Skeletal muscle myofibrillar mRNA expression in heart failure: relationship to local and circulating hormones

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Toth, Michael J., Philip A. Ades, Martin M. LeWinter, Russell P. Tracy, and André Tchernof. Skeletal muscle myofibrillar mRNA expression in heart failure: relationship to local and circulating hormones. J Appl Physiol 100: 35–41, 2006. First published September 1, 2005; doi:10.1152/japplphysiol.00570.2005.—Chronic heart failure is characterized by changes in skeletal muscle that contribute to exercise intolerance and muscle weakness. To determine whether changes in the quantity and isoform distribution of key myofibrillar proteins are related to altered gene expression, we measured skeletal muscle myofibrillar mRNA abundance in nine heart failure patients (mean ± SE; 63 ± 3 yr) and nine controls (70 ± 3 yr). In addition, we assessed the relationship of circulating levels of anabolic and catabolic hormones, as well as local expression of insulin-like growth factor (IGF)-I, to myofibrillar mRNA abundance. Heart failure patients were characterized by lower abundance of mRNA encoding the myosin heavy chain (MHC) I isoform (P < 0.01), whereas MHC IIa and MHC IIx mRNA did not differ between groups. Actin mRNA was also lower in heart failure patients compared with controls (P < 0.001). The expression of each MHC isoform transcript correlated with its respective protein product (MHC I: r = 0.656, P < 0.01; MHC IIa: r = 0.489, P < 0.05; MHC IIx: r = 0.505, P < 0.05; n = 18 for all). In addition to changes in myofibrillar transcripts, we found lower (P < 0.01) skeletal muscle IGF-IEx mRNA content in heart failure patients. Myofibrillar mRNA levels were positively associated with skeletal muscle IGF-IEx transcript levels (range of r values: 0.663–0.765; P values: <0.01 to <0.001) and modestly associated with circulating markers of immune activation (range of r values: −0.487 to −0.555; P values: <0.05 to <0.03). Our findings suggest that alterations in skeletal muscle MHC content and isoform distribution in heart failure may derive, in part, from changes in MHC gene expression. The relationships of myofibrillar mRNA content to both local and circulating hormones further suggest that alterations in the balance between anabolic and catabolic hormones in heart failure patients may influence skeletal muscle myofibrillar protein phenotype by altering gene expression.

Heart failure patients are characterized by changes in skeletal muscle protein quantity and isoform distribution that contribute to muscle wasting, exercise intolerance, and muscle weakness. Of particular importance for the maintenance of muscle mass and function are the myofibrillar proteins myosin and actin. They comprise a large percentage of total muscle protein (43) and are directly responsible for muscle contraction. Several studies have reported alterations in skeletal muscle myofibrillar protein in heart failure, including a reduction in myosin heavy chain (MHC) protein content (37, 40) and a shift in MHC isoform distribution toward a more fast-twitch phenotype (38, 40, 41). These changes in MHC correlate to exercise intolerance and muscle contractile dysfunction (38, 40, 41), suggesting they may contribute to physical disability. The mechanisms underlying changes in the quantity and isoform distribution of key myofibrillar proteins, however, have not been defined.

Recent work has suggested a role for alterations in anabolic/catabolic hormone balance in the skeletal muscle adaptations to heart failure (4, 7). Studies have shown that reduced circulating levels of anabolic hormones and/or increased concentrations of catabolic hormones are correlated to reduced muscle mass and function (5, 6, 30). More recent reports have raised the possibility that changes in the local production of anabolic and/or catabolic hormones could alter skeletal muscle protein metabolism via autocrine/paracrine pathways (16, 21). One mechanism whereby these hormones could regulate myofibrillar protein content and isoform distribution is through changes in gene expression. Indeed, several of the hormones altered in heart failure are known to affect skeletal muscle gene expression (1, 2). No study, however, has examined the relationship of circulating and locally expressed hormones to skeletal muscle gene expression in heart failure.

The objectives of this study were twofold. First, we sought to determine whether heart failure alters the abundance of mRNA for key skeletal muscle myofibrillar proteins. To address this question, we measured skeletal muscle mRNA levels for the three isoforms of MHC (I, IIa, and IIx) and actin by real-time RT-PCR in a cohort of heart failure and controls. Based on studies in animal models (36, 37), we hypothesized that MHC mRNA would be lower in HF but that actin mRNA would not differ. Moreover, the pattern of differences in mRNAs for individual MHC isoforms would parallel the shift in protein isoform distribution in HF toward a more fast-twitch phenotype. A second objective of our study was to evaluate the potential role of anabolic/catabolic hormone balance in controlling skeletal muscle gene expression. We measured circulating anabolic and catabolic hormone levels, as well as skeletal muscle insulin-like growth factor (IGF)-I mRNA abundance, and assessed their relationship to myofibrillar gene expression. We hypothesized that lower abundance of MHC mRNA would be associated with lower levels of anabolic hormones and greater concentrations of catabolic hormones.

METHODS

Subjects. Nine male volunteers with chronic heart failure were recruited from the Heart Failure Clinic of the Cardiology Unit of the University of Vermont (left ventricular ejection fraction: 29 ± 4% by
echocardiography). Heart failure was due to coronary artery disease in six patients, defined by history of myocardial infarction and/or multivessel coronary obstructions at cardiac catheterization, idiopathic dilated cardiomyopathy in two patients, and dilated cardiomyopathy secondary to severe hypertension in one patient. New York Heart Association (NYHA) functional class averaged 2.4 ± 0.2 with six patients in class II, two in class III, and one in class IV. Heart failure patients were taking the following medications: diuretics (n = 9; 100%), digoxin (n = 6; 67%), angiotensin-converting enzyme inhibitors (n = 9; 100%), and adrenergic blocking agents (n = 7; 78%). Two of the nine patients were characterized by diabetes mellitus: one insulin dependent and one noninsulin dependent. At the time of testing, patients were clinically stable and free of peripheral edema.

Nine male volunteers were recruited to serve as controls. Seven of these volunteers were healthy and free of disease, had no signs or symptoms of heart disease, and normal rest and exercise electrocardiograms. The control group also included two individuals with known coronary artery disease but no other chronic diseases. Both had normal left ventricular contraction patterns and did not have exercise-induced ischemia, as demonstrated by a normal electrocardiographic stress test to exhaustion. Both volunteers were treated with aspirin, one with a Ca2+ channel blocker, and the other with an 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Because data from these two controls did not impact group differences or associations among variables (see RESULTS), we decided to retain them in the control group. Other medications in the control group were 5-reductase inhibitor (n = 1) and nonsteroidal anti-inflammatory (n = 1). Controls had no history of diabetes mellitus, normal fasting blood glucose (<6.22 mM) and normal glucose tolerance (glucose <7.77 mM 2 h after 75-g oral glucose load). The nature, purpose, and possible risks of the study were explained to each subject before giving written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont. Data on these volunteers describing the effects of heart failure on skeletal muscle myofibrillar protein content, synthesis, and isoform expression have been published previously (40).

Experimental protocol. Each volunteer was tested during outpatient and inpatient visits to the research center. Body composition and exercise capacity were measured on an outpatient basis at least 1 wk before skeletal muscle biopsies. Skeletal muscle tissue was obtained under lidocaine anesthesia using the percutaneous biopsy technique the morning after an inpatient visit to the General Clinic Research Center. For 3 days before admission, all subjects were provided a standardized, weight-maintenance diet (60% carbohydrate, 25% fat, 15% protein). The last meal of the standardized diet was consumed by 1900 the evening of admission, and subjects fasted until completion of testing the following day. Medications were maintained for all volunteers per normal dosing regimens.

Body composition. Body mass was measured on a digital scale (Scale-Tronix, Wheaton, IL). Fat mass, fat-free mass, and bone mass were measured by dual-energy X-ray absorptiometry, using a Lunar DPX-L densitometer (Lunar, Madison, WI). Bone mass data are not presented. Appendicular skeletal muscle mass was measured as described by Heymsfield et al. (22).

Exercise capacity. Peak oxygen consumption (V_{O2peak}) was measured during a graded, treadmill test to volitional fatigue. Briefly, a comfortable initial walking speed was found for each volunteer and was maintained throughout the test. The grade was increased 2.5% every 2 min until volitional fatigue. Peak V_{O2} was defined as the highest 30-s average V_{O2} value measured during the last 2 min of the test. One heart failure patient with severe symptoms (NYHA class IV) did not attempt the exercise test.

Echocardiography. Echocardiography was used to evaluate left ventricular systolic function in heart failure. Two-dimensional echocardiographic images were obtained in sequential fashion from the parasternal long-axis, parasternal short-axis, apical four-chamber, and apical two-chamber views. Three to five cardiac cycles were digitized at end systole (time of smallest cavity area) and end diastole (R-wave peak). Ejection fraction was calculated according to standard guidelines (18).

Skeletal muscle gene expression. Total RNA was isolated from 25 mg of muscle tissue using Trizol (GIBCO, Burlington, Ontario, Canada), following the manufacturer’s recommendations. First-strand cDNA synthesis was accomplished using 1.5 μg of the isolated RNA in a reaction containing 200 units of Superscript II Reverse Transcriptase (Invitrogen, Burlington, Ontario, Canada), 300 ng of oligo (dT)18, 500 μM dNTP, 10 mM DTT, and 34 units of porcine RNase inhibitor (Amersham Pharmacia, Uppsala, Sweden) in a final volume of 50 μl. The resulting products were treated with 1 μg of RNase A for 30 min at 37°C and purified thereafter with Qiapquick PCR purification kits (Qiagen, Santa Clara, CA). For quantitative PCR analyses, a light-cycler PCR (Roche Diagnostics; Lewes, UK) was used to measure the mRNA abundance of MHC I (NM_000257), MHC IIA (NM_017534), MHC IIX (NM_005963), and actin (NM_001100). The following sets of primers were used (forward, reverse): MHC I: 5’-TGCCGAGTCCAGGTAACG-3’ and 5’-TGGG-GCTTTGGACACCTC-3’; MHC IIA: 5’-GATCGACCTGGCGGCCACCT-3’ and 5’-GGGGCCACT-3’; MHC IIX: 5’-GCCAACATGAGAGGAAATCAGACACAC-3’ and 5’-CCG-GAATTTGGAGAGGTTGACGTT-3’; and actin: 5’-CGTCACTGTCGGGGGCACCA-3’ and 5’-GCCGCGCTCCTGTACACTCTCGC-3’.

In addition, two isoforms of IGF-I were measured: IGF-IEIA (X56773) and IGF-IEIC (also known as mechano growth factor; U48070). The following sets of primers were used: IGF-IEIA: 5’-CAATGACGAGAGGATGCGAAGA-3’ and 5’-ACAGCAAATCTACCAACTCCAGGACCA-3’; and IGF-IEIC: 5’-CGAGATCTCAGGAGGAAGAAG-3’ and 5’-ACAGGTAACTCGTGCAGAGC-3’. Primers were designed with GeneTools software (BioTools, Edmonton, Alberta, Canada), and their specificity was verified by blasting in the GeneBank database. The FastStart SYBR Green kit (Roche Diagnostics, Lewes, UK) was used in a final reaction volume of 20 μl containing 4 mM MgCl2, 20 ng of each primer, and 20 ng of the cDNA template. PCR was carried out according to the following conditions: 95°C/10 min; 40 cycles of (95°C/10 min, 65°C/1 s, 72°C/1 s, 78°C/3 s) with a temperature transition of 3°C/s. Glucose-6-phosphate dehydrogenase (NM_000492) was used as a housekeeping gene and was measured using the following primers: 5’-CAGCGCTCAACAGCCACAT-3’ and 5’-AAGGGCTTTCGCCAGTATGC-3’. All PCR products were sequenced using BigDye Terminator (version 3.1 cycle sequencing, ABI Prism) and analyzed on an ABI 3730 automated DNA sequencer (PE Applied Biosystems, Foster City, CA) to verify the amplification of the target mRNA. The crossing points (Cp) to calculate the amount of copies in initial cDNA specimens were determined with the double derivative method, as described previously (24). For each sample, the Cp value of each gene was divided by that of the housekeeping gene. To further minimize interassay variability, this Cp ratio was then multiplied by the average Cp generated for housekeeping gene amplifications of all samples examined in the present experiment (24). A universal standard curve was generated using subunit O of ATPase (Atp5o) from an amplification with perfect efficiency (i.e., efficiency coefficient = 2.00), using cDNA amounts of 0, 103, 104, 105, and 106 copies and was used to calculate copy number for the mRNAs of interest. We chose a universal standard curve, rather than a standard curve for each gene, in light of recent reports showing that the use of the second derivative approach to calculate the Cp value of a wide variety of genes yields limited variability in efficiency coefficients (24). More importantly, variability in amplification efficiency among genes will not impact the comparison of individual genes among groups. PCR data are expressed as corrected copy number normalized to total RNA. Data from individual MHC isoforms were summed to derive total MHC mRNA copy number.

Hormone analysis. C-reactive protein was measured by ELISA (25) with an interassay coefficient of variation (CV) ranging from 2 to

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4%. Tumor necrosis factor (TNF) and interleukin (IL)-6 plasma concentrations and soluble receptors (TNF-RII and IL-6 sR, respectively) were measured by ultra-sensitive ELISA assays (R & D Systems, Minneapolis, MN) with interassay CVs of 16 and 6% for TNF and IL-6 concentration and 9 and 10% for their respective receptors. IGF-1 was measured by radioimmunoassay (ALPCO, Windham, NH) with an interassay CV of 5%. Insulin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO) with an interassay CV of 7.4%. Cortisol was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA) with an interassay CV of 8.7%.

**Statistical analysis.** Differences between groups were determined by unpaired, Student’s t-tests or Mann-Whitney U-test, depending on the distributional characteristics of the variable. Relationships between variables were assessed using Pearson’s correlation coefficients. Variables that were not normally distributed (e.g., cytokines), were log10 transformed before correlation analyses. Distributional assumptions of log10 transformed variables were tested before analysis using the Shapiro-Wilk test. All analyses were conducted with SPSS software 9.0 (SPSS, Chicago, IL). All values are means ± SE, unless otherwise specified.

**RESULTS**

Physical characteristics are shown in Table 1. No differences in age, body size, or muscle mass were observed between groups.

Skeletal muscle total MHC and actin mRNA copy numbers are shown in Fig. 1. Total MHC mRNA tended (P = 0.07) to be lower in heart failure compared with controls (71.9 ± 13.8 vs. 105.6 ± 10.1 10^6 copies/µg total RNA), whereas, actin mRNA was lower (P < 0.001) in heart failure (HF: 51.1 ± 8.6 vs. C: 105.9 ± 9.9 10^6 copies/µg total RNA). The ratio of MHC to actin mRNA tended (P = 0.08) to be higher in heart failure compared with controls (heart failure: 1.41 ± 0.15 vs. controls: 1.05 ± 0.12). Total RNA content was not different between groups (heart failure: 0.164 ± 0.025 vs. controls: 0.184 ± 0.008 µg/mg wet wt; data not shown). Exclusion of the two controls with coronary artery disease did not affect group differences in MHC (heart failure: 71.9 ± 13.8 vs. controls: 99.2 ± 11.2 10^6 copies/µg total RNA; P = 0.16) or actin (heart failure: 51.1 ± 8.6 vs. controls: 104.7 ± 13.8 10^6 copies/µg total RNA; P < 0.01) mRNA content.

mRNA levels for genes encoding different MHC isoforms are shown in Fig. 2. The trend toward lower total MHC mRNA in heart failure noted above was due to lower (P < 0.01) MHC I mRNA (heart failure: 27.7 ± 3.9 vs. controls: 54.7 ± 8.9; P < 0.01), MHC IIA (heart failure: 40.5 ± 10.0 vs. C: 42.2 ± 9.8) or MHC IX (heart failure: 3.71 ± 1.19 vs. controls: 2.80 ± 1.06 10^6 copies/µg total RNA) mRNA abundance.

To examine the potential contribution of these differences in myofibrillar gene expression to skeletal muscle protein phenotype, we examined the relationship of myofibrillar mRNA levels to protein content and synthesis rate data reported previously in these patients (40) (Table 2). Of note, mRNA abundance for individual MHC isoforms correlated with their physical characteristics are shown in Table 1. No differences in age, body size, or muscle mass were observed between groups.

Table 1. Physical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Heart Failure</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Age, yr</td>
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<td>70±4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176±1</td>
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<tr>
<td>Body mass, kg</td>
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<td>Appendicular skeletal muscle mass, kg</td>
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</tbody>
</table>

Data are means ± SE.

![Fig. 1. Differences in total myosin heavy chain (MHC; top) and actin mRNA (bottom) in skeletal muscle tissue from heart failure patients (HF; closed bars) and controls (open bars). mRNA data are normalized to GAPDH mRNA levels and are expressed as copy number relative to total RNA. Values are means ± SE. *P < 0.001; †P = 0.07.](http://jap.physiology.org/)

![Fig. 2. Effect of heart failure on mRNA levels for skeletal muscle MHC isoforms (I, IIA, IX). Closed bars, heart failure; open bars, controls. mRNA data are normalized to GAPDH mRNA levels and are expressed as copy number relative to total RNA. Values are means ± SE. *P < 0.01.](http://jap.physiology.org/)
Table 2. Relationship of myofibrillar mRNA levels to protein content and synthesis rates in heart failure patients and controls

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total MHC</th>
<th>MHC I</th>
<th>MHC IIA</th>
<th>MHC IIX</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MHC</td>
<td>0.164</td>
<td>0.376</td>
<td>−0.342</td>
<td>−0.414</td>
<td>0.562*</td>
</tr>
<tr>
<td>MHC I</td>
<td>−0.186</td>
<td>0.656*</td>
<td>−0.628*</td>
<td>−0.577*</td>
<td>0.049</td>
</tr>
<tr>
<td>MHC IIA</td>
<td>0.235</td>
<td>−0.501*</td>
<td>0.489*</td>
<td>0.395</td>
<td>0.081</td>
</tr>
<tr>
<td>MHC IIX</td>
<td>0.037</td>
<td>−0.517*</td>
<td>0.484*</td>
<td>0.505*</td>
<td>−0.179</td>
</tr>
<tr>
<td>Actin</td>
<td>0.149</td>
<td>−0.087</td>
<td>0.107</td>
<td>−0.036</td>
<td>0.192</td>
</tr>
<tr>
<td>MHC FSR</td>
<td>0.309</td>
<td>0.377</td>
<td>−0.380</td>
<td>−0.208</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Values represent Pearson correlation coefficients. The units for total myosin heavy chain (MHC) and actin mRNA and for protein content are 10^6 copies/g total RNA and arbitrary densitometric units/g total protein, respectively. For individual MHC isoforms, both mRNA abundance and protein content data are expressed as a percentage of total MHC. FSR, fractional synthesis rate in %/day. *P < 0.05; †P < 0.01.

respective protein product reported previously (40) (P < 0.05 to P < 0.01). In contrast, neither total MHC nor actin mRNA abundance correlated with their respective protein content data. Moreover, there were no relationships between any index of MHC mRNA and MHC protein synthesis rate.

In heart failure, MHC I mRNA levels showed a trend (MHC I: r = −0.598; P = 0.09) toward being negatively related to NYHA class. Actin mRNA levels were negatively related to NYHA class (r = −0.657; P < 0.05). Although negative relationships were noted between NYHA class and mRNA levels for total MHC mRNA and the other MHC isoforms (total MHC: r = −0.523; P = 0.15; MHC IIA: −0.448; P = 0.23; MHC IIX: −0.387; P = 0.30), these relationships did not reach significance. Neither MHC nor actin mRNA levels were related to ejection fraction or peak VO2 in heart failure.

Skeletal muscle IGF-IeA mRNA levels were lower (P < 0.01) in heart failure compared with controls (heart failure: 17.9 ± 7.7 vs. controls: 36.2 ± 6.1 10^3 copies/μg total RNA). Although the IGF-Ie transcript [also known as mechano growth factor (17)] was detectable, its abundance was sufficiently low as to not allow for quantitation. In correlation analyses, lower IGF-IeA expression was related to lower total MHC (r = 0.663; P < 0.01), MHC I (r = 0.674; P < 0.01), and actin (r = 0.765; P < 0.001) mRNA abundance but not MHC IIA (r = 0.392; P = 0.11) or MHC IIX (r = −0.059; P = 0.82). Significant correlations are shown in Fig. 3. Exclusion of the two controls with coronary artery disease did not alter the group differences in IGF-IeA mRNA levels (heart failure: 17.9 ± 7.7 vs. controls: 37.1 ± 7.8 10^3 copies/μg total RNA; P < 0.01) or relationships to total MHC (r = 0.647; P < 0.01), MHC I (r = 0.669; P < 0.01), and actin (r = 0.759; P < 0.001) mRNA abundance. Similarly, exclusion of the one NYHA class IV heart failure patient did not alter relationships between the expression of IGF-IeA and total MHC (r = 0.540; P < 0.05), MHC I (r = 0.601; P < 0.02), and actin (r = 0.707; P < 0.01) mRNA abundance.

Heart failure patients were characterized by greater circulating levels of IL-6 (heart failure: 4.13 ± 0.83 vs. controls: 2.11 ± 0.45 pg/ml; P < 0.03), IL-6 soluble receptor (heart failure: 39.2 ± 1.2 vs. controls: 31.2 ± 1.6 ng/ml; P < 0.01), and TNF-RII (heart failure: 3.541 ± 325 vs. controls: 2.748 ± 179 pg/ml; P < 0.05). No differences in C-reactive protein (heart failure: 5.3 ± 1.4 vs. controls: 2.1 ± 0.7 μg/ml), TNF (heart failure: 1.9 ± 0.3 vs. controls: 1.6 ± 0.2 pg/ml), IGF-I (heart failure: 151 ± 20 vs. controls: 110 ± 9 ng/ml), cortisol (heart failure: 19.4 ± 1.5 vs. controls: 18.3 ± 1.3 μg/dl), or insulin (heart failure: 13.3 ± 1.5 vs. controls: 11.6 ± 1.7 μIU/ml) were found.

The relationship of circulating anabolic and catabolic hormones to myofibrillar mRNA levels are show in Table 3. IL-6 levels were negatively related to both total MHC and actin mRNA levels (P < 0.05). In addition, IL-6sR was negatively related to MHC I mRNA (P < 0.05). After exclusion of the two controls with coronary artery disease, the strength and statistical significance of the correlations of IL-6 to both total
MHC ($r = -0.442; P = 0.09$) and actin ($r = -0.436; P = 0.09$) mRNA and of IL-6sR to MHC I ($r = -0.436; P = 0.09$) were diminished. Exclusion of the one NYHA class IV heart failure patient did not alter relationships of IL-6 to total MHC ($r = 0.583; P < 0.02$) and actin ($r = 0.510; P < 0.04$) mRNA abundance or the relationship of IL-6sR to MHC I ($r = -0.563; P < 0.02$).

**DISCUSSION**

Numerous studies have shown that heart failure affects skeletal muscle and that these changes, in turn, contribute to exercise intolerance and muscle weakness. To further characterize the molecular mechanisms underlying adaptations in myocardial protein content and isoform distribution, we measured mRNA abundance for MHC isoforms and actin in heart failure and controls. We found a trend toward lower total MHC mRNA in heart failure. This was due to lower mRNA abundance of the MHC I isoform, with no group differences in MHC IIa or IIx transcripts. Actin mRNA abundance was also lower in heart failure. In addition to these differences in myofibrillar transcripts, we observed lower expression of IGF-I$\alpha$ mRNA in skeletal muscle in heart failure. Lower levels of myofibrillar mRNA levels were associated with lower skeletal muscle IGF-I$\alpha$ expression and were modestly related to greater circulating concentrations of immune markers. Collectively, our results suggest the hypothesis that alterations in MHC protein content and isoform distribution in heart failure are due, in part, to changes in MHC gene expression. Furthermore, we speculate that these changes in gene expression may be mediated by hormonal regulators acting via autocrine, paracrine, and/or endocrine pathways. Our findings are discussed in the context of changes in skeletal muscle myofibrillar protein content and isoform distribution reported previously (40).

The trend toward lower ($-32\%$) total MHC mRNA in heart failure relative to controls was due to a lower expression of MHC I transcripts in heart failure. In contrast, no group differences in MHC IIa or IIx mRNA levels were found. These results are qualitatively similar to findings from our laboratory and others at the protein level showing decreased MHC protein content (37, 40) and a shift in MHC isoform distribution toward a more fast-twitch phenotype (38, 40, 41) in heart failure. In fact, the relative expression of mRNA for each MHC isoform correlated significantly with its respective protein product (Table 2), suggesting the hypothesis that heart failure affects MHC protein through alterations in the availability of MHC mRNA. More specifically, differences in MHC content and isoform distribution appear to arise from lower expression of MHC I. Differences in MHC mRNA levels could result from changes in MHC gene transcription, the stability of MHC transcripts, or a combination of these factors. The lower abundance of MHC transcripts would be expected to alter MHC protein content and isoform distribution by limiting MHC protein translation. However, we did not find relationships between MHC transcripts and protein synthesis rates. Moreover, although we have previously noted a $12\%$ lower MHC protein synthesis rate in heart failure compared with controls, this difference did not reach statistical significance (40). Our results in human heart failure are similar to data from Simonini et al. (37) in a rat model of failure. In this study, rats with heart failure were found to have lower MHC protein content and lower MHC I mRNA abundance. Despite lower protein content and transcript levels, differences in MHC protein synthesis rates ($17\%$ lower in rats with heart failure) did not reach statistical significance. The inability to demonstrate a link between MHC mRNA abundance and synthesis rates in both of these studies may be due to the fact that measurements of MHC synthesis rate represents the average rate of synthesis of all MHC isoforms. Thus differences in the synthesis rate of individual MHC isoforms, such as MHC I, might not be detected. Nonetheless, our findings are the first to suggest that the skeletal muscle MHC phenotype of heart failure may derive from alterations in gene expression.

In contrast to MHC, group differences in actin mRNA and protein levels did not correlate. We found lower actin mRNA abundance in heart failure compared with controls and a stepwise decrease with increasing NYHA functional class, whereas no such differences or relationships were found in actin protein levels (40). Thus it appears that actin protein content is not directly related to actin gene expression. We should stress that this dissociation between actin mRNA and protein content is not unprecedented. A recent report by Haddad et al. (19) showed a marked reduction in actin mRNA with muscular inactivity without a corresponding change in actin protein content. Thus it would appear as if actin protein expression is regulated differently than MHC. This notion is supported by various studies showing differing effects of physiological and pathophysiological stimuli on MHC and actin protein and mRNA levels (1, 19). That actin protein levels were maintained in the face of reduced actin transcript may be explained by increased stability of actin mRNA and/or greater translational efficiency. Alternatively, actin protein content could be regulated by processes distal to gene expression and translation, such as protein breakdown.

To further explore the potential mechanisms underlying reductions in skeletal muscle myofibrillar protein transcripts,
we examined the relationship of myofibrillar mRNA levels to local and circulating hormones. Lower mRNA abundance for total MHC, MHC I, and actin were associated with reduced skeletal muscle IGF-I mRNA. To our knowledge, this is the first demonstration of a relationship between skeletal muscle MHC and IGF-I gene expression in humans. These relationships are in keeping with the well-described anabolic effects of IGF on muscle (3) and studies in animal models showing a similar relationship between skeletal muscle MHC and IGF-I gene expression (42). Considering that IGF-I eae mRNA abundance was markedly lower in heart failure compared with controls, in agreement with prior work in humans and animals (21, 35), these findings suggest the possibility that decreased MHC gene expression may arise from alterations in local IGF-I production. One mechanism whereby IGF-I may regulate MHC expression is through the calcineurin signaling pathway. Skeletal muscle calcineurin, which is activated by local IGF-I expression (27, 28), has been shown to play an important role in controlling MHC gene expression and isoform distribution (32, 33). Activation of this pathway promotes a slow-twitch phenotype (14, 29), whereas inhibition causes a shift in MHC isoforms toward a fast-twitch phenotype (8, 11). In fact, some studies have shown that calcineurin modulates MHC isoform distribution specifically by regulating MHC I gene expression (26). In this context, and considering that group differences in MHC mRNA content and isoform distribution are due to alterations in MHC I mRNA expression, these findings suggest the hypothesis that reduced MHC protein content and the shift toward a more fast-twitch phenotype in heart failure may be due to reduced MHC I gene expression secondary to decreased local IGF-I production. Recent findings in an animal model of heart failure showing that growth hormone administration, a maneuver that would increase skeletal muscle IGF-I production (9, 15), shifts MHC protein isoform distribution toward a more slow-twitch phenotype further supports this hypothesis (12).

Lower abundance of myofibrillar transcripts were also modestly related to circulating markers of immune activation, such as IL-6 and its soluble receptor. These relationships are in keeping with studies showing that inflammatory cytokines downregulate myofibrillar gene expression and protein content in striated muscle (1, 20, 34). Recent studies have also shown that heart failure patients are characterized by increased expression of inflammatory cytokines in skeletal muscle (16). Thus it is plausible to hypothesize a role for local and/or circulating inflammatory cytokines in alterations in myofibrillar gene expression and protein content in heart failure.

Another potential factor contributing to group differences in myofibrillar gene expression is the fact that heart failure patients are characterized by insulin resistance relative to controls (31, 39). In our cohort, two heart failure patients had overt diabetes, and, although not directly measured, we assume that the majority of heart failure patients were characterized by some degree of insulin resistance (31, 39). The question of interest, therefore, is whether differences in insulin levels and/or sensitivity could explain the effect of heart failure on muscle gene expression. Although we are aware of no studies that have examined the relationship between tissue insulin sensitivity and MHC gene expression, Houmard et al. (23) showed that acute hyperinsulinemia upregulated MHC IIX gene expression. However, because we did not find differences in fasting insulinemia or MHC IIX mRNA abundance between groups, we do not believe that group differences in MHC gene expression are the result of differences in insulin levels or action. Furthermore, studies have shown no effect of insulin levels or insulin resistance on MHC protein synthesis rates (10) or myofibrillar protein content (13). Thus we do not believe that the myofibrillar protein phenotype in heart failure is due to differences in insulin levels or sensitivity.

In summary, our results show that heart failure patients are characterized by lower abundance of MHC I and actin mRNA and a trend toward reduced total MHC mRNA content. These differences in myofibrillar gene expression may, in part, explain alterations in MHC protein content and isoform distribution (40). The relationships between myofibrillar mRNA abundance and local and circulating hormonal markers suggest that adaptations in myofibrillar protein content and isoform distribution in response to heart failure may arise from changes in skeletal muscle gene expression secondary to altered anabolic/catabolic hormone balance.

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