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Analysis of Skeletal Muscle Gene Expression Patterns and the Impact of Functional Capacity in Patients With Systolic Heart Failure

DANIEL E. FORMAN, MD^{1,2,3}, KARLA M. DANIELS, MS³, LAWRENCE P. CAHALIN, PhD, PT⁴, ALEXANDRA ZAVIN, BS¹, KELLY ALLSUP, BS¹, PEIRANG CAO, PhD⁵, MAHALAKSHMI SANTHANAM, PhD⁵, JACOB JOSEPH, MD^{2,3}, ROSS ARENA, PhD, PT⁶, ANTONIO LAZZARI, MD^{7,8}, P. CHRISTIAN SCHULZE, MD, PhD⁹, and STEWART H. LECKER, MD, PhD⁸

¹New England Geriatric Research, Education, and Clinical Center, Veterans Affairs Boston Healthcare System, Boston, Massachusetts

²Division of Cardiovascular Medicine, Veterans Affairs Boston Healthcare System, Boston, Massachusetts

³Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical, School, Boston, Massachusetts

⁴Department of Physical Therapy, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida

⁵Division of Nephrology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

⁶Department of Physical Therapy and Integrative Physiology Laboratory e College of Applied Health Sciences, University of Illinois Chicago, Chicago, Illinois

⁷Division of Rheumatology, Veterans Affairs Boston Healthcare System, Boston, Massachusetts

⁸Boston University School of Medicine, Boston, Massachusetts

⁹Division of Cardiovascular Medicine, Columbia University Medical Center, New York, New York.

Abstract

Background—Declining physical function is common among systolic heart failure (HF) patients and heralds poor clinical outcomes. We hypothesized that coordinated shifts in expression of ubiquitin-mediated atrophy-promoting genes are associated with muscle atrophy and contribute to decreased physical function.

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Reprint requests: Daniel E. Forman, MD, Division of Cardiovascular Medicine, Brigham and Women's Hospital, 75 Francis St, Boston, MA 02115. Tel: 857-307-1989; Fax: 857-307-1955. deforman@partners.org.

Disclosures

None.

Methods—Systolic HF patients (left ventricular ejection fraction [LVEF] 40%) underwent skeletal muscle biopsies (nondominant vastus lateralis) and comprehensive physical assessments. Skeletal muscle gene expression was assessed with the use of real-time polymerase chain reaction. Aerobic function was assessed with the use of cardiopulmonary exercise and 6-minute walk tests. Strength capacity was assessed with the use of pneumatic leg press (maximum strength and power). Serologic inflammatory markers also were assessed.

Results—54 male patients (66.6 ± 10.0 years) were studied: 24 systolic HF patients (mean LVEF $28.9 \pm 7.8\%$) and 30 age-matched control subjects. Aerobic and strength parameters were diminished in HF versus control. FoxO1 and FoxO3 were increased in HF versus control (7.9 ± 6.2 vs 5.0 ± 3.5 , 6.5 ± 4.3 vs 4.3 ± 2.8 relative units, respectively; $P = .05$ in both). However, atrogin-1 and MuRF-1 were similar in both groups. PGC-1 α was also increased in HF (7.9 ± 5.4 vs 5.3 ± 3.6 relative units; $P < .05$). Muscle levels of insulin-like growth factor (IGF) 1 as well as serum levels of tumor necrosis factor α , C-reactive protein, inter-leukin (IL) 1 β , and IL-6 were similar in HF and control.

Conclusion—Expression of the atrophy-promoting genes FoxO1 and FoxO3 were increased in skeletal muscle in systolic HF compared with control, but other atrophy gene expression patterns (atrogin-1 and MuRF-1), as well as growth promoting patterns (IGF-1), were similar. PGC-1 α , a gene critical in enhancing mitochondrial function and moderating FoxO activity, may play an important counterregulatory role to offset ubiquitin pathway-mediated functional decrements.

Keywords

Heart failure; skeletal muscle; gene expression

Functional decline is typical among heart failure (HF) patients.¹ Alterations to skeletal function, including muscle weakening and atrophy, influence the functional decline, particularly among older adults who, because of old age and a relatively sedentary lifestyle, are also prone to sarcopenia.^{2,3} Although molecular mechanisms underlying HF-related skeletal muscle changes have been notably studied in animal models, human studies remain rare.⁴ Clarification of the molecular mechanisms by which skeletal muscle atrophies and weakens may ultimately facilitate therapies that better restore or preserve physical function in today's growing population of older HF patients who are prone to disability, dependency, and diminished quality of life.

In animal models, changes in expression of key genes (termed “atrogenes”) have been demonstrated to underlie the induction of the adenosine triphosphate-dependent ubiquitin (Ub) proteasome pathway (UPP) in atrophying muscle.^{5–7} Two muscle-specific E3-ligases, atrogin-1 (also called muscle atrophy F-box protein 1 [MAFbx-1]) and muscle ring-finger protein 1 (MuRF-1) are highly expressed in atrophying muscle and thought to drive the loss of muscle mass.^{8,9} Key components of the Ub-proteasome system, as well as proteasomal subunits and ubiquitin itself, have also been shown in animal models to be induced in processes of muscle loss.^{5,6} Some of these transcriptional changes, especially increased expression of atrogin-1 and MuRF-1, have been found in states of muscle loss in humans.¹⁰

In a parallel pathway, myostatin, by binding to the type IIB activin receptor, signals through Smad 2/3 to suppress inhibition of muscle growth and has been correlated with reduced muscle in animals and humans.^{7,11} It is unclear if and how myostatin-and ubiquitin-mediated pathways interrelate.

Insulin-like growth factor (IGF) 1 plays a key regulatory role in skeletal muscle physiology. IGF-1 promotes phosphorylation of protein kinase B (PKB/AKT) and mammalian target of rapamycin (mTOR), key intracellular signaling proteins that promote skeletal muscle growth and performance.^{12,13} Inversely, IGF-1-mediated Forkhead box O (FoxO) phosphorylation renders this pathway inactive and unable to promote transcription of a panel of atrophy-promoting genes.

Consequently, low levels of IGF-1 are associated with reduced PKB/AKT and mTOR, as well as increased transcription factor FoxO. Dephosphorylated FoxO is translocated into the nucleus, where it induces transcription of atrogen-1, MuRF-1, and other genes that lead to muscle protein breakdown, therefore linking reduced AKT/FoxO signaling to increased expression of atrophy-related genes.^{14,15}

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a transcriptional coactivator that promotes mitochondrial biogenesis and improved muscle bioenergetics.¹⁶ Although PGC-1α primarily interacts with the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR-γ), which permits the interaction with multiple transcription factors, it also plays a key role in feedback loops that moderate atrogen expression and protects muscle from atrophy.¹⁷

In a cross-sectional study, we analyzed skeletal muscle gene expression patterns in relation to physical function (aerobic and strength attributes) and body tissue composition (ie, fat vs lean body mass) in adult men with systolic HF versus age- and sex-matched control subjects. Concurrent assessments of atrophic and anabolic gene expression patterns in skeletal muscle of HF patients compared with control subjects have not been performed in earlier investigations. We hypothesized that skeletal muscle performance (quantified by aerobic and strength capacity) would be reduced in HF patients compared with control subjects, and that disease-related differences in skeletal muscle atrogen expression would be related to these functional differences. Similarly, we hypothesized that lean mass would be reduced in HF patients, even in those who were noncachectic, consistent with increased atrogen activity.

Methods

Subjects

Clinically stable male HF patients aged 50 years with a clinical diagnosis of systolic HF from a Veterans Administration (VA) Heart Failure program were compared with age-matched control subjects also treated at the VA hospital. All HF patients were on a standard regimen of evidence-based HF therapy, including a beta-blocker and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (unless their providers indicated specifically that they were not tolerated). HF and/or control candidates were excluded if they had any neurologic, severe pulmonary, or musculoskeletal problems, which may have

otherwise confounded the functional assessments. Candidates with severe valvular disease or cancer also were ineligible. A physical exam was completed to ensure euvolemic volume status, both as a safety and performance standard and to avoid confounding effects of water retention on dual-energy X-ray absorptiometry (DXA) scans. Echocardiography within 6 months of enrollment confirmed that the left ventricular ejection fraction (LVEF) was 40% in HF patients. Control subjects were screened to exclude HF or other cardiovascular disease. Control subjects were screened also to exclude those who exercised on a regular basis (>150 min per week in the preceding 3 months) to avoid any potentially confounding effects of exercise conditioning on performance indices and possible effects on gene expression. Comorbidities in the HF and control populations were ascertained by the VA electronic medical record. This study was approved by the VA Institutional Review Board, and every subject signed an informed consent before participation.

Functional Evaluations

Cardiopulmonary exercise testing (CPX) was completed with the use of a modified Balke¹⁸ protocol on a motorized treadmill. The ventilatory expired gas analysis system (Breezesuite; Medgraphics, St Paul, Minnesota) was calibrated according to manufacturer specifications before each test. Continuous gas exchange, telemetry, blood pressure, and oxygen saturation were assessed for each subject 1 minute before, during, and after the exercise test in standard clinical fashion.

CPX assessments included peak oxygen consumption (VO_2), ventilatory anaerobic threshold (VAT), and the minute ventilation to carbon dioxide production (VE/VCO_2) slope. Peak VO_2 was defined as the 30-second averaged value during the last stage of the exercise test. Ten-second averaged VE and VCO_2 values, acquired from the initiation of exercise to maximal exertion, were entered into spreadsheet software (Excel; Microsoft Corp, Bellevue, Washington) to calculate the VE/VCO_2 slope via least squares linear regression ($y = mx + b$; $m = \text{slope}$).

VAT was determined as a 10-second average by the V-slope method.¹⁹ All subjects achieved a peak respiratory exchange ratio (RER) of 1.0, an accepted indication of at least satisfactory exercise effort among subjects who were often unaccustomed to exercising.¹⁹

A 6-minute walk test (6MWT) was completed in a standardized fashion.²⁰ Subjects were asked to walk as far as possible in 6 minutes along a 25-meter corridor. Subjects were instructed that if they felt short of breath or exhausted that they could slow down or stop to rest as needed. The test was scored in meters walked in 6 minutes, rounding to the nearest meter.

Muscular strength and endurance were measured with the use of a pneumatic leg press (A420, Keiser, Fresno, California). To assess maximal strength capacity, participants performed a leg press (measured by the full range of motion, ie, knee extension and flexion) using a baseline weight of 45 kg. The weight was incrementally increased until subjects were no longer able to complete a full repetition or maintain proper form. The final successfully completed repetition was recorded as the 1-repetition maximum (1RM). To assess submaximal power (hereafter labeled as “power”), the weight was set to 60% of the

1RM and subjects completed up to 30 continuous repetitions. Power was assessed as work per time (W), based on an average of all repetitions.²¹

Body Composition

In addition to simple calculation of the body mass index (BMI; kg/m²), a DXA scanner (Healthcare Lunar iDXA Bone Densitometer 5th Generation with Encore software version 13.6; GE, Madison, Wisconsin) was used to assess body composition by quantifying lean and fat mass. Total lean and fat mass were assessed as well as anthropometric measures (ie, legs and arms). The DXA scanner was calibrated according to the manufacturer's specifications²² before each scan.

Skeletal Muscle Biopsies

Within 1 week after the CPX, 6MWT, DXA, and strength/ endurance assessments, a skeletal muscle biopsy of the vastus lateralis muscle in the nondominant leg was performed in standard fashion. Patients on aspirin therapy withheld the medication for 3 days before the procedure. Patients treated with warfarin for atrial fibrillation underwent bridging therapy or withheld warfarin for 3 days before the biopsy, with this procedural choice determined by their primary cardiologists. Biopsy sites were prepared in sterile fashion. A 5-mm Bergstrom muscle biopsy needle was inserted through a 0.5-cm incision and advanced into the muscle. Suction induced with the core of the Bergstrom needle was used to draw skeletal muscle into the needle's cutting window and increase yield of the biopsy specimen.²³ The wound site was closed with Steri-Strips and a pressure bandage was applied. Reassessments in 2-3 days confirmed normal healing.

Serum

Serum was obtained from all subjects during the same visit as the skeletal muscle biopsy. Standard phlebotomy technique in sterile fashion was used. Whole venous samples were centrifuged and stored until batch analyses were completed.

Gene Expression Analyses

Total skeletal muscle mRNA was extracted from muscle biopsies with the use of Trizol reagent and well established protocols. Typically 5-10 µg total RNA is obtained from approximately one-half of a biopsy specimen. Samples were stored in nuclease-free water at -80° C. Total RNA was converted to cDNA by reverse transcription with the use of Superscript reverse transcriptase (Stratagene) and oligo dT primers in standard reactions according to the manufacturer's recommendations.

Gene expression levels were determined from diluted cDNA samples (1:100) by real-time polymerase chain reaction (PCR) using the Applied Biosystems StepOne real-time PCR analyzer with a standardized quantification method (Taqman Cytokine Gene Expression Plate 1 protocol; Applied Biosystems). Multiplexed amplification reactions were performed with the use of 18s as an endogenous control (18s primers/VIC-labeled probe #4310884E; Applied Biosystems) and the Taqman Universal PCR Master Mix reagents Kit (#4304437; Applied Biosystems). Expression of the following genes associated with atrophying muscle, as validated in animal model systems and in humans, were analyzed with the use of FAM-

labeled probe/primer sets purchased from Applied Biosystems: protein degradation atrogenes: atrogen-1/MAFbx (forward: 5'- CACTGGTCCAAAGAGTCGGC; reverse: 5'- CGTCTCCATCCGATACACCC; Taqman probe: 5'- AGTCTGTGCTGGTCGGGAACATTAACATG), MuRF-1 (Hs00261590_m1), ubiquitin B (UBB; Hs004320290_m1), proteasome subunit alpha-1 (PSMA1; Hs01027360_m1), FoxO1 (Hs01054576_m1), FoxO3 (Hs00921424_m1), and myostatin (Hs00976237_m1); molecular signaling related to growth: PGC-1 α (Hs01016724_m1), IGF-1 (Hs01547656_m1), and IGFBP-5 (Hs00181213_m1). Gene expression levels are given as mean $\pm$ SD relative to the expression of 18S RNA performed in the same sample of cDNA.

Statistical Analysis

Statistical Package for the Social Sciences statistical software version 20 (SPSS; Armonk, New York) was used to analyze the data, and values are reported as mean $\pm$ SD unless otherwise indicated. Comparisons of HF and control were determined with the use of nonpaired *t* tests, and Pearson correlations were used to evaluate the correlation coefficients. A principal component analysis was performed to identify significant patterns in the gene data in an attempt to reduce dimensions and compress the data for subsequent analyses.²⁴ A comparison of gene expression and other characteristics were performed in subjects with and without diabetes in both HF and control groups to determine the inclusion or exclusion of subjects with diabetes. HF patients were also dichotomized into 2 groups (high vs low aerobic performance) to further clarify pertinent gene expression patterns.²⁵ High aerobic performance was identified by peak $\text{VO}_2 > 14 \text{ mL kg}^{-1} \text{ min}^{-1}$ or VE/VCO_2 slope < 34 , and low aerobic performance was identified by peak $\text{VO}_2 \leq 14 \text{ mL kg}^{-1} \text{ min}^{-1}$ or VE/VCO_2 slope ≥ 34 . A P value of $< .05$ was used to define statistical significance for all tests.

Results

Fifty-four subjects completed the study; including 24 systolic HF patients (mean age 67.2 ± 9.3 years) and 30 age-matched control subjects (mean age 65.9 ± 10.6 years). Baseline characteristics of the HF and control groups are presented in Table 1. Although no significant differences were observed in mean age, height, weight, or BMI between the 2 groups, significantly more HF patients were taking HF-related medications (beta-blockers, angiotensin-converting enzyme inhibitors, statins, and/or diuretics). HF patients had higher prevalence of cardiac and noncardiac morbidities (ie, myocardial infarction, coronary artery bypass graft, percutaneous coronary interventions, implantable cardioverter-defibrillators, peripheral artery disease, chronic kidney disease, and diabetes), but control patients were matched regarding age and other chronic conditions (hypertension, hyperlipidemia, chronic obstructive pulmonary disease). Whereas HF patients had an average of 11 cumulative comorbidities, age-matched control patients were also burdened with significant morbidity, with an average of 8 comorbid conditions (a profile of significant multimorbidity is common among patients at a Veterans Administration hospital). C-Reactive protein (CRP) and other inflammatory markers were similar between the 2 groups. Despite elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high comorbidity, the average New York Heart Association (NYHA) HF functional class was between I and III (namely, 2.4).

Table 2 compares the HF and control groups regarding body composition. BMI as well as DXA indices of lean body mass, fat mass, and distribution (legs and arms) were similar between the 2 groups. Table 3 lists aerobic performance and strength measurements for the HF and control groups. Peak VO_2 and VAT were lower and the VE/VCO_2 slope greater in the HF cohort compared with the control subjects. HF patients also had reduced muscular strength and endurance as demonstrated by lower values of 1RM and power.

Table 4 presents patterns of gene expression in HF and control patients. Expression of FoxO1 and FoxO3 as well as ubiquitin (UBB and PSMA1) was increased in the HF cohort. Expression of other atrogenes (ie, atrogin-1 and MuRF-1) was similar in HF and control groups. PGC-1 α expression was significantly increased in the moderate HF cohort in this study, and IGFBP-5 trended higher in the HF group. IGF-1 expression was similar in HF and control groups.

Because of the known impact of diabetes on skeletal muscle, we analyzed our data with and without including those with diabetes. The analyses showed no significant difference in gene expression or subject characteristics between HF patients with and without diabetes; MuRF-1 was the only characteristic that was significantly different between subjects with and without diabetes in the control group (2.0 vs 3.4 relative units, respectively; $P < .05$).

Figures 1 and 2 illustrate the relationships between gene expression patterns and peak VO_2 . Figure 1 shows the relationships between peak VO_2 and FoxO-1 as well as FoxO3 for the control and HF groups; peak VO_2 correlated significantly with FoxO1 and FoxO3 only in the control group. Figure 2 shows the relationship between peak VO_2 and IGFBP-5 as well as PGC-1 α for the control and HF groups; peak VO_2 correlated significantly with IGFBP-5 and PGC-1 α only in the HF group.

In the HF group, other significant modest positive relationships were found between peak VO_2 and atrogin-1 ($r = 0.49$), UBC ($r = 0.48$), and myostatin ($r = 0.45$), and a significant modest negative relationship was observed between the VE/VCO_2 slope and IGFBP-5 ($r = -0.44$). In the control group, MuRF-1 correlated negatively with peak VO_2 ($r = -0.42$) and no significant relationship was observed between gene expression and the VE/VCO_2 slope. No significant correlations between atroгене expression and strength parameters (1RM and power) were detected in the HF patients, and only IGF-1 was significantly correlated with power in the control group ($r = -0.39$). The results of the principal component analysis found no significant patterns in the gene data and no subsequent analyses were performed.

The results of analyses in HF patients based on both peak VO_2 and the VE/VCO_2 slope found that PGC-1 α was significantly different between HF patients with higher and lower aerobic performance. PGC-1 α was 9.2 ± 5.9 relative units in 15 HF patients with peak $\text{VO}_2 > 14 \text{ mL kg}^{-1} \text{ min}^{-1}$ versus only 4.5 ± 1.9 in 9 patients with peak $\text{VO}_2 \leq 14 \text{ mL kg}^{-1} \text{ min}^{-1}$ ($P < .01$). Likewise, PGC-1 α was 9.7 ± 6 in 14 HF patients with a VE/VCO_2 slope ≥ 34 versus 5.3 ± 3.4 in 10 HF patients with a VE/VCO_2 slope < 34 ($P < .05$). Furthermore, IGFBP-5 was significantly greater in HF patients with a peak $\text{VO}_2 > 14 \text{ mL kg}^{-1} \text{ min}^{-1}$, and IGFBP-5 as well as IGF-1 were significantly greater in HF patients with a VE/VCO_2 slope < 34 . HF patients with a peak $\text{VO}_2 > 14 \text{ mL kg}^{-1} \text{ min}^{-1}$ and VE/VCO_2 slope < 34 also had

significantly greater atrogin-1, MuRF-1, UBB, and myostatin compared with HF patients with a peak VO_2 $14 \text{ mL kg}^{-1} \text{ min}^{-1}$ and a VE/VCO_2 slope 34 .

Discussion

In a cross-sectional study of community-dwelling systolic HF patients and age-matched control subjects with significant comorbid disease, we showed physical functional decrements associated with HF. Whereas expression patterns of many genes associated with enhanced muscle proteolysis were elevated in HF patients (eg, FoxO1, FoxO3, UBB, PSMA1), gene expression of atrogin-1 and MuRF-1 were similar to control subjects. Moreover, PGC-1 α skeletal muscle gene expression was unexpectedly increased in HF. These data demonstrate both significant variability in proteolytic patterns in HF and the possible role of enhanced PGC-1 α expression as a counterregulatory mechanism that may help preserve physical function despite the presence of chronic cardiac disease.

The systolic HF patients included in this study had relatively mild disease severity (average NYHA functional class 2.4) but still demonstrated reduced aerobic and strength functional capacities compared with age-matched control subjects. However, proteolytic gene expression patterns were relatively heterogeneous compared with earlier animal^{4,5} and human^{10,26,27} studies, suggesting greater complexity of disease in this cohort than what has been previously reported. Notably, HF patients in this study were also noncachectic, distinguishing our data regarding atrophy and metabolism from HF trials where subjects tended to be cachectic and frail.²⁸

Although it is possible that our data merely represent insufficient power to demonstrate the originally anticipated patterns of gene expression in relation to HF, it seems that activation of proteolytic pathways in skeletal muscle may have distinctive timing in different patients and/or clinical circumstances. Inconsistencies with earlier studies suggest that kinetics of skeletal muscle loss is not uniform, with only some studies reflecting loss of muscle mass and progressive muscle atrophy. Consistently, the anticipated HF-related decrements of skeletal muscle IGF-1 as well as HF-related increases in skeletal muscle myostatin and serum inflammatory markers were not detected in the present analysis.

In contrast to earlier studies, which have tended to show reduced IGF-1 in HF cohorts,^{29,30} we demonstrated similar IGF-1 expression in HF and control patients. Because similar IGF-1 signaling was present in HF and control, the equivalent downstream similarities of MuRF-1 may be secondary to the similar IGF-1 expression in each population.^{14,15}

Even more likely, the high degree of comorbidity among the age-matched control subjects stands out as a feature of this study that may have obscured many of the HF-specific distinctions that have been reported previously. In other words, earlier assessments of disease-related differences³¹ in skeletal muscle gene signaling patterns may have been more distinct when control patients were relatively healthier and less affected by comorbid disease than the control subjects in our study. Consistently, the control group in this study also had high levels of circulating tumor necrosis factor (TNF) α , suggesting that high comorbidities

in each population also gave rise to similar proinflammatory phenotypes with equivalent effects on skeletal muscle signaling patterns.³²

The most novel aspect of our analysis compared with previous studies focusing on skeletal muscle atrophy is that PGC-1 α expression was also assessed, expanding the perspective regarding mitochondrial biogenesis as a counter-regulatory pathway. PGC-1 α is a known master regulator of metabolic pathways, and its expression has been linked to a more oxidative phenotype of skeletal muscle.^{33,34} Because PGC-1 α has been demonstrated to be decreased in animal models of HF,^{35,36} the fact that PGC-1 α was elevated in skeletal muscle of HF patients seems to be counterintuitive. One possibility is that PGC-1 α may balance atrogene expression, offsetting muscle atrophy and subsequent debility that might otherwise develop from unchecked atrogene expression. The unexpected similarities in atrogin-1 and MuRF-1 expression in HF, despite increases in expression of FoxO1, FoxO3, UBB, and PSMA1, might therefore correspond to the relative increases in PGC-1 α .

DXA data showed similar lean and fat tissue in the HF and control cohorts. The similarities in body composition corroborate assumptions that higher atrogene expression may be balanced by expression of regulatory genes such as PGC-1 α preserving tissue composition. Furthermore, our population of noncachectic, community-dwelling HF patients with predominantly NYHA functional class II symptoms seems likely to reflect a balance of pernicious disease versus compensating factors. This suggests that at an early stage of HF, skeletal muscle may mediate the capacity to counteract catabolic influences through yet unknown pathways.

Gielen et al recently published a study demonstrating increased MuRF-1 expression in HF compared with age-matched control subjects at baseline, with no differences in atrogin-1 expression between the HF and control populations.²⁷ Moreover, Gielen et al and others^{27,29,30,37} showed reduced muscle gene expression of IGF-1 in HF versus control patients whereas TNF- α and myostatin were relatively greater. Those studies focused on the utility of aerobic exercise to modify these relationships, ie, exercise-induced reductions in skeletal muscle. MuRF-1 expression²⁷ as well as exercise-induced increases in skeletal muscle IGF-1 and reductions in serum TNF- α .^{27,29,37}

Our study expands on previous investigations by assessing expression of a broader selection of atrophy-related genes in skeletal muscle relative to functional performance. We also included assessment of body composition. Although we did not perform an exercise training intervention, we compared gene expression patterns with functional indices with the expectation that higher proteolytic gene expression and reduced lean body mass are related to lower physical function. Likewise, we anticipated increased anabolic gene expression and increased lean body mass in subjects with higher physical function.

Although our study showed that higher expression of MuRF-1, FoxO1, and FoxO3 negatively correlated with peak VO₂, these correlations reached significant levels only within the control cohort and not in the HF population. In fact, among HF patients, atrogin-1 and myostatin expression correlated with higher peak VO₂, which seems to be counterintuitive. It seems likely that our original hypothesis regarding the capacity for

atrogenes to determine skeletal muscle mass and structure, and to thereby have distinct effects on physical function, may be only partly true.

Muscle function and physical function were reduced in HF compared with age-matched control subjects, but with ambiguous correlation with atrophy genes and lean body mass. Counterregulatory pathways and/or heterogeneity based on comorbid disease, nutrition, or other individual idiosyncrasies seem pertinent.

Study Limitations

As a VA-based study, this study was oriented to male HF patients because they predominate in the VA HF population. Such omission of women is a significant limitation in a study focused on muscle and functional differences, which may differ between women and men.

Also, although age-matched control subjects were screened to exclude HF or other cardiovascular disease, the group as a whole was not as “healthy” as was anticipated. Many control subjects shared the same comorbidities and prescription medications as the HF participants. The high TNF- α level in both populations supports the premise that the control population was vulnerable to many of the same intrinsic risks of skeletal muscle atrophy as the HF patients.

Although the high prevalence of comorbidity is primarily a study limitation, we also see the high comorbidity among HF and control patients to be a study strength. We enrolled a population that is typical of an older community-dwelling HF population and demonstrated significant skeletal muscle gene patterns that determined physical function capacities despite the confounding effects of comorbidity, medications, diet, and other potentially obscuring dynamics. We think that this corroborates the importance of skeletal muscle gene expression as a valuable consideration in promoting physical function in older HF patients, particularly in early stages of the disease.

Finally, although the study methods included screening out high baseline activity of control patients, similar enrollment constraints were not systematically applied to HF patients. We assumed that the HF cohort was sedentary (and our assumptions were corroborated by high BMI and low functional indices, including CPX, 6MWT, and strength assessments), but it remains possible that HF subjects may have been more active than we appreciated, thereby confounding the analyses of gene expression in HF versus control subjects.

Conclusion

This study demonstrates that patients with HF have decreased functional capacity compared with age-matched control subjects without HF, but that anticipated UPP gene expression patterns (particularly MuRF-1), myostatin, and muscle atrophy were not increased in this cohort. Moreover, PGC-1 α gene expression was unexpectedly increased in HF patients, and this merits further investigation. These data highlight the complex nature of muscle gene transcription in systolic HF patients as well as the variability between individual patients.

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Fig. 1. Scatterplots of the relationships between peak VO_2 and FoxO1 for the (A) control and (B) heart failure (HF) groups as well as peak VO_2 and FoxO3 for the (C) control and (D) HF groups. VO_2 , oxygen consumption; Fox, forkhead box protein.

Fig. 2.

Scatterplots of the relationship between peak VO_2 and IGFBP-5 for the (A) control and (B) heart failure (HF) groups as well as peak VO_2 and PGC-1 α for the (C) control and (D) HF groups. VO_2 , oxygen consumption; IGFBP, insulin-like growth factor-binding protein; PGC, peroxisome proliferator-activated receptor gamma coactivator.

Table 1**Subject Characteristics**

	Heart Failure (n = 24)	Control (n = 30)	Significance
Patient demographics			
Age (y)	67.2 ± 9.3	65.9 ± 10.6	.64
Height (m)	1.73 ± 0.1	1.72 ± 0.1	.68
Weight (kg)	88.3 ± 29.3	87.4 ± 15.9	.88
BMI (kg/m ²)	29.4 ± 8.0	29.8 ± 5.2	.81
GFR (mL min ⁻¹ 1.73 m ⁻²)	77.7 ± 23.7	85.7 ± 21.0	.20
NYHA functional class	I: n = 1 (4%) II: n = 16 (67%) III: n = 7 (29%) Mean 2.4 (range 1–3)		
Serologic markers			
NT-proBNP (pg/mL)	1518.3 ± 1810.5	185.3 ± 421.8	.002
TNF-α (pg/mL)	14.4 ± 4.4	13.3 ± 8.1	.56
CRP (mg/dL)	0.53 ± 0.52	0.49 ± 0.60	.77
IL-1β (pg/mL)	2.6 ± 4.8	1.7 ± 1.7	.31
IL-6 (pg/mL)	6.2 ± 3.0	7.0 ± 10.6	.72
Medications, n (%)			
Beta-blocker	18 (75)	2 (7)	<.0001
ACE inhibitor	19 (79)	5 (17)	<.0001
ARB	2 (8)	2 (7)	.82
Statin therapy	20 (83)	14 (47)	<.01
Diuretic	17 (71)	4 (13)	<.01
Comorbidities, n (%)			
CABG	6 (25)	0 (0)	<.01
CKD	5 (21)	0 (0)	<.05
COPD	1 (4)	1 (3)	.88
DM	11 (46)	6 (20)	<.05
HTN	14 (58)	11 (37)	.11
Hyperlipidemia	15 (63)	12 (40)	.10
MI	5 (21)	0 (0)	<.05
ICD	7 (29)	0 (0)	<.005
PAD	3 (13)	0 (0)	<.05

BMI, body mass index; GFR, glomerular filtration rate; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TNF, tumor necrosis factor; CRP, C-reactive protein; IL, interleukin; CABG, coronary artery bypass graft; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HTN, hypertension; MI, myocardial infarction; ICD, implanted cardioverter-defibrillator; PAD, peripheral arterial disease.

Table 2

Differences in Body Composition Between Heart Failure and Control Groupss

	Heart Failure	Control	Significance
Clinical exam			
BMI	29.4 ± 8.0	29.8 ± 5.2	.81
DXA			
Total lean (kg)	54.9 ± 10.2	56.6 ± 8.0	.49
Total Fat (kg)	30.5 ± 18.5	28.1 ± 9.6	.58
Lean body mass as % of body weight	64.6 ± 9.0	65.4 ± 5.9	.69
Fat body mass as % of body weight	32.2 ± 8.6	31.6 ± 6.1	.78
Legs lean (kg)	18.0 ± 4.1	19.4 ± 3.2	.17
Arms lean (kg)	6.6 ± 1.4	6.9 ± 1.7	.58
Legs fat (kg)	8.3 ± 5.5	7.5 ± 2.4	.54
Arms fat (kg)	2.8 ± 1.7	2.8 ± 1.2	.97
Android	7.5 ± 3.3	7.1 ± 1.9	.54
Gynoid	12.2 ± 4.1	12.3 ± 2.5	.87
Android-gynoid ratio	1.2 ± 0.20	1.3 ± 0.18	.10

BMI, body mass index; DXA, dual-energy X-ray absorptiometry.

Table 3

Differences in Peak Aerobic and Strength Parameters Between Heart Failure and Control Groups

	Heart Failure	Control	Significance
Aerobic indices			
Peak VO_2 ($\text{mL O}_2 \text{ kg}^{-1} \text{ min}^{-1}$)	14.9 ± 3.3	22.8 ± 6.6	<.0001
VAT ($\text{mL O}_2 \text{ kg}^{-1} \text{ min}^{-1}$)	9.9 ± 3.5	13.5 ± 4.3	<.0001
VE/ VCO_2 slope	34.4 ± 9.2	28.9 ± 5.0	.007
6MWT (m)	391.9 ± 108.8	491.1 ± 100.7	.001
Peak heart rate (beats/min)	104.3 ± 22.1	131.0 ± 19.4	<.001
Strength indices			
1RM (kg)	155.7 ± 53.1	195.2 ± 58.8	.015
Power (W)	239.3 ± 93.3	297.5 ± 122.8	.068

VO_2 , oxygen consumption; VAT, ventilatory anaerobic threshold; VE/ VCO_2 , minute ventilation to carbon dioxide ratio; 6MWT, 6-minute walking test; 1RM, 1-repetition maximum.

Table 4

Gene Expression Between Heart Failure and Control Groups

	Heart Failure	Control	Significance
Catabolic			
FoxO1	7.9 ± 6.2	5.0 ± 3.5	.041
FoxO3	6.5 ± 4.3	4.3 ± 2.8	.042
Atrogin-1/MAFbx	4.2 ± 1.8	3.6 ± 2.1	.28
MuRF-1	3.5 ± 1.4	3.1 ± 1.5	.34
UBB	5.0 ± 2.4	3.7 ± 1.7	.033
PSMA1	4.4 ± 2.0	3.5 ± 1.6	.05
Myostatin	10.4 ± 8.6	9.1 ± 6.6	.52
Anabolic			
IGF-1	5.2 ± 3.1	4.3 ± 2.8	.28
IGFBP-5	19.0 ± 13.2	13.1 ± 8.6	.06
PGC-1 α	7.9 ± 5.4	5.3 ± 3.6	.049

FoxO1, forkhead box protein O1; FoxO3 = forkhead box protein O3; MAFbx, muscle atrophy F-box; MuRF-1, muscle ring-finger protein 1; UBB, ubiquitin B; PSMA1, proteasome (prosome, macropain) subunit alpha-1; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; PGC, peroxisome proliferator-activated receptor gamma coactivator. Gene expression levels are given as mean ± SD relative to the expression of 18S RNA performed in the same sample of cDNA.