Skeletal muscle myofibrillar protein metabolism in heart failure: relationship to immune activation and functional capacity

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Toth, Michael J., Dwight E. Matthews, Philp A. Ades, Marc D. Tischler, Peter Van Buren, Michael Previs, and Martin M. LeWinter. Skeletal muscle myofibrillar protein metabolism in heart failure: relationship to immune activation and functional capacity. Am J Physiol Endocrinol Metab 288: E685–E692, 2005. First published November 23, 2004; doi:10.1152/ajpendo.00444.2004.—Chronic heart failure is characterized by changes in skeletal muscle that contribute to physical disability. Most studies to date have investigated defects in skeletal muscle oxidative capacity. In contrast, less is known about how heart failure affects myofibrillar protein metabolism. Thus we examined the effect of heart failure on skeletal muscle myofibrillar protein metabolism, with a specific emphasis on changes in myosin heavy chain (MHC) protein content, synthesis, and isoform distribution in 10 patients with heart failure (63 ± 3 yr) and 11 controls (70 ± 3 yr). In addition, we examined the relationship of MHC protein metabolism to inflammatory markers and physical function. Although MHC and actin protein content did not differ between groups, MHC protein content decreased with increasing disease severity in heart failure patients (r = −0.748, P < 0.02), whereas actin protein content was not related to disease severity. No difference in MHC protein synthesis was found between groups, and MHC protein synthesis rates were not related to disease severity. There were, however, relationships between C-reactive protein and both MHC protein synthesis (r = −0.442, P = 0.05) and the ratio of MHC to mixed muscle protein synthesis (r = −0.493, P < 0.03). Heart failure patients showed reduced relative amounts of MHC I (P < 0.05) and a trend toward increased MHC IIX (P = 0.06). In regression analyses, decreased MHC protein content was related to decreased exercise capacity and muscle strength in heart failure patients. Our results demonstrate that heart failure affects both the quantity and isoform distribution of skeletal muscle MHC protein. The fact that MHC protein content was related to both exercise capacity and muscle strength further suggests that quantitative alterations in MHC protein may have functional significance.

sarcopenia; cardiac cachexia; myosin heavy chain isoform; cytokine; actin

RESEARCH OVER THE PAST TWO DECADES has identified an important role for skeletal muscle in reduced functional capacity in heart failure patients. Several abnormalities intrinsic to skeletal muscle, including muscle atrophy (7, 30) and diminished oxidative capacity (35), have been cited as factors contributing to physical disability. The mechanisms underlying these defects and their contribution to exercise intolerance and reduced muscle strength, however, remain unclear. The majority of studies that have examined the effect of heart failure on skeletal muscle have focused on oxidative capacity and/or mitochondrial function. In contrast, less is known about the impact of heart failure on myofibrillar proteins and their relationship to reduced functional capacity.

Protein is the primary structural and functional macromolecule in skeletal muscle. From both quantitative and functional perspectives, myosin heavy chain (MHC) is the most important protein in skeletal muscle. It comprises ~25% of cellular protein mass (36) and is a key structural component of myofibrils. In addition, together with actin and associated regulatory proteins, MHC is responsible for the generation of mechanical work. Alterations in MHC protein content and/or isoform distribution, therefore, can have significant effects on the morphology, structural integrity, and functionality of skeletal muscle.

Several studies have examined the effect of heart failure on skeletal muscle MHC protein metabolism. The majority of work in this area has focused on MHC isoform distribution. In general, human studies show a reduction in MHC I and a corresponding increase in MHC IIX isoform expression (25, 33). In contrast to isoform data, considerably less is known about how heart failure affects MHC protein content. To our knowledge, only one study has examined the effect of heart failure on MHC protein content. In a rat model of failure, Simonini et al. (22) observed reduced skeletal muscle MHC protein in rats with heart failure compared with controls. No study, however, has measured skeletal muscle MHC protein content in human heart failure patients. Moreover, the relative contribution of quantitative vs. qualitative (i.e., isoform distribution) changes in MHC to reduced functional capacity in heart failure patients has not been examined.

Numerous studies suggest that immune activation contributes to the skeletal muscle myopathy of heart failure (2, 3, 17), specifically that inflammatory mediators, such as cytokines, promote skeletal muscle wasting. Although the ability of cytokines to promote muscle atrophy has been well characterized (12, 15), their role in muscle catabolism in heart failure has not been clearly defined. Anker et al. (4) have shown that increased circulating cytokine levels were related to reduced skeletal muscle mass in heart failure patients (4). More recently, Larsen et al. (13) showed a negative relationship between interleukin-6 (IL-6) levels and muscle fiber size. Whether these relationships reflect a direct catabolic effect of cytokines on skeletal muscle or an indirect effect of cytokines (e.g., weight loss-induced muscle atrophy), however, is not known. In support of the latter interpretation, studies from our laboratory (18, 30, 31) suggest that muscle wasting is explained largely by weight loss secondary to reduced dietary energy intake. A
A clearer understanding of the role that cytokines play in muscle wasting would be gained by examining their relationship to the biochemical processes that regulate muscle mass (e.g., skeletal muscle protein synthesis). To date, however, no study has examined the relationship of cytokines to skeletal muscle protein metabolism in heart failure patients. Moreover, there is no information regarding the relationship of cytokines to heart failure-induced alterations in the quantity or isoform distribution of key myofibrillar proteins, such as MHC.

In the present study, our objective was threefold. First, we sought to evaluate the effects of heart failure on skeletal muscle myofibrillar protein metabolism, with a specific emphasis on changes in MHC protein quantity and isoform distribution. Second, we examined the relationship of alterations in myofibrillar protein metabolism to circulating cytokine levels. Finally, we evaluated quantitative and qualitative changes in MHC protein as determinants of reduced exercise capacity and muscle strength in heart failure patients. To accomplish these objectives, we measured skeletal muscle myofibrillar protein content, isoform expression and protein synthesis, circulating cytokines and markers of immune activation [i.e., C-reactive protein (CRP)], exercise capacity, and knee extensor muscle strength in heart failure patients and elderly controls. We hypothesized that heart failure patients would be characterized by reduced MHC protein content and a shift in MHC protein isoform toward a more fast-twitch phenotype. The reduction in MHC protein content would be accompanied by reduced MHC protein synthesis rates, which, in turn, would be related to increased circulating cytokine levels. Finally, quantitative changes in MHC protein would predict exercise intolerance and muscle weakness in heart failure patients.

MATERIALS AND METHODS

Subjects. Ten 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: 32 ± 4% by echocardiography). New York Heart Association (NYHA) functional class averaged 2.5 ± 0.2, with six patients in class II, three in class III, and one in class IV. Heart failure was due to coronary artery disease in seven patients, defined by history of myocardial infarction and/or multivessel coronary obstructions at cardiac catheterization, idioventricular dilated cardiomyopathy in two patients, and dilated cardiomyopathy secondary to severe hypertension in one patient. Heart failure patients were taking the following medications: diuretics (n = 10, 100%), digoxin (n = 7, 70%), angiotensin-converting enzyme inhibitors (n = 9, 90%) and β-adrenergic blocking agents (n = 8, 80%). Additionally, one patient had insulin-dependent diabetes mellitus, and two patients had non-insulin-dependent diabetes mellitus and were treated with oral hypoglycemic drugs. At the time of testing, patients were clinically stable and free of peripheral edema.

Eleven male volunteers were recruited to serve as controls. Nine of these volunteers were healthy and free of disease, had no signs or symptoms of heart disease, and had 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 exertional ischemia, as demonstrated by a normal electrocardiographic stress test to exhaustion. Both patients were treated with aspirin, one with a Ca2+ channel blocker, and the other with a hydroxymethylglutaryl-CoA reductase inhibitor. Because inclusion of data from these two controls did not impact group differences or associations among variables (see RESULTS), we decided to include them in the control group. Other medications in the control group were anti-epileptic (n = 1), anti-anxiety (n = 1), 5α-reductase inhibitor (n = 1), and nonsteroidal anti-inflammatory (n = 1). Controls had no history of diabetes mellitus and had normal fasting blood glucose (<6.22 mmol/l) and normal glucose tolerance (glucose <7.77 mmol/l 2 h after a 75-g oral glucose load). The nature, purpose, and possible risks of the study were explained to each subject before written consent was given to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

Experimental protocol. Each volunteer was tested during outpatient and inpatient visits to the General Clinical Research Center (GCRC). Body composition, exercise capacity, and muscle strength were measured on an outpatient basis and were separated from skeletal muscle protein synthesis measurements by ~1 wk. Skeletal muscle protein synthesis was measured during an inpatient visit to the GCRC. 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 regardless of dosing regimens.

On the following morning, the subject was awakened, and Teflon catheters were placed in an antecubital vein and a dorsal hand vein. At ~0630 (0 min), a primed (4.8 μmol/kg), continuous (5.6 μmol·kg−1·min−1) infusion of [1,2-13C2]leucine was started, and the bicarbonate pool was primed (3.1 μmol/kg) with sodium [13C]bicarbonate. Muscle tissue (100–150 mg) was taken from the vastus lateralis muscle under lidocaine anesthesia by percutaneous needle biopsy at 90 min and from the contralateral leg at 480 min. Biopsy samples were cleaned of adipose and connective tissue, frozen in liquid nitrogen, and stored at ~70°C until analysis. Blood samples were drawn at 0, 300, 360, 420, and 480 min.

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. (9). We have shown previously (30) that there is no effect of heart failure on total body water or the hydration of fat-free tissue in heart failure patients on stable diuretic therapy and no signs or symptoms of edema. Thus we do not believe that alterations in fluid homeostasis had any impact on group differences in muscle mass or their association to cytokines in the present study.

Exercise capacity. Peak oxygen consumption (V̇O2 peak) 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. The grade was increased 2.5% every 2 min until volitional fatigue. V̇O2 peak was defined as the highest 30-s average V̇O2 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.

Skeletal muscle strength. Isometric and isokinetic knee extensor strength was measured using a multijoint dynamometer (Lido Active; Loredan Biomedical, Sacramento, CA). The right leg was tested in all subjects. The volunteer was seated and positioned so that the lateral femoral epicondyle was aligned with the central axis of the dynamometer. Exaneous movement was restricted by a velcro strap placed across the abdomen and a padded restraint at the distal point of the thigh. The lever arm of the dynamometer was attached just proximal to the lateral malleolus. Following instructions, volunteers were allowed to perform several practice trials for each condition at moderate intensity (~25%) to ensure familiarity with the procedure. For isometric measurements, the lever arm was fixed at 55°. Volunteers performed three brief (5-s) maximal voluntary contractions, each separated by 1 min of rest. The highest torque (Nm) value for each contraction was recorded. The average from the three trials was calculated. Isokinetic measurements were performed at 90°/s. The range of motion was set from 0 to 90° flexion relative to full knee.
extension. Volunteers performed 15 consecutive contractions. The average of the three highest torque values from the first five contractions was recorded and averaged.

**MHC content and isoform expression.** MHC and actin content were determined by SDS-PAGE, as described by Haddad et al. (6), with minor modifications. Muscle tissue (~15 mg) was homogenized in buffer (50 mM HEPES, pH 7.4, 150 mM KCl, 1 mM EGTA, 1 mM benzamidine, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml chymostatin, 1 µg/ml leupeptin, 1 µg/ml antipain, 1 µg/ml pepstatin) with a glass homogenizer. The homogenate was analyzed for protein content (total muscle protein content) using bovine serum albumin as a standard (Bio-Rad Laboratories, Hercules, CA). An aliquot of the muscle homogenate was added to loading buffer (2% SDS, 62.5 mM Tris, 10% glycerol, 0.001% bromophenol blue, pH 6.8, with 5% β-mercaptoethanol), heated for 5 min at 100°C, and analyzed by SDS-PAGE. For each muscle sample, 2.5 µg of protein were loaded per lane. The stacking gel contained 4% acrylamide-N,N′-methylene-bis-acrylamide (bis) and the resolving gel 10% acrylamide-bis. Gels were run at constant current (60 mA) for 2.5 h at 22°C and then stained with Coomassie blue. MHC and actin band intensities were determined by densitometry (Quantity One, Bio-Rad Laboratories) and were expressed as densitometric units per microgram of protein loaded. All densitometric measurements were done by the same assessor, who was blinded to group status.

The relative distribution of MHC isoforms was determined according to the method of Klitgaard et al. (11), with minor modifications. Briefly, ~15 mg of muscle tissue were homogenized in buffer (20 mM KCl, 2 mM K2HPO4, 1 mM EGTA, pH 6.8) and incubated on ice for 15 min. The homogenate was centrifuged at 12,000 g for 15 min, washed with homogenization buffer, and centrifuged for 1 min at 12,000 g. The supernatant was decanted and the pellet extracted with 40 mM Na3P04.1, 1 mM MgCl2, and 1 mM EGTA, pH 9.5, on ice for 15 min. The supernatant was assayed for protein content with bovine serum albumin as a standard (Pierce Biotechnology, Rockford, IL) added to loading buffer, and heated for 5 min at 100°C. The stacking gel contained 37.5% glycerol (wt/vol) and 4% acrylamide-bis (50:1), and the resolving gel contained 37.5% glycerol and 6% acrylamide-bis (50:1). Gels were run at 70 V for 16 h followed by 4 h at 200 V at 6°C. Gels were stained with Coomassie blue, and the relative distribution of MHC isoforms was quantified by densitometry (Quantity One). All densitometric measurements were done by the same assessor, who was blinded to group status.

**Skeletal muscle protein synthesis.** Mixed muscle protein synthesis measurements were performed as described previously (18). Briefly, muscle tissue (~20 mg) was homogenized in solubilization buffer (100 mM sodium pyrophosphate, 1% SDS, and 4 mM EGTA, pH 7.4), the resulting homogenate was centrifuged and the supernatant decanted, trichloroacetic acid (TCA) was added (10% final concentration), and the sample was allowed to precipitate overnight (~14 h) and then centrifuged. The precipitate was washed first with 10% TCA, then with distilled water, and finally with petroleum ether. The ether was evaporated under N2. 6 ml of 6 N HCl was added, and the tube was capped and heated for 24 h at 110°C. Amino acids in the hydrolysate were isolated by ion exchange chromatography and derivatized to their ECF derivative, and ECF-leucine enrichment was measured by GC-C-IRMS.

**Mixed muscle protein synthesis.** MHC and nonmyofibrillar protein synthesis measurements were performed according to the methods of Hasten et al. (8). Briefly, muscle tissue (~50 mg) was homogenized in sucrose buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, 20 mM imidazole, pH 6.8), the homogenate was centrifuged, and the supernatant was discarded. The pellet was suspended in Triton X-100 buffer (175 mM KCl, 0.5% Triton X-100, pH 6.8), homogenized, and centrifuged. The supernatant, which contains nonmyofibrillar proteins (23), was decanted. KCl buffer (150 mM KCl, 20 mM Tris base, pH 7.0) was added to the pellet, the pellet was homogenized, and the resulting supernatant was added to the nonmyofibrillar fraction. This process was repeated, TCA was added (10% final concentration) to the nonmyofibrillar fraction, and the sample was allowed to precipitate overnight (~14 h) and then centrifuged. Further processing and measurement of leucine enrichment in the nonmyofibrillar protein fraction were similar to those of mixed muscle protein described above, excluding the petroleum ether wash. The pellet, which contained myofibrillar proteins, was solubilized in loading buffer (2% SDS, 62.5 mM Tris, 10% KCl, 0.001% bromophenol blue, pH 8.8, with 5% β-mercaptoethanol), vortexed, and heated at 100°C for 5 min.

MHC was isolated by SDS-PAGE. The stacking gel contained 4% acrylamide-bis (37.5:1) and the resolving gel 7% acrylamide-bis (37.5:1). The myofibrillar protein fraction was loaded onto the gel and run at 150 V until the dye front reached the resolving gel and then was increased to 220 V until the dye front reached the end of the gel (~4 h). The gels were stained with Coomassie blue and destained with 10% acetic acid. The MHC band was excised from the gel and placed into a test tube, 4 ml of 6 N HCl were added, and the mixture was heated at 110°C for 48 h. Amino acids were isolated and derivatized to their ECF derivative, and ECF-leucine enrichment was measured by GC-C-IRMS.

The fractional synthesis rates (ks) of mixed muscle, MHC, and nonmyofibrillar protein were calculated as: ks = (E3 - E1)/(E3 - E2), where E2 and E1 are the enrichments (MPE) of leucine in skeletal muscle protein from biopsies taken at 480 and 90 min, respectively, and t is the difference in time (in days) between these biopsies. Plasma α-keratinocaprate was used as a proxy for intracellular precursor pool enrichment (E2, 5, 34). MHC protein synthesis measurements was not completed on one heart failure patient due to technical problems.

**Hormone analysis.** CRP was measured by enzyme-linked immunosorbent assay [ELISA (16)], with an interassay coefficient of variation (CV) ranging from 2 to 4%. Tumor necrosis factor-α (TNF-α) and IL-6 plasma concentrations and soluble receptors (TNF-α RII and IL-6 sR, respectively) were measured by ultrasensitive ELISA assays (R&D Systems; Minneapolis, MN) with interassay CVs of 16 and 6% for TNF-α and IL-6 concentration and 9 and 10% for their respective receptors.

**Statistical analysis.** Differences between groups were determined by unpaired Student’s t-tests. Relationships between variables were assessed using Pearson correlation coefficients. Variables that were not normally distributed (e.g., cytokines), were log10 transformed before correlation analysis. Distributional assumptions of log10-transformed variables were tested before analysis by use of the Shapiro Wilk test. All analyses were conducted with SPSS software version 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, body composition, or total or regional muscle mass were observed between groups.

Skeletal muscle MHC and actin protein content data are shown in Fig. 1. Neither MHC content [heart failure (HF): 142 ± 9 vs. control (C): 179 ± 23 arbitrary units (AU)/µg protein, P = 0.16] nor actin content (HF: 172 ± 11 vs. C: 166 ± 14 AU/µg protein) differed between groups. Similarly, the actin-to-myosin ratio did not differ between groups (HF: 1.24 ± 0.10 vs. C: 1.04 ± 0.10, P = 0.15). Exclusion of the two controls with coronary artery disease did not affect group differences in MHC (HF: 142 ± 9 vs. C: 179 ± 25 AU/µg protein) or actin (HF: 172 ± 11 vs. C: 164 ± 15 AU/µg protein) protein content.
MHC content was negatively related to NYHA class in heart failure patients ($r = -0.748$, $P < 0.02$; Fig. 2). In contrast, no relationship was found between actin content and NYHA class ($r = -0.07$, not significant; Fig. 2). To further evaluate whether the negative relationship between MHC content and NYHA class was due to the one class IV patient, we compared class II and III patients. There was a 23% reduction in MHC protein content in class III patients compared with class II patients (class II: $159 \pm 9$ vs. class III: $122 \pm 12$ AU/µg protein; $P < 0.05$). In addition, no difference in actin content was found (class II: $164 \pm 16$ vs. class III: $170 \pm 20$ AU/µg). Neither MHC ($r = 0.224$) nor actin ($r = -0.184$) protein content was related to ejection fraction.

Skeletal muscle protein synthesis data are shown in Fig. 3. No differences in mixed muscle (HF: $0.89 \pm 0.06$ vs. C: $0.84 \pm 0.06$%/day), MHC (HF: $0.65 \pm 0.06$ vs. C: $0.74 \pm 0.08$%/day), or nonmyofibrillar (HF: $1.29 \pm 0.12$ vs. C: $1.21 \pm 0.09$%/day) protein synthesis were observed. No difference in the ratio of MHC to mixed muscle protein synthesis (HF: $0.73 \pm 0.12$ vs. C: $0.87 \pm 0.05$%/day) was found. Exclusion of the two controls with coronary artery disease did not alter differences in mixed muscle (HF: $0.89 \pm 0.06$ vs. C: $0.79 \pm 0.06$%/day), MHC (HF: $0.65 \pm 0.06$ vs. C: $0.72 \pm 0.09$%/day), or nonmyofibrillar (HF: $1.29 \pm 0.12$ vs. C: $1.16 \pm 0.09$%/day) protein synthesis rates. No relationship was found between any index of skeletal muscle protein synthesis and either NYHA class or ejection fraction in heart failure patients.

The relationships of various markers of immune activation to muscle protein synthesis measurements in the entire cohort are shown in Table 2. Of note, MHC protein synthesis rates were negatively related to circulating CRP levels ($r = -0.443$, $P = 0.05$). The relationship between CRP and MHC protein synthesis was not significant when examined in heart failure patients ($r = -0.356$, $P = 0.35$) or controls ($r = -0.471$, $P = 0.144$) separately. CRP was negatively related to the ratio of MHC to mixed muscle protein synthesis in the combined cohort ($r = -0.493$, $P < 0.03$) and when heart failure patients were analyzed separately ($r = -0.683$, $P < 0.05$) but not in controls ($r = -0.132$). Scatterplots for the relationship of CRP to muscle protein synthesis are shown in Fig. 4. In addition, IL-6 was negatively related to mixed muscle protein synthesis in controls ($r = -0.626$, $P < 0.05$), and TNF-α levels were positively related to nonmyofibrillar protein synthesis in heart failure patients ($r = 0.637$, $P < 0.05$). Cytokine measurements were not related to MHC or actin protein content or MHC.
isof orm distribution in the total sample or when heart failure patients and controls were analyzed separately.

Figure 5 shows skeletal muscle MHC isoform expression. A representative gel showing the effect of heart failure on MHC isoform expression is provided in Fig. 5A. Figure 5B illustrates group mean differences in MHC isoform expression. Heart failure patients were characterized by reduced (P < 0.05) expression of MHC I (HF: 50.1 ± 6.8 vs. C: 66.5 ± 3.8%) and a trend (P = 0.06) toward increased expression of MHC IIx (HF: 11.0 ± 4.3 vs. C: 2.4 ± 1.1%). No difference in expression of MHC Ila was found (HF: 38.7 ± 4.5 vs. C: 31.1 ± 3.4%). Exclusion of the two controls with coronary artery disease did not affect group differences in MHC isoform expression. Heart failure isoform distribution measurements and either NYHA class or ejection fraction in heart failure patients.

\[ \text{Values are Pearson correlation coefficients. For myosin heavy chain (MHC) measurements, } n = 20. \text{ C-reactive protein, interleukin-6, and tumor necrosis factor-} \alpha \text{ were log}_{10} \text{ transformed before correlation analysis.} \]

\[ * P < 0.05; † P = 0.06. \]

\[ \text{Table 2: Correlations between markers of immune activation and muscle protein synthesis rates in heart failure patients and controls} \]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mixed Muscle Protein</th>
<th>MHC</th>
<th>Nonmyofibrillar Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td>0.065</td>
<td>-0.442*</td>
<td>0.060</td>
</tr>
<tr>
<td>Interleukin-6 sR</td>
<td>-0.148</td>
<td>-0.275</td>
<td>0.069</td>
</tr>
<tr>
<td>Tumor necrosis factor-(\alpha)</td>
<td>-0.028</td>
<td>-0.281</td>
<td>0.423†</td>
</tr>
<tr>
<td>Tumor necrosis factor-(\alpha) RII</td>
<td>-0.119</td>
<td>-0.164</td>
<td>0.308</td>
</tr>
</tbody>
</table>

\[ \text{Fig. 5. Effect of heart failure on skeletal muscle MHC isoform expression. } A: \text{ representative gel for the 3 isoforms of skeletal muscle MHC (I, Ila, and IIx) in controls (C) and HF patients. B: group mean data for HF patients (filled bars) and controls (open bars). Data are expressed as % total MHC expression. Values are means ± SE.} * P < 0.05; † P = 0.06. \]

\[ \text{\(V_{\text{O}_2 \text{peak}}\) was 36% lower (HF: 1.62 ± 0.22 vs. C: 2.55 ± 0.19 l/min, } P < 0.01) \text{ in heart failure patients compared with controls. This reduction in exercise capacity persisted after } \text{\(V_{\text{O}_2 \text{peak}}\) data were expressed relative to body mass (HF: 20.2 ± 1.7 vs. C: 31.9 ± 1.8 ml·kg body wt}^{-1} \text{·min}^{-1}, \text{ } P < 0.01) \text{ or fat-free mass (HF: 26.9 ± 2.5 vs. C: 43.3 ± 2.0 ml·kg FFM}^{-1} \text{·min}^{-1}, \text{ } P < 0.01). \text{ Similarly, both isokinetic (HF: 88 ± 10 vs. C: 121 ± 11 Nm) and isometric (HF: 144 ± 13 vs. C: 190 ± 15 Nm) knee extensor strength were reduced (both } P < 0.05) \text{ in heart failure patients compared with controls. The relationship of quantitative and qualitative aspects of skeletal muscle myofibrillar protein to functional capacity measurements in heart failure patients are shown in Table 3. MHC protein content was positively related to aerobic capacity (} P < 0.01) \text{ and isokinetic knee extensor strength (} P < 0.05). \text{ In contrast, no relationship was found between MHC isoform distribution measurements and either aerobic capacity or muscle strength. The scatterplots for the relationship of MHC protein content to aerobic capacity and isokinetic knee extensor strength are shown in Fig. 6.} \]

\[ \text{Values represent Pearson correlation coefficients. Peak oxygen consumption (} V_{\text{O}_2 \text{peak}}\text{) represents } V_{\text{O}_2 \text{peak}} \text{ expressed relative to fat-free mass (i.e., ml·kg FFM}^{-1} \text{·min}^{-1}). \text{ *} P = 0.05; † P < 0.01. \text{ All correlations are based on } n = 10 \text{ heart failure patients, except } V_{\text{O}_2 \text{peak}}, \text{ which was } n = 9. \]

\[ \text{Table 3: Relationship of myofibrillar protein quantity and quality measurements to functional capacity in heart failure patients} \]

<table>
<thead>
<tr>
<th>MHC protein</th>
<th>(V_{\text{O}_2 \text{peak}})</th>
<th>Isokinetic Strength</th>
<th>Isometric Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC</td>
<td>0.870†</td>
<td>0.628*</td>
<td>0.488</td>
</tr>
<tr>
<td>Actin protein</td>
<td>0.135</td>
<td>0.222</td>
<td>0.506</td>
</tr>
<tr>
<td>MHC I</td>
<td>0.005</td>
<td>-0.208</td>
<td>-0.231</td>
</tr>
<tr>
<td>MHC Ila</td>
<td>-0.586</td>
<td>-0.032</td>
<td>0.257</td>
</tr>
<tr>
<td>MHC IIx</td>
<td>0.513</td>
<td>0.364</td>
<td>0.101</td>
</tr>
</tbody>
</table>

\[ \text{Fig. 4. Relationship of log}_{10} \text{ C-reactive protein (log CRP) levels to MHC protein synthesis rate (} n = 20; \text{ } r = -0.443, P = 0.05) \text{ and the ratio of MHC to MMP synthesis rate (} n = 20; \text{ } r = -0.493, P < 0.03). } }\]
muscle protein was 21% lower in heart failure patients (Fig. 2). We should note that this type of selective MHC protein depletion, without changes in actin protein content, is not unprecedented. Previous studies in animal models and humans have observed a selective loss of skeletal muscle MHC under conditions of muscle disuse (6) and muscle disuse combined with increased catabolic hormones (14, 21). Previous studies from our laboratory have shown that heart failure patients are profoundly inactive (31). Moreover, heart failure is characterized by increased circulating concentrations of several catabolic hormones (2). Thus the combination of muscle disuse and a catabolic hormonal milieu may promote selective MHC protein depletion in heart failure. That both physical inactivity and catabolic hormone levels are potentiated with disease progression (26, 28, 29) may explain the negative relationship between MHC protein content and NYHA functional class.

To further explore the potential mechanisms underlying differences in MHC protein content among heart failure patients, we measured skeletal muscle protein synthesis rates, including the synthesis rate of MHC protein (19, 20). No differences, however, were observed in MHC, mixed muscle, or nonmyofibrillar protein synthesis rates between heart failure patients and controls. These findings are similar to recent data from our laboratory in an animal model of heart failure (18). In addition, protein synthesis rates were not related to NYHA functional class or ejection fraction in heart failure patients. Our results agree with those of Simonini et al. (22), who, despite finding reduced MHC protein content, did not observe a reduction in MHC protein synthesis rates in rats with heart failure. The reason for the disparity between MHC protein content and synthesis rates is not clear but may relate to the temporal context of each measurement. For example, in the present study, protein synthesis was measured under postabsorptive conditions over a relatively short period of time (8 h). Therefore, synthesis measurements are indicative of protein metabolism at one discrete time point along the disease continuum. In contrast, MHC protein content measurements reflect the net balance between protein synthesis and breakdown over months or years of exposure to heart failure. We may be limited in our ability to detect reduced protein synthesis rates that contributed to MHC protein depletion unless heart failure patients were actively losing muscle protein at the time of measurement.

Despite the lack of group differences in MHC protein synthesis, we did observe negative correlations between MHC protein synthesis and CRP, a marker of immune activation. Because heart failure patients are characterized by increased circulating levels of several cytokines (1) in proportion to the severity of their disease (26, 27), this finding suggests that immune activation may contribute to the reduction of skeletal muscle MHC protein content by decreasing MHC protein synthesis. Although we cannot discern cause and effect from correlation analysis, the inhibitory effects of cytokines on

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 alterations in skeletal muscle that may contribute to reduced functional capacity, the present investigation examined the effect of heart failure on skeletal muscle myofibrillar protein metabolism, with a specific focus on changes in MHC protein content, synthesis, and isoform distribution. We found no difference in MHC or actin protein content between heart failure patients and controls. Despite there being no group differences, MHC protein content was negatively related to disease severity. In contrast, no relationship was found between actin content and disease severity. MHC protein synthesis rates and the ratio of MHC to mixed muscle protein synthesis rates were negatively related to CRP. Qualitative changes in MHC protein were also noted; specifically, heart failure patients were characterized by a shift in MHC isoform toward a more fast-twitch phenotype. Although heart failure was characterized by changes in both MHC quantity and isoform distribution, variation in MHC content was a stronger correlate of reduced exercise capacity and muscle strength in heart failure patients.

MHC and actin protein content were not significantly different between heart failure patients and controls. We should point out, however, that MHC content expressed per unit total muscle protein was 21% lower in heart failure patients ($P = 0.16$). In contrast, actin protein content was nearly identical between groups. Although differences did not reach significance, it is difficult not to ascribe some physiological significance to such a marked depletion of MHC. Indeed, recent work by Larsson et al. (14) has shown a direct relationship between MHC protein content and single muscle fiber contractile performance. A reduction in MHC protein content with heart failure has been documented by other laboratories. In an animal model of failure, Simonini et al. (22) showed that soleus MHC protein content was reduced by 30% in rats with heart failure compared with control animals. Thus reduced MHC protein content may be a novel feature of the skeletal muscle myopathy of heart failure.

Perhaps more important than group differences, we found that variation in MHC content was negatively correlated to disease severity in heart failure patients, as indicated by NYHA functional class, whereas actin content was not (Fig. 2). We should note that this type of selective MHC protein depletion, without changes in actin protein content, is not unprecedented. Previous studies in animal models and humans have observed a selective loss of skeletal muscle MHC under conditions of muscle disuse (6) and muscle disuse combined with increased catabolic hormones (14, 21). Previous studies from our laboratory have shown that heart failure patients are profoundly inactive (31). Moreover, heart failure is characterized by increased circulating concentrations of several catabolic hormones (2). Thus the combination of muscle disuse and a catabolic hormonal milieu may promote selective MHC protein depletion in heart failure. That both physical inactivity and catabolic hormone levels are potentiated with disease progression (26, 28, 29) may explain the negative relationship between MHC protein content and NYHA functional class.

To further explore the potential mechanisms underlying differences in MHC protein content among heart failure patients, we measured skeletal muscle protein synthesis rates, including the synthesis rate of MHC protein (19, 20). No differences, however, were observed in MHC, mixed muscle, or nonmyofibrillar protein synthesis rates between heart failure patients and controls. These findings are similar to recent data from our laboratory in an animal model of heart failure (18). In addition, protein synthesis rates were not related to NYHA functional class or ejection fraction in heart failure patients. Our results agree with those of Simonini et al. (22), who, despite finding reduced MHC protein content, did not observe a reduction in MHC protein synthesis rates in rats with heart failure. The reason for the disparity between MHC protein content and synthesis rates is not clear but may relate to the temporal context of each measurement. For example, in the present study, protein synthesis was measured under postabsorptive conditions over a relatively short period of time (8 h). Therefore, synthesis measurements are indicative of protein metabolism at one discrete time point along the disease continuum. In contrast, MHC protein content measurements reflect the net balance between protein synthesis and breakdown over months or years of exposure to heart failure. We may be limited in our ability to detect reduced protein synthesis rates that contributed to MHC protein depletion unless heart failure patients were actively losing muscle protein at the time of measurement.
skeletal muscle protein synthesis have been well characterized (12, 15). In fact, certain cytokines may have an effect to selectively deplete MHC (15). That cytokines may have a similar effect in humans is implied by the negative relationship between CRP and the ratio of MHC to mixed muscle protein synthesis rates. From these relationships, we suggest the hypothesis that immune activation contributes to the selective depletion of skeletal muscle MHC protein in heart failure by reducing MHC protein synthesis.

Heart failure patients were characterized by a shift in MHC isoform distribution toward a more fast-twitch phenotype. Our results are in accord with prior studies (24, 33). Despite the agreement among studies, the mechanism underlying altered skeletal muscle MHC isoform distribution in heart failure has not been identified. Because circulating cytokine levels were not correlated with MHC isoform content, our data argue against the hypothesis that shifts in MHC distribution are related to immune activation. Instead, we favor the hypothesis that isoform shifts are due primarily to muscle disuse. We should acknowledge, however, that some studies indicate that factors other than inactivity contribute to MHC isoform shifts (32).

A novel finding of our study was that MHC content was correlated to both VO2 peak and isokinetic muscle strength in heart failure patients. These relationships are logical given that the power output of a muscle is determined by the number of MHC molecules. In fact, Larsson et al. (14) have demonstrated recently that single muscle fiber contractile function is directly related to MHC protein content. We should acknowledge, however, that the relationships between MHC protein content and physical function variables may be due to their shared association with another variable, such as physical inactivity, which is a predominant feature of the heart failure syndrome (31). Indeed, it is axiomatic that aerobic capacity and muscle strength are reduced with physical inactivity. Additionally, selective loss of MHC protein content has been observed in animal models of muscle disuse (6). Because MHC content is negatively related to disease severity, it could also be argued that these relationships reflect the fact that both MHC content and functional capacity measurements decline with increasing disease severity. This possibility seems unlikely, however, because statistical control for NYHA class did not diminish the relationship of MHC content to VO2 peak (partial r = 0.771, P < 0.03), although it did lessen the correlation with muscle strength (partial r = 0.372, P = 0.33). Nonetheless, the fact that MHC protein content is reduced with increased severity of failure and is related to strength and endurance measurements suggests that quantitative changes in MHC may contribute to reduced functional capacity.

In summary, our results suggest that heart failure is associated with both quantitative and qualitative changes in skeletal muscle MHC protein. Importantly, these alterations were observed in a cohort of heart failure patients not characterized by skeletal muscle wasting. Thus changes in myofibrillar protein metabolism are likely due to heart failure per se and not the skeletal muscle atrophy that often accompanies the disease. A novel finding of our study is that heart failure patients were characterized by a selective loss of skeletal muscle MHC protein with increasing disease severity. That MHC protein synthesis rates were negatively related to CRP suggests a possible role for immune activation in the depletion of MHC protein in heart failure patients. Finally, a reduction in MHC protein content was related to exercise intolerance and reduced muscle strength. Taken together, these findings identify a new feature of the skeletal muscle myopathy of failure, reduced MHC protein content, which is correlated with both the severity of the disease and immune activation and may contribute to reduced functional capacity.

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GRANTS

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