) to weigh less. Lower body mass in HS rats was related to a 16% reduction (P < 0.01) in food intake. No differences in muscle protein or DNA content, the protein-to-DNA ratio, or muscle protein synthesis were found. Finally, no differences in skeletal muscle gene expression were found to suggest increased protein breakdown or apoptosis in HS rats. Our results suggest that muscle wasting in this model of heart failure is not associated with alterations in skeletal muscle metabolism. Instead, muscle atrophy was related to reduced body weight secondary to decreased food intake. These findings argue against the notion that heart failure is characterized by a skeletal muscle myopathy that predisposes to atrophy.

A number of hypotheses have been put forth to explain muscle atrophy in heart failure (4, 37, 45). Each of the mechanisms forwarded in these hypotheses must ultimately affect the regulation of skeletal muscle protein or cell mass. Muscle atrophy can occur during net negative protein balance, in which protein breakdown exceeds synthesis, during net negative muscle cell balance, in which the loss of muscle cells exceeds replacement, or during a combination of these two events. Studies in both humans and animal models suggest that heart failure is associated with alterations in the regulation of skeletal muscle protein synthesis (40), protein breakdown (36), and apoptosis (47) that may predispose to skeletal muscle atrophy. The relative importance of changes in these regulatory systems to skeletal muscle atrophy, however, has not been rigorously examined.

The objective of the present study was to examine the effect of heart failure on those physiological systems that regulate skeletal muscle mass to further define the mechanisms underlying atrophy. To accomplish this objective, we studied the Dahl salt-sensitive rat, a well-characterized strain that, when fed a high-salt (HS) diet, develops left ventricular hypertrophy and failure secondary to combined pressure and volume overload. In this model, rats develop compensated hypertrophy after 6 wk of HS diet and progress to failure between 8 and 12 wk (26). Typically, rats remain in the failing state for several weeks before death (25). We hypothesized that heart failure would be characterized by changes in the regulation of skeletal muscle protein synthesis, protein breakdown, and apoptosis that would predispose to muscle atrophy. As a corollary, we explored whether changes in muscle protein metabolism would be accompanied by increased muscle cytokine expression.

MATERIALS AND METHODS

Animals. Male and female Dahl salt-sensitive rats were obtained (Taconic, Germantown, NY) at 6 wk of age and were housed singly in wire-bottom cages. All rats were maintained on a 12:12-h light-dark cycle in a temperature-controlled room. Tap water and a low-salt (0.6%) chow were available ad

IN BOTH HUMAN AND ANIMAL MODELS, heart failure is frequently characterized by skeletal muscle atrophy (14, 22, 33, 40, 43). Loss of skeletal muscle contributes to exercise intolerance (20, 43), a cardinal symptom of heart failure, and increases morbidity (1, 39) and mortality (5). Knowledge of the mechanisms underlying muscle atrophy in heart failure, however, is limited.

Address for reprint requests and other correspondence: M. J. Toth, Health Science Research Facility 126B, Univ. of Vermont, Burlington, VT 05405 (E-mail: mtoth@zoo.uvm.edu).

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libitum before initiation of the study. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Protocol. At 7 wk of age (time 0), rats were divided into populations that received either HS (8% NaCl) or low-salt (LS; 0.6% NaCl) diet (percentages of energy provided: 20% protein, 6% fat, 74% carbohydrate). Body mass was measured twice per week and food intake once per week throughout the experiment. Starting at the 6th wk of diet, echocardiography was performed once per week in HS rats to assess left ventricular (LV) function. Myocardial failure was considered to be present if LV fractional shortening was \( \leq 30\% \). Skeletal muscle studies were performed within 1 wk of detection of heart failure. LS rats were studied after a similar period on diet to match HS rats.

Skeletal muscle studies were performed within 1 wk of dieting with unlabeled phenylalanine (32 mg/100 g body wt; Sigma, St. Louis, MO) was injected via tail vein. Exactly 10 min later, the right hindlimb was stripped of skin, and skeletal muscles were dissected, weighed, and frozen in liquid nitrogen for protein synthesis measurements. The left hindlimb was stripped of skin, and skeletal muscles were dissected, weighed, and frozen in liquid nitrogen for protein and DNA analysis.

For RNA analysis, soleus and gastrocnemius muscles were collected from an additional group of HS (n = 16) and LS (n = 11) rats (all male). These rats underwent the same protocol as just described except that they were housed three per cage in plastic-bottom cages, neither body mass nor food intake was measured during the experimental diet, and protein synthesis measurements were not performed.

Body mass and food intake measurements. Body mass was measured twice weekly after initiation of the diet with a digital scale (Ohaus, Florham Park, NJ). Food intake was measured over a 24-h period once per week to monitor alterations in food intake, as described previously (46).

Echocardiography. Rats were briefly (\( \sim 5 \) min) anesthetized with isoflurane (1%) and placed in the supine position on top of a warming pad, and their precordium was shaved. Echocardiography was performed using a Sequoia system (B/W, in the two-dimensional mode, with a 15L8 linear array transducer (15.0 MHz; Acuson, Mountain View, CA). Briefly, in the two-dimensional mode, the left ventricle was imaged at the tip of the papillary muscle. This allowed positioning of the M-mode cursor perpendicular to the LV septum and posterior LV wall. M-mode images were recorded. LV dimensions [end-diastolic diameter (LVEDd) and end-systolic diameter (LVESd)] were measured using the leading edge-to-leading edge convention. Posterior wall thickness was measured at end diastole. Values from all measured beats were averaged. Fractional shortening (\( \% \)) was calculated as \( \frac{\text{LVEDd} - \text{LVESd}}{\text{LVEDd}} \times 100 \).

Protein and DNA content. Muscle protein and DNA content were measured in a sample of muscle tissue \((\sim 50 \) mg) homogenized in TNE buffer (10 mM Tris, 0.1 M NaCl, 1.0 mM EDTA). Protein concentration was determined using a commercial available kit (Bio-Rad, Hercules, CA) according to the method of Lowry (32), with bovine serum albumin as a standard. DNA concentration was measured using a fluorometric assay for the DNA-binding fluorochrome bisbenzimide H-33258 (Sigma), with calf thymus DNA (Sigma) as a standard (27). For both protein and DNA, total muscle content was derived from the product of the concentration and the weight of the muscle.

Skeletal muscle protein synthesis. Samples of muscle tissue \((\sim 50 \) mg) were homogenized in solubilization buffer (100 mM sodium pyrophosphate, 1% SDS, and 4 mM EGTA, pH 7.4). The resulting homogenate was centrifuged. The supernatant was decanted, treated with ice-cold 10% trichloroacetic acid (TCA), allowed to pre-warm overnight (\( \geq 14 \) h), and then centrifuged. The supernatant, which contained tissue-free amino acids, was collected and stored for later analysis. The precipitate was washed several times with 10% TCA, and the supernatant was added to the tissue-free amino acid fraction. The precipitate was washed with petroleum ether, the ether was evaporated under \( N_2 \), 6 M HCl was added, and the tube was capped and heated for 24 h at 110°C. An aliquot (0.5 ml) of the protein hydrolyzate was removed and dried under \( N_2 \). The sample was reacidified with 1 M acetic acid. Amino acids were isolated by ion exchange chromatography and derivatized to their N-(O,S)-ethoxycarbonyl ethyl ester derivative (24). Briefly, the dried amino acid sample was treated with 100 \( \mu \)l of ethylchloroformate (ECF) solvent (60:32:8, \( \mathrm{H}_2\mathrm{O}\)-ethanol-ethyl chloroform), and 10 \( \mu \)l of ECF and the sample were vortexed. Thereafter, 100 \( \mu \)l of a 10% ECF solution (90:10, chloroform-ECF) were added, and the sample was vortexed. The ECF-derivatized amino acids were removed from the organic phase, and ECF-phenylalanine isotopic enrichment (mole percent excess) was measured by gas chromatography-combustion-isotope ratio mass spectrometry. Tissue-free amino acids were derivatized to their N-heptafluorobutyl, \( n \)-propyl derivative and measured by gas chromatography-mass spectrometry, as previously described (34). We used tissue-free phenylalanine enrichment as a proxy of the enrichment of the precursor pool for protein synthesis.

The fractional synthesis rate \( (k_s) \) of mixed skeletal muscle protein was calculated as:

\[
 k_s = \frac{E^m \cdot 100}{t \cdot E^p}
\]

where \( E^m \) is the enrichment of phenylalanine in mixed muscle protein, 100 is a constant to convert \( k_s \) to a percentage, \( t \) is the incorporation time in days, and \( E^p \) is the enrichment of tissue-free phenylalanine. The \( k_s \) is expressed in units of percent per day. Absolute protein synthesis rates were calculated as muscle protein content (mg) multiplied by the fractional synthesis rate (%/day).

Expression of genes for the ubiquitin proteasome pathway. RNA was isolated from soleus muscles by use of the method of Chomczynski and Sacchi (11) with NaCl separation (17). Northern blots were performed by electrophoresis of 10 \( \mu \)g of each sample in 1% agarose containing formaldehyde followed by transfer to nylon membranes and UV cross-linking of the RNA to the membrane. The membrane was hybridized at 65°C with the \( ^{32} \)P-labeled cDNA of interest, washed at the same temperature, and subjected to autoradiography. Probes encoding ubiquitin, the 14-kDa ubiquitin carrier protein \( \text{E2[14a]; Ref. (49), rat proteasome subunits C2 (15) and C8 (42), and murine ubiquitin ligases muscle RING Finger 1 (MuRF-1) (10) and atrogin/muscle atrophy F-box (7, 18) were employed. Individual transcripts were quantified by densitometric scanning of the autoradiographs. All transcripts were expressed relative to 18S RNA. Smaller quantities of some of the samples were loaded on the gel to verify that quantification of the bands was linear.

Expression of genes for apoptotic signals and cytokines. RNA was isolated from soleus and gastrocnemius muscles by following the method of Chomczynski and Sacchi (11). The
expression of cytokines (rCK-1, rCK-3β templates) and apoptotic markers (rAPO-1 template) was assessed using ribonuclease protection assay (Ribonquant; Pharmigen, San Diego, CA), according to the manufacturer’s instructions. In these studies, 5 μg of RNA were hybridized to a 32P-labeled probe set encoding the specific complementary sequences for multiple genes of interest and housekeeping genes for normalization of loading. Briefly, the in vitro transcribed and radio-labeled probe was hybridized to sample mRNA, treated with RNase A, and then separated on an acrylamide-urea gel. Each protected, hybridized product migrated according to size, allowing identification of individual bands consistent with specific mRNA products. The expression of mRNAs was quantified by phosphoimager analysis of the radioactive gels. For normalization, each phosphoimagined gene of interest was divided by the corresponding L32 measurement for that sample and then multiplied by 10,000. Statistical analysis. Differences between groups were determined by unpaired Student’s t-test. All data are expressed as means ± SE, unless otherwise specified.

RESULTS

At the time of echocardiographic detection of heart failure, LV fractional shortening was lower (P < 0.01) in HS compared with LS rats (28 ± 1 vs. 42 ± 1%). LVEDd (8.4 ± 0.2 vs. 6.5 ± 0.1 mm; P < 0.01), LVESd (6.1 ± 0.1 vs. 3.8 ± 0.1 mm; P < 0.01), and posterior wall thickness (1.46 ± 0.06 vs. 0.70 ± 0.16 mm; P < 0.01) were all greater in HS rats, indicating LV dilation and hypertrophy. Imaging data were confirmed by greater (P < 0.01) heart mass expressed per unit body mass in HS compared with LS rats (6.1 ± 12 vs. 3.7 ± 0.8 mg/g). Increased wet weight of the lung (6.9 ± 0.8 vs. 4.9 ± 0.4 mg/g; P < 0.05) and liver (49.8 ± 1.8 vs. 35.9 ± 1.8 mg/g; P < 0.01) in HS rats expressed per unit body mass provided further evidence of heart failure. The average time on diet was similar between HS (9.9 ± 0.5 wk) and LS (9.9 ± 0.5 wk) groups.

Changes in body mass and food intake during the experimental diet up to the average time on diet (i.e., 10 wk) are shown in Fig. 1. Data shown in A and B represent 15 animals per group through 6 wk. Thereafter, sample sizes decrease as animals were studied. Body mass was lower (P < 0.05) in HS rats after 1 wk of diet. Thereafter, no significant differences were found. However, there were trends (P < 0.10) toward a lower body mass in the HS group at 4, 7, and 9 wk. Food intake was significantly lower in HS rats during weeks 2 (P < 0.05), 6 (P < 0.05), and 7 (P < 0.01). Average food intake during the diet was 16% lower (P < 0.01) in HS (19.0 ± 0.8) than in LS rats (22.5 ± 0.6 g/day).

Final body mass and skeletal muscle weights are shown in Table 1. Although the difference in final body mass was not significant, there was a trend toward a lower (13%) body mass in the HS group (P = 0.094). Gastrocnemius and plantaris muscles weighed less (both P < 0.01) in HS compared with LS rats. After muscle weights were expressed per unit of body mass, these differences were abolished. No differences in soleus or tibialis anterior muscles were found either on an absolute basis or after expression per unit body mass.

Protein and DNA content of soleus and gastrocnemius muscles are shown in Table 2. No differences

Table 1. Final body mass and skeletal muscle weights of HS and LS rats

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>LS</th>
<th>n (HS/LS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>5/10</td>
<td>5/10</td>
<td>15/15</td>
</tr>
<tr>
<td>Final body mass, g</td>
<td>322 ± 15</td>
<td>370 ± 23</td>
<td>15/15</td>
</tr>
<tr>
<td>Soleus mg</td>
<td>136 ± 6</td>
<td>150 ± 8</td>
<td>15/15</td>
</tr>
<tr>
<td>Soleus mg/g</td>
<td>0.42 ± 0.01</td>
<td>0.42 ± 0.02</td>
<td>15/15</td>
</tr>
<tr>
<td>Gastrocnemius mg</td>
<td>1,708 ± 99</td>
<td>2,044 ± 92</td>
<td>15/15</td>
</tr>
<tr>
<td>Gastrocnemius mg/g</td>
<td>5.29 ± 0.106</td>
<td>5.69 ± 0.279</td>
<td>15/15</td>
</tr>
<tr>
<td>Tibialis anterior mg</td>
<td>641 ± 30</td>
<td>782 ± 59</td>
<td>13/11</td>
</tr>
<tr>
<td>Tibialis anterior mg/g</td>
<td>2.05 ± 0.06</td>
<td>2.11 ± 0.21</td>
<td>13/11</td>
</tr>
<tr>
<td>Plantaris mg</td>
<td>349 ± 18</td>
<td>445 ± 25</td>
<td>13/10</td>
</tr>
<tr>
<td>Plantaris mg/g</td>
<td>3.01 ± 0.09</td>
<td>2.71 ± 0.18</td>
<td>13/10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats on each diet. Muscle weight data are shown as absolute values (mg) and relative to final body mass (mg/g). HS and LS, high- and low-salt diet, respectively. *P < 0.01.
were found in protein or DNA content of skeletal muscles when expressed on an absolute basis or relative to muscle mass. No differences in the protein-to-DNA ratio were found in either soleus or gastrocnemius muscles.

The fractional synthesis rates for gastrocnemius and soleus muscle protein are shown in Fig. 2. No differences in either gastrocnemius (HS: 8.38 ± 0.99 vs. LS: 7.47 ± 0.71%/day; n = 13) or soleus (HS: 13.35 ± 0.85 vs. LS: 12.15 ± 1.23%/day; n = 13) muscle protein synthesis were found. In addition, no differences in the absolute rates of protein synthesis were found in either the gastrocnemius (HS: 11.9 ± 1.9 vs. LS: 11.5 ± 1.5 mg/day) or soleus (HS: 1.15 ± 0.12 vs. LS: 1.18 ± 0.16 mg/day) muscle.

The expression of genes encoding components of the ubiquitin proteasome pathway for protein breakdown in soleus muscle of 9 HS and 7 LS rats (all male) is shown in Fig. 3. No differences in the level of mRNA (arbitrary units relative to 18S RNA) for ubiquitin (HS: 32 ± 5 vs. LS: 37 ± 8), E214k (HS: 0.586 ± 0.077 vs. LS: 0.614 ± 0.046), or C2 subunit (HS: 2.82 ± 0.36 vs. LS:

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### Table 2. Protein and DNA content of gastrocnemius and soleus muscles of HS and LS rats

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>LS</th>
<th>n = (HS/LS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg protein</td>
<td>8.52 ± 0.40</td>
<td>9.81 ± 0.68</td>
<td>15/11</td>
</tr>
<tr>
<td>mg/g</td>
<td>63 ± 2.2</td>
<td>64 ± 2.2</td>
<td>15/11</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>139 ± 20</td>
<td>169 ± 20</td>
<td>15/15</td>
</tr>
<tr>
<td>mg protein</td>
<td>80 ± 8</td>
<td>81 ± 8</td>
<td>15/15</td>
</tr>
<tr>
<td>mg/g</td>
<td>0.992 ± 0.009</td>
<td>0.109 ± 0.013</td>
<td>15/11</td>
</tr>
<tr>
<td>Soleus</td>
<td>mg DNA</td>
<td>0.67 ± 0.06</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.26 ± 0.85</td>
<td>1.39 ± 0.27</td>
<td>15/15</td>
</tr>
<tr>
<td>mg DNA</td>
<td>0.73 ± 0.11</td>
<td>0.65 ± 0.10</td>
<td>15/15</td>
</tr>
<tr>
<td>Protein/DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>103 ± 9</td>
<td>95 ± 5</td>
<td>15/11</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>240 ± 23</td>
<td>272 ± 20</td>
<td>15/15</td>
</tr>
</tbody>
</table>

Protein and DNA data (means ± SE) are shown as absolute values (mg) and relative to muscle mass (mg/g). Protein/DNA, ratio of protein to DNA.

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**Fig. 2. Fractional protein synthesis rates (%/day) of gastrocnemius (A) and soleus (B) muscle protein in HS (●; n = 13) and LS (○; n = 13) rats. Values are means ± SE.**

**Fig. 3. Expression of genes encoding components of the ubiquitin proteasome pathway in skeletal muscle in HS (●; n = 9) and LS (○; n = 7) rats. All data are expressed relative to 18S RNA. Values are means ± SE. *P < 0.05.**
2.67 ± 0.20) were found. The expression of the C8 subunit of the proteasome complex was lower (P < 0.05) in HS (0.553 ± 0.051) than in LS (0.669 ± 0.064) rats. Recently, two ubiquitin protein ligases, MuRF-1 and atrogin, have been shown to be markedly induced in skeletal muscle under various atrophic conditions (7, 18). Analysis of our samples showed that these ligases were expressed in soleus muscle of both HS and LS rats, but only at low levels. Furthermore, no measurable differences were found between groups.

The expression of genes for various apoptotic signals for 7 HS and 4 LS rats (all male) is shown in Table 3. There were no differences in the expression of apoptotic genes (arbitrary units, relative to L32 mRNA and multiplied by 10,000) between HS and LS rats with the exception of the proapoptotic gene bax, which was 29% lower (P < 0.01) in HS rats.

The expression of cytokine genes for 7 HS and 4 LS rats (all male) is shown in Table 4. Tumor necrosis factor-α (TNF-α) mRNA was not detected in either gastrocnemius or soleus muscles. Similarly, interleukin-1β (IL-1β) mRNA and IL-6 mRNA were not detected in the gastrocnemius muscle. No differences were found in the expression of other cytokines in either the gastrocnemius or soleus muscles.

### DISCUSSION

Heart failure is frequently characterized by profound skeletal muscle atrophy (14, 22, 33, 40, 43), which may contribute to muscle weakness (8, 30), exercise intolerance (20, 43), and poor prognosis (5). The goal of our study was to examine the effect of heart failure on those physiological systems that regulate skeletal muscle mass to further define the mechanism(s) underlying atrophy. To accomplish this, we measured skeletal weight and composition, muscle protein synthesis, and the expression of genes encoding components of the ubiquitin proteasome pathway for muscle protein breakdown and pro- and anti-apoptotic signals in the gastrocnemius or soleus muscles.

| Table 3. Expression of pro- and anti-apoptotic genes in gastrocnemius and soleus muscles of HS and LS rats |
|-----------------|-----------------|-----------------|
|                | **HS**          | **LS**          |
| **Gastrocnemius** |                |                |
| Fas             | 7,241 ± 317     | 6,953 ± 584    |
| Fas ligand      | 8,410 ± 423     | 6,839 ± 662    |
| Bax             | 6,352 ± 703     | 5,661 ± 731    |
| Bcl-2           | 11,056 ± 1,843  | 9,434 ± 1,974  |
| Bcl-xl          | 18,421 ± 1,432  | 13,805 ± 2,570 |
| Caspase-3       | 7,248 ± 437     | 6,449 ± 703    |
| **Soleus**      |                |                |
| Fas             | 98 ± 11         | 105 ± 9        |
| Fas ligand      | 183 ± 15        | 166 ± 10       |
| Bax             | 316 ± 23        | 444 ± 29       |
| Bcl-2           | 125 ± 12        | 117 ± 4        |
| Bcl-xl          | 1,359 ± 204     | 1,427 ± 198    |
| Caspase-3       | 173 ± 11        | 200 ± 7        |

Values (means ± SE) are for 7 HS and 4 LS rats. All data are expressed relative to L32 mRNA and multiplied by 10,000. *P < 0.01.

Dahl salt-sensitive rat model of heart failure. Although atrophy was observed in several muscles, we found no effect of heart failure on skeletal muscle protein synthesis or on the expression of genes for protein breakdown machinery, apoptotic signals, or cytokines. Instead, the muscle atrophy observed in this model was explained primarily by reduced body weight secondary to decreased food intake.

Gastrocnemius and plantaris muscle weights were lower in HS compared with LS rats. In contrast, soleus and tibialis anterior muscles did not differ. After muscle weight data were expressed relative to body mass to control for differences in body size, no differences in muscle mass were found. From these results, we conclude that differences in muscle mass between HS and LS groups were primarily related to variation in body size. Therefore, the question of interest becomes: what factors contribute to differences in body size? Prior studies in our laboratory (43, 44) and others (33) suggest that reduced food intake is a primary determinant of weight loss in human heart failure. In agreement with this notion, food intake was, on average, 14% lower in HS than in LS rats. The reduced food intake in the HS group may be a consequence of the palatability of the LS diet or a manifestation of the pressure/volume overload state. Irrespective of the mechanism, our results suggest that reduced muscle mass in this model of heart failure was due to differences in body size that occur secondary to reduced food intake.

Measurement of muscle mass alone may not be an accurate reflection of functional tissue mass, since HS rats experience fluid overload. That is, muscle weights may overestimate functional tissue mass and, in turn, underestimate the degree of atrophy if edema is present. To address this problem, we measured the protein content of the soleus and gastrocnemius muscles as a proxy of functional tissue mass. Although the absolute protein content of both the soleus and the gastrocnemius was lower in HS rats (both ~13%), this difference did not reach statistical significance. Moreover, when protein content data were expressed rela-
tive to muscle mass, no differences were observed between HS and LS groups, suggesting that minor differences in the total amount of protein were related to differences in muscle size. Finally, the protein-to-DNA ratio, an index of the amount of protein per cell unit, did not differ between groups. Thus, similar to gross muscle weights, protein content data suggest that the effect of heart failure on skeletal muscle mass is minimal after correction for differences in body or muscle size.

Our results in this animal model of heart failure agree with findings from our laboratory in human heart failure. We have previously shown that skeletal muscle atrophy is largely confined to those patients that experience weight loss during the course of the disease (43). Moreover, we showed that reduced food intake was the primary determinant of weight loss in these patients (44). Together, these findings from animals and humans suggest that energy imbalance is a major contributor to muscle atrophy in heart failure. It is important to note, however, that although weight loss may be a major impetus for muscle atrophy, factors other than energy balance are likely to contribute. Weight loss in heart failure is associated with a preferential loss of lean tissue mass (43) that is greater than what might be expected from caloric deprivation alone (16). This pattern of tissue loss is similar to wasting in other chronic diseases, such as cancer (21), and suggests that alterations in skeletal muscle metabolism may exacerbate weight loss-induced atrophy.

To explore other mechanisms that may contribute to muscle atrophy, we examined the effect of heart failure on the regulation of skeletal muscle protein synthesis, protein breakdown, and apoptosis.

Previous studies suggest that muscle wasting in a number of chronic diseases is related to reduced skeletal muscle protein synthesis (38). We found no differences in skeletal muscle fractional synthesis rate in either gastrocnemius or soleus muscles. Similar results were observed by Simonini et al. (40) in a rodent coronary ligation model of heart failure. Despite differences in soleus muscle mass that persisted after adjustment for body weight, these authors found no differences in the fractional synthesis rate of either total muscle protein or myosin heavy chain. The failure to observe an effect of heart failure on protein synthesis in the study of Simonini et al. may relate to the chronic nature of their heart failure model; animals were studied 20 wk after coronary ligation surgery. One could postulate that reduced muscle protein synthesis was present earlier in the course of the disease and caused muscle atrophy. However, after muscle atrophy occurred and a new steady-state muscle mass was reached, protein synthesis rates returned to normal. Our results argue against this notion, because in our acute model of failure, no evidence for decreased skeletal muscle protein synthesis was found. Thus decreased muscle protein synthesis does not appear to be an early manifestation of the heart failure syndrome.

To assess the role of protein breakdown in heart failure-related muscle atrophy, we measured the expression of genes encoding components of the ubiquitin proteasome pathway. This pathway is responsible for the majority of proteolysis in skeletal muscle (41) and has been implicated in muscle wasting in several disease states (29, 35). Increased expression of genes for various components of this pathway has been observed in several muscle-wasting conditions (6, 23, 49) and has been shown to correlate with muscle protein breakdown (6). No differences in the expression of ubiquitin, E2, E100k, C2 subunit, or E3 ligases were found between HS and LS rats in this study. Paradoxically, expression of the C8 subunit of the proteasome complex was reduced in HS rats. Our results agree with those of Dalla Libera et al. (13), who found no effect of heart failure on the amount of ubiquitin protein in soleus muscle. Collectively, these findings argue against a role for protein breakdown in muscle atrophy in heart failure. We should acknowledge, however, that no study, including our own, has measured muscle protein breakdown directly in an animal model of heart failure.

Several studies in humans (3, 47) and animal models (13, 48) suggest that heart failure is associated with increased skeletal muscle apoptosis. Apoptosis may contribute to muscle atrophy and has been linked to increasing disease severity (47) and exercise intolerance (3). To evaluate the effect of heart failure on skeletal muscle apoptosis, we measured the expression of pro- and anti-apoptotic genes in skeletal muscle. No differences were found in mRNA levels of pro-apoptotic genes to suggest that heart failure is associated with increased apoptotic drive. Measurement of apoptosis in this model of heart failure by in situ techniques will be required to verify this conclusion, as we have measured only gene expression of apoptotic signals. Our results differ from those of Vescovo et al. (48) and Dalla Libera and colleagues (12, 13), who found increased amounts of pro-apoptotic (caspase 3) and reduced amounts of anti-apoptotic (Bcl-2) proteins in rats with right ventricular failure due to monocrotaline. In addition, they found increased rates of apoptosis in skeletal muscle of animals with heart failure. Differences between studies may relate to the animal models used and the different type of failure induced (i.e., right vs. left ventricular failure). The monocrotaline model of failure is a rapidly progressing model in which animals typically develop right ventricular failure between 25 and 30 days after administration and die shortly thereafter. Skeletal muscle apoptosis observed in this model, therefore, may reflect the effect of rapidly progressing, end-stage failure. Parenthetically, the level of bax mRNA was reduced in the soleus muscle of HS compared with LS rats. Bax is a pro-apoptotic protein that is thought to bind and inactivate the anti-apoptotic protein Bcl-2 (19). This difference in Bax expression may partially explain reduced rates of apoptosis and muscle atrophy observed in the soleus muscle of animals with heart failure in the present study and others (13).

Heart failure is characterized by immune activation and increased circulating and tissue levels of cytokines (2). Cytokines can induce skeletal muscle atrophy by...
inhibiting muscle protein synthesis (28), increasing muscle protein breakdown (31), or inducing apoptosis (9). We found no differences in proinflammatory cytokine mRNA levels between HS and LS rats in either gastrocnemius or soleus muscles, suggesting that heart failure is not associated with increased skeletal muscle cytokine expression. Our inability to detect mRNA for several cytokines was related to the sensitivity of the ribonuclease protection assay and not to the absence of transcripts. Although we were able to demonstrate the presence of TNF-α transcripts in soleus muscles by use of competitive RT-PCR, no difference was found between HS and LS groups (Toth MJ, unpublished results). The absence of differences in muscle cytokine expression between LS and HS rats may relate to the lack of immune activation in this acute model of heart failure.

In conclusion, our results suggest that heart failure is not characterized by alterations in skeletal muscle protein metabolism or the expression of genes regulating muscle protein breakdown or apoptosis. These results are not consistent with the hypothesis that the presence of heart failure, per se, is associated with a skeletal muscle myopathy that contributes to atrophy. Instead, our findings suggest, in agreement with human studies (43, 44), that reduced body weight secondary to decreased food intake is a primary determinant of muscle atrophy in heart failure.

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