Chronic heart failure reduces Akt phosphorylation in human skeletal muscle: relationship to muscle size and function

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Heart failure (HF) is the final common pathway for many chronic cardiac diseases and is presently the only cardiac diagnosis continuing to increase in prevalence in the United States. Patients suffering from HF report high rates of physical disability, as defined by an inability to perform simple daily activities (43). Although the reason for their physical disability is unclear, most research has focused on aerobic exercise intolerance in these patients (23). This is logical considering that the hallmark symptom of HF is exertional dyspnea and because of the widespread use of aerobic capacity as a diagnostic tool (36). Diminished aerobic capacity, however, does not greatly limit the ability of patients to perform most daily activities (42). Instead, performance of many activities of daily living is strongly dependent on muscle strength (1, 4, 44), which is determined by the size of the muscle and its contractile properties. HF patients often experience muscle atrophy and weakness during the course of the disease, which may contribute to their physical disability. The mechanisms whereby HF alters skeletal muscle size and function, however, have not been clearly defined.

The mass and function of skeletal muscle are largely dictated by its protein expression, which is determined by opposing anabolic and catabolic stimuli. Insulin-like growth factor-1 (IGF-1) is believed to be a key regulator of protein metabolism, stimulating anabolic (45) and inhibiting catabolic (51) pathways. One of the key mechanisms whereby IGF-1 mediates these effects downstream of receptor activation is through phosphorylation and activation of Akt (3). The anabolic effects of Akt are mediated, in part, through activation of mammalian target of rapamycin (mTOR) (37) and inhibition of glycogen synthase kinase-3β (GSK-3β) (9). Activation of mTOR stimulates protein translation through its effects on p70 ribosomal S6 kinase (p70 S6K) and eukaryotic translation initiation factor 4E binding protein-1 (eIF4E-BP) (15), whereas Akt-mediated phosphorylation of GSK-3β (9) stimulates protein translation (45) by diminishing its inhibitory phosphorylation of eIF2B (59). Additionally, Akt activation reduces protein breakdown via phosphorylation of forkhead box O (FOXO) transcription factors (46). In their phosphorylated form, FOXOs are excluded from the nucleus, where they would otherwise stimulate transcription of E3 ubiquitin ligases important for muscle proteolysis (46,51). Thus IGF-1, working through the activation of Akt, promotes muscle protein anabolism through reciprocal regulation of protein synthesis and breakdown.

Early studies suggested that HF reduces circulating IGF-1 levels (41), but few studies have evaluated the potential effect of these alterations on skeletal muscle. In animal models of HF, reduced skeletal muscle expression of IGF-1 was found and was related to decreased muscle fiber size (48). Moreover, administration of growth hormone (10), which stimulates muscle IGF-1 expression, or muscle-specific transgenic overexpression of IGF-1 (47) inhibits muscle atrophy and improves contractile function. In human HF, skeletal muscle IGF-1 mRNA abundance (20, 53) and protein expression (20) are reduced and are correlated with decreased muscle size (20) and myofibrillar gene expression (53). Based on these results, one might expect corresponding downregulation of signaling pathways downstream of IGF-1 receptor activation. However, the one study that has examined these downstream signaling events in humans found no effect of HF (27). In addition to the paucity of knowledge about signaling distal to receptor activation, none of these studies have accounted for the fact that HF...
patients have low levels of physical activity (55). Muscle use positively regulates skeletal muscle IGF-1 expression and activation of downstream signaling molecules (22, 30). Thus it is unclear whether diminished local IGF-1 expression observed in prior studies (20, 48, 53) is due to HF or is a consequence of muscle disuse that accompanies the disease. Considering these caveats, the unique effect of the HF syndrome on these variables remains undefined.

The conventional wisdom is that diminished circulating IGF-1 and muscle IGF-1 expression in HF patients (20, 41, 53) promote muscle atrophy and dysfunction, but virtually no studies have evaluated signaling pathways within skeletal muscle that would translate the effect of IGF-1 on muscle size and function. Thus the primary objective of the present study was to evaluate the effect of HF on skeletal muscle IGF-1 expression and the phosphorylation status of signaling molecules downstream of IGF receptor activation. The present series of experiments focused on examining the phosphorylation status of the Akt/mTOR and Akt/GSK-3β pathways since we recently found no effect of HF on FOXO-regulated genes, atrogin and MuRF1, or markers of skeletal muscle protein breakdown (39). To examine the unique effects of HF, we recruited sedentary healthy controls to match patients for activity level and studied HF patients at least 6 mo following any hospitalization to minimize any influence of disease exacerbation or muscle disuse associated with bed rest. We hypothesized that HF would be characterized by reduced skeletal muscle IGF-1 expression and, correspondingly, altered phosphorylation of Akt, mTOR, GSK-3β, and downstream signaling molecules. Moreover, decreased IGF-1 expression and signaling molecule phosphorylation would be correlated to reduced muscle size and function.

METHODS

Subjects. Patients (n = 11; 7 men, 4 women) with chronic HF were recruited from the Heart Failure Clinic of the Cardiology Unit at the University of Vermont. The population consisted of patients with systolic dysfunction (left ventricular ejection fraction <40%; n = 6) and those with preserved systolic function (ejection fraction >40%; n = 5). The average New York Heart Association (NYHA) functional class was 2.27 ± 0.65, with one class I patient, six class II patients, and four class III patients. The etiology of HF was ischemic in three volunteers and nonischemic in eight. In addition, four patients had Type II diabetes mellitus. All patients were clinically stable and had not been hospitalized for at least 6 mo before testing. None had signs or symptoms of severe hepatic (i.e., cirrhosis) or renal disease (i.e., plasma creatinine >3 mg/dl), peripheral vascular disease, or an active neoplastic process, and none were smokers or were taking sex steroid replacement therapy (estrogen or estrogen/progesterin therapy in women or testosterone in men). All patients were on stable doses of HF medications, including angiotensin-converting enzyme inhibitors/receptor blockers (100%), β-blockers (91%), and diuretics (64%). In addition, five patients were taking HMG CoA reductase inhibitors (i.e., statins), and one female patient was on a stable regimen of levofloxacin.

Controls (n = 11; 6 men, 5 women) were recruited who self-reported being sedentary to minimally physically active (≤2 sessions of ≥30 min of exercise/wk) and not currently participating in any organized exercise training or weight loss programs. These criteria were established to obtain a cohort of nondiseased controls who would show similar levels of physical activity compared with HF patients. To verify that our approach yielded controls with similar activity levels as HF patients, we assessed activity patterns using accelerometry (see below). Controls were also nonsmokers and had a stable body weight (±2 kg) for 6 mo before testing. They had no signs or symptoms of HF, coronary heart disease, or diabetes (fasting blood glucose >112 mg/dl), normal left ventricular function by echocardiography (ejection fraction >55%), normal blood counts and biochemistry values and were not taking sex steroid replacement therapy (estrogen or estrogen/progesterin therapy in women or testosterone in men). Four patients had a history of hypertension: three were treated with diuretics and one with angiotensin-converting enzyme inhibitor. All controls were normotensive at the time of testing, and none showed evidence of left ventricular hypertrophy or atrial enlargement by echocardiography. In an attempt to frequency match HF patients for medications that might influence skeletal muscle metabolism, we included three control volunteers who were taking statins to treat hyperlipidemia and one female on a stable regimen of levofloxacin. Written informed consent was obtained from each volunteer, and the protocol was approved by the Committees on Human Research at the University of Vermont. Data from patients and controls in this study for whole muscle and single fiber function and single fiber protein content and structure have been published previously (38, 39, 57).

Experimental protocol. Each volunteer was tested during outpatient and inpatient visits. Eligibility was determined during two outpatient screening visits at which time medical history, physical exam, blood samples, a treadmill test, strength testing, and echocardiography were performed. At least 1 wk following any treadmill or strength testing, eligible volunteers underwent further evaluation during an inpatient visit. Medications were maintained for all volunteers per normal dosing regimens before the inpatient visit, except coumadin (n = 1 HF patient), which was stopped 5 days before admission to minimize bleeding during the biopsy procedure. Volunteers were fasted after 1900 the evening of admission. On the following morning, body composition was measured, muscle tissue was obtained via percutaneous biopsy of vastus lateralis muscle, and computed tomography was performed. Approximately one-third of the biopsy material was cleaned of visible fat and connective tissue, blotted dry, and frozen in liquid N2 until analysis. The remainder was used for single muscle fiber structural and functional measurements (38, 39).

Total and regional body composition. Body mass was measured on a digital scale (ScaleTronix, Wheaton, IL). Leg fat-free mass, a proxy of muscle mass, was measured by dual-energy x-ray absorptiometry using a GE Lunar Prodigy densitometer (GE Lunar, Madison, WI), as described previously (2). Of note, although computed tomography was performed to assess thigh muscle cross-sectional area, as stated in Experimental protocol, data were unavailable on two volunteers due to technical problems. Thus we decided not to present these data in lieu of using leg fat-free mass as an index of muscle mass and to adjust leg strength measures for muscle size.

Peak oxygen consumption. Peak oxygen consumption (peak VO2) was determined using a treadmill test to volitional exhaustion, as described previously (2), while volunteers were taking their medications (detailed in Subjects).

Isometric knee extensor torque. Peak torque of knee extensor muscles was measured under isometric conditions at 70° knee flexion relative to full knee extension, which is the angle at which the greatest peak torque is achieved, as described (57). Briefly, two trials (each contraction held for minimum of 4 s) were performed separated by 2 min of rest. The peak torques were averaged and adjusted statistically for leg fat-free tissue mass before comparison between groups.

Accelerometry. Free-living physical activity was estimated using a single-plane accelerometer (Caltrac; Muscle Dynamics Fitness Network, Torrance, CA), as described previously (2).

Western blot analysis. Skeletal muscle tissue was homogenized in ice-cold (4°C) buffer (50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 0.5% Igepal-CA630, 1 mM NaVO3, 5 mM NaF, 1 mM β-glycerophosphate, 1 mM Na2PO4, 1 mM DTT, 10 μg/ml leupeptin, 1% aprotinin, 1 mM PMSF, pH 7.4), incubated on ice for 30 min and centrifuged (16,000 g at 4°C for 30 min); the protein content was
determined (Bio-Rad DC Protein Assay, Hercules, CA), and the supernatant was diluted in sample pH buffer (2% SDS, 62.5 mM Tris, 10% glycerol, 0.005% bromophenol blue, 100 mM DTT, pH 6.8). Proteins were separated by SDS-PAGE [4–15% for mTOR; 10% for Akt, GSK-3β, eIF2Bε, and p70 S6K; 15% with reduced bisacrylamide concentration (acylamide:bisacrylamide, 30:0.19%) for eIF4E-BP, as described (35), transferred to nitrocellulose (Akt, GSK-3β) or PVDF (mTOR, eIF2Bε, eIF4E-BP, p70 S6K), and blocked with nonfat milk or BSA in Tris-buffered saline-0.1% Tween. Blots were incubated overnight with antibodies (all from Cell Signaling, Beverly, MA, unless otherwise noted) for Akt (1:1,000), phospho-Akt (S473; 1:2,000), mTOR (1:2,000), phospho-mTOR (S2448; 1:1,000), p70 S6K (1:1,000), phospho-p70 S6K (T389; 1:500), GSK-3β (1:1,000), phospho-GSK-3β (S9; 1:1,000), eIF4E-BP (1:1,000), phospho-eIF4E-BP (T37/46; 1:1,000), eIF2Bε (1:1,000; Abcam; Cambridge, MA), and phospho-eIF2Be (S540; 1:1,000; GeneTex; San Antonio, TX). After washing, blots were incubated with anti-mouse (Amer sham) or anti-rabbit (Cell Signaling) conjugated to horseradish peroxidase for chemiluminescent detection (Pierce ECL Western blotting substrate or Super Signal West Femto; Thermo Scientific, Carlsbad, CA). Bands were quantified by densitometry (Quantity One; Bio-Rad; Hercules, CA). The ratio of the amount of phospho-specific to total protein content for each signaling molecule was used as a relative index of its activation, although data for phospho- and total protein content for each analyte are provided. All samples were run on the same gel to obviate the need to account for gel-to-gel variability, with protein content for each signaling molecule used as a relative measure (35). Bands were quantified by densitometry (Quantity One; Bio-Rad; Hercules, CA). An aliquot of the supernatant was added to loading buffer (2% SDS, 62.5 mM Tris, 10% glycerol, 0.001% bromophenol blue, 5% β-mercaptoethanol, pH 6.8), heated for 2 min at 65°C and stored at –80°C until analysis. Myofibrillar proteins were isolated by SDS-PAGE (resolving gel of 7.5%); gels were stained with Coomassie blue and scanned; and MHC band intensity determined by densitometry (Quantity One; Bio-Rad). MHC protein content data are expressed as densitometric units per microgram of protein loaded. MHC content measurements were not performed on one HF patient because of lack of tissue. Finally, the relative content of MHC I, IIA, and IIX isoforms was determined by gel electrophoresis, as previously described (39).

Statistics. All data are reported as means ± SE. Unpaired Student t-tests were used to compare groups. Analysis of covariance was used to evaluate differences in knee extensor torque after statistical adjustment for leg muscle mass. Relationships between variables were determined using Pearson correlation coefficients. For knee extensor isometric torque, leg muscle mass was entered as a covariate in correlation analysis to remove any potentially confounding effects of variation in muscle size. Normality of distribution was assessed by Shapiro-Wilk test and log_{10} transformation imposed to restore normality in variables with skewed distributions. All analyses were conducted with SPSS software version 15 (SPSS; Chicago, IL).

RESULTS

Physical characteristics for HF patients and controls are shown in Table 1. Groups were similar for age, body size, leg muscle mass, knee extensor strength, and physical activity level (range of P values: 0.07–0.88). As an aside, the strong trend (P = 0.07) for group differences was noted for knee extensor torque, which we have previously shown to differ with HF in more carefully matched cohorts (57). Peak V_{O2} was lower (P < 0.01) in patients compared with controls when expressed relative to body mass, which is the method of data expression most commonly employed in the HF literature. However, it remains lower (P < 0.01) in patients when

<table>
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<th>Table 1. Clinical characteristics and physical activity levels of controls and heart failure patients</th>
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<td><strong>Control</strong></td>
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| Peak oxygen consumption, ml·kg⁻¹·min⁻¹ | 23.4 ± 12 | 13.7 ± 1.2 *
| Knee extensor isometric torque, N·m⁺ | 159 ± 11 | 129 ± 11 |
| Physical activity level, kcal/day | 247 ± 40 | 215 ± 44 |

Data are means ± SE for n = 11 controls and n = 11 heart failure (HF) patients. Peak oxygen consumption data are expressed relative to body mass (kg). *Knee extensor isometric torque was adjusted for leg fat-free mass statistically using analysis of covariance, which yields group adjusted means with units of N·m. Physical activity level reflects average daily energy expenditure for physical activity as calculated from an accelerometer worn at the wrist for an average of 7 days. *P < 0.01.
The abundance of IGF-1 mRNA (Fig. 1) expressed relative to GAPDH did not differ between controls (C) and patients (HF): 100 ± 23.2% ± 18%. Similar results were observed when IGF-1 mRNA data were indexed against 18S as the housekeeping gene (C: 100 ± 25.4% vs. HF: 70.0 ± 8.1%; P = 0.22).

Figure 2 shows phospho- and total Akt (Fig. 2A), mTOR (Fig. 2B), and GSK-3β (Fig. 2C) data. There was a strong trend (P = 0.06) for reduced phospho-Akt (S473) in HF patients compared with controls (C: 100 ± 11.0% vs. HF: 61.5 ± 16.5%), whereas there was no difference in total Akt protein content between groups (C: 100 ± 3.2% vs. HF: 110.0 ± 14.6%; P = 0.46). Accordingly, the ratio of phospho-Akt to total Akt was reduced in patients compared with controls (C: 100 ± 10.2% vs. HF: 56.5 ± 13.5%; P = 0.02). These differences between groups may be explained by variation in fiber type since HF has previously been shown to decrease the proportion of MHC I fibers (56) and the fact that MHC I fibers may have greater Akt expression and phospho-Akt levels (49).

This does not likely explain our results, however, since evaluation of fiber type distributions via MHC isoform expression showed lower MHC I (C: 41.9 ± 3.0% vs. HF: 25.8 ± 2.7%; P < 0.01) and greater MHC IIX expression (C: 31.9 ± 6.2% vs. HF: 40.3 ± 4.1%; P < 0.05) in controls, with no group differences in MHC IIA (C: 39.7 ± 3.5% vs. HF: 35.6 ± 2.4%; P = 0.37). Parenthetically, the absence of a shift in MHC isoform distribution toward a more fast-twitch phenotype, as observed in our prior work (56), likely relates to the careful matching of patients and controls for physical activity level. Although we tried to assess phosphorylation of Akt on T308, preliminary experiments were unable to obtain measurable signals for this analyte. No differences in phospho-mTOR (S2448) (C: 100 ± 12.4% vs. HF: 87.5 ± 16.4%; P = 0.55), total mTOR (C: 100 ± 14.0% vs. HF: 104.3 ± 16.6%; P = 0.84), or the ratio of phospho- to total mTOR (C: 100 ± 26.4% vs. HF: 63.2 ± 13.5%; P = 0.28) were found. Finally, no group differences were found in phospho-GSK-3β (S9) (C: 100 ± 11.6% vs. HF: 112.2 ± 6.9% P = 0.45), total GSK-3β (C: 100 ± 8.1% vs. HF: 103.9 ± 3.7%; P = 0.72), or the ratio of phospho-GSK-3β to total GSK-3β (C: 100 ± 8.8% vs. HF: 110.5 ± 7.3%; P = 0.41).

Figure 3 shows phospho- and total p70 S6K (Fig. 3A), eIF4E-BP (Fig. 3B), and eIF2Be (Fig. 3C) data. No group differences were found in phospho-p70 S6K (T389) (C: 100 ± 33.5 vs. HF: 40.3 ± 23.5%; P = 0.19), total p70 S6K (C: 100 ± 11.6% vs. HF: 98.8 ± 12.6%; P = 0.95), or the ratio of phospho-p70 S6K to total p70 S6K (C: 100 ± 34.3% vs. HF: 36.3 ± 21.2%; P = 0.16). Because of the difficulty in assessing site-specific phospho-p70 S6K in the rested, postabsorptive condition, we also utilized a gel shift assay to examine the possibility that HF alters the general phosphorylation status of p70 S6K. Similar to measurements using site-specific antibodies, we found no differences between patients and controls in the level of phospho-p70 S6K (C: 100 ± 23.6% vs. HF: 114 ± 37.4%; P = 0.75; data not shown in figure). No group differences were found in phospho-eIF4E-BP (T37/46) (C: 100 ± 18.0% vs. HF: 100.0 ± 16.2%; P = 0.98), total eIF4EB (C: 100 ± 28.3% vs. HF: 110.3 ± 25.8%; P = 0.79), or the ratio of phospho-eIF4E-BP to total eIF4EB (C: 100 ± 37.8% vs. HF: 59.7 ± 8.6%; P = 0.31). Of note, the disparity between groups in the phospho-to-total eIF4EB was driven by an outlier in the control group, who had high phospho-eIF4EB relative to total. Removal of her data, however, still yielded no difference in the phospho-to-total ratio of eIF4EB (C: 100 ± 39.1% vs. HF: 36.3 ± 22.0%; P = 0.17).

Data are means ± SE and are expressed as a percentage of the average value for controls.
21.9 vs. HF: 90.2 ± 14.9%; P = 0.71). To further clarify the effect of HF on phosphorylation of eIF4E-BP, we analyzed the shift in electrophoretic mobility of eIF4E-BP (evident in blots in Fig. 3B) to examine the possibility that HF alters the general phosphorylation status of eIF4E-BP. Similar to measurements using site-specific antibodies, we found no differences between patients and controls in the level of phospho-eIF4E-BP (C: 100 ± 31.2 vs. HF: 105.3 ± 27.1%; P = 0.90; data not shown in figure). Finally, no group differences were found in phospho-eIF2Be (C: 100 ± 2.7 vs. HF: 103.0 ± 2.4%; P = 0.42), total eIF2Be (C: 100 ± 2.4 vs. HF: 99.0 ± 2.2%; P = 0.78) or the ratio of phospho-eIF2Be to total eIF2Be (C: 100 ± 1.8 vs. HF: 104.1 ± 2.5%; P = 0.20).

To examine the potential role of IGF-1 expression and phosphorylation of signaling molecules on muscle size and function, we evaluated the relationship of IGF-1 mRNA levels and phospho-to-total ratio of each signaling molecule to indexes of muscle size and function (Table 2). Phospho-Akt/Akt (P < 0.02) was related to MHC protein content (Fig. 4), and phospho-mTOR/mTOR was related to knee extensor strength (P < 0.05).

**DISCUSSION**

The present study examined IGF-1 expression and activation of signaling molecules downstream of receptor activation in skeletal muscle from HF patients and sedentary controls. HF patients were characterized by diminished phospho-Akt/Akt, whereas the expression of IGF-1 mRNA, Akt protein, and the total and phospho- to total ratio of mTOR, p70 S6K, eIF4E-BP, and eIF2Be protein did not differ between groups. The potential physiological relevance of reduced Akt phosphorylation is suggested by its relationship to decreased MHC protein content, a molecular marker of muscle size and functionality, as well as the similarity of the pattern of signaling molecule phosphorylation to preclinical HF models characterized by muscle atrophy and dysfunction (47). An important strength of our study is our recruitment criteria for both HF patients and controls that sought to minimize the confounding effects of a variety of disease-related factors. Because these considerations yielded groups closely matched for age, muscle

![Fig. 3. Protein expression and phosphorylation of p70 ribosomal S6 kinase (p70 S6K; T389; A), eukaryotic translation initiation factor (eIF) 4E binding protein-1 (4E-BP; T37/46; B), and eIF2Be (S540; C) in controls (C; closed bars) and patients (HF; open bars). Data are n = 9/group, except p70 S6K, where n = 7 in the HF group. Representative blots of total and phospho-proteins are inset, with black lines demarcating where intervening lanes have been spliced out. Group data are presented for average (means ± SE) total and phospho-protein and their ratio expressed as a percentage of the average value for controls.](image1)

![Fig. 4. Relationship of skeletal muscle myosin heavy chain (MHC) protein content to levels of phospho- to total Akt (r = 0.602; P < 0.02) in controls (n = 9; closed squares) and HF patients (n = 6; open squares). Data for Akt are expressed as a ratio of the amount of the site-specific (S473) phospho-protein relative to total Akt. AU, arbitrary units.](image2)
Prior studies that have shown reduced skeletal muscle IGF-1 expression (20, 53) in HF patients have not controlled for the profound inactivity of this population (55). Because IGF-1 expression in humans is influenced by muscle activity (14, 21), a portion of the reduced IGF-1 expression, and its relationship to reduced muscle size and gene expression, may relate to muscle disuse. Our present study supports this notion since no group differences in IGF-1 expression were found when comparing patients and controls matched for physical activity level. We acknowledge that the difference noted in our study may be realized as significant in larger cohorts of patients. Nonetheless, based on relative group differences, our results suggest that a substantial portion of the effect of heart failure observed in prior reports in humans (20, 53) is likely explained by muscle disuse. In contrast, the severity of disease in HF patients likely does not explain variation among studies considering that similar reductions in IGF-1 expression have been found in cohorts of differing disease severity (20, 53) and the similarity in disease severity between our present and prior (53) cohorts. Ultimately, alterations in circulating or locally expressed growth factors in HF patients will integrate with other anabolic and catabolic effectors downstream of receptor activation. Thus, in the present study, we focused our efforts on evaluating the effect of HF on downstream signaling events.

Phospho-Akt (S473)/Akt was reduced in HF patients compared with controls. A similar decrease in Akt phosphorylation has been found in an animal model of HF (47) and in angiotensin II-induced muscle wasting, a purported surrogate of HF-induced muscle atrophy (50). In contrast, prior studies in humans have shown no effect of HF on skeletal muscle Akt phosphorylation (27). The fact that patients in this later study had nonsymptomatic HF and were younger than those in the present study may explain divergent results. It could be argued that our inclusion of two diabetic patients, or the insulin resistance inherent to the HF population (52), might explain our finding of reduced Akt phosphorylation, since insulin also signals through Akt (3). This is unlikely, however, since studies have failed to show an effect of insulin resistance or diabetes on Akt phosphorylation or kinase activity under postabsorptive conditions (7, 28). Akt is a central node in cell signaling downstream of numerous anabolic and catabolic stimuli (16, 40). Because of this fact, and in the setting of complex human disease states such as HF, it is impossible to define the exact proximal factor contributing to reduced Akt activation. However, considered together with studies in humans showing reduced Akt phosphorylation with age (31), amyotrophic lateral sclerosis (33), and detraining (30), conditions that are accompanied by muscle atrophy and dysfunction, we conservatively interpret our finding of decreased phosphorylation of Akt in HF patients as indicative of a general reduction in anabolic and/or an increase in catabolic tone in the muscle.

Interestingly, despite reduced Akt phosphorylation, we did not observe diminished phosphorylation of mTOR, GSK-3β, p70 S6K, eIF4E-BP or eIF2Bε, which is somewhat surprising since all of these signaling molecules lie downstream of Akt (37). This could be explained by the hypothesis that site-specific phosphorylation of Akt directs its effects on downstream signaling components (18, 26); specifically, that S473 phosphorylation directs Akt regulation of FOXO and protein kinase C, whereas T308 phosphorylation directs Akt regulation of S6K, eIF4E-BP, and GSK-3β (18, 26). Although our data appear to support this notion, there are some important inconsistencies. For example, we would predict that reduced S473 phosphorylation in HF patients would diminish FOXO phosphorylation and increase FOXO-regulated gene transcription, leading to increased proteolysis. However, we previously reported no evidence for increased expression of genes regulated by FOXO, such as atrogin and MuRF1, or upregulation of protein breakdown (39). This type of discordance between S473 phosphorylation and its purported downstream signaling targets is not unique to our study, as others have shown that increased and decreased phosphorylation of Akt on S473 does not lead to predictable changes in FOXO nuclear localization or atrogin/MuRF1 expression in human skeletal muscle (12, 31, 32). Thus, within the range of variation in Akt S473 phosphorylation in human skeletal muscle, there is limited evidence for a relationship to activation of specific downstream signaling components (e.g., FOXO, atrogenes).

An alternative explanation for the disparity between Akt phosphorylation and activation of downstream signaling molecules is that our measurements were performed under rested, postabsorptive (i.e., basal) conditions. Studies that have defined the mechanisms underlying the activation of these molecules and their position in the signaling cascades have been conducted primarily on cultured muscle cells using potent anabolic stimuli [e.g., genetic manipulation, hormones, nutrients, stretch (45, 51)]. Under basal conditions, the propagation of activation down the signaling cascade may be interrupted or modified if there are predominating effects of other modulators [e.g., AMPK-activated protein kinase downregulation of mTOR (8, 19)]. That such a disconnect between Akt and downstream signaling molecules occurs under basal conditions is supported by results obtained from humans under a variety of physiological and pathophysiological conditions (12, 31, 33). Building on this notion, it could be argued that we might have observed altered phosphorylation status of these molecules if we compared the response to anabolic stimuli (e.g., exercise, nutrition, hormones) between patients and controls. Although this may be true, the fact that diminished Akt phosphorylation was evident under basal conditions suggests a particularly robust effect of the HF syndrome on this signaling component.

Buttressing our observations of signaling molecule activation in human HF are data from a preclinical model of chronic left ventricular dysfunction/HF showing a similar pattern of phosphorylation of signaling molecules (47). The fact that this preclinical model is associated with muscle atrophy and contractile dysfunction (34, 47) lends support to the notion that diminished phosphorylation of Akt may contribute to muscle atrophy and dysfunction in human HF. Interestingly, in a model of more severe muscle atrophy due to angiotensin II infusion, which is purported to reflect HF-related muscle wasting, downregulation of Akt phosphorylation was accompanied by reduced mTOR, p70 S6K, and GSK-3β phosphorylation (50). The more wide-ranging inhibition of signaling molecules in this model may be explained by the pronounced elevations in circulating angiotensin II levels, which are not observed in the coronary artery ligation model (47) or clinically stable
patients (13). In this context, the angiotensin II model may reflect adaptations in muscle signaling pathways that occur during acute, decompensated HF, where there is profound activation of the renin-angiotensin system and elevated levels of angiotensin II (13). The degree of phosphorylation of signaling molecules downstream of receptor activation, therefore, may vary with severity of the HF syndrome and its attendant hormonal disruptions, which may partially explain why we observed alterations in Akt phosphorylation, whereas studies in patients with less severe disease did not (27).

To evaluate the potential significance of variation in the expression of IGF-1 or phosphorylation of downstream signaling molecules, we examined their association to indexes of skeletal muscle size and function. The novel finding from these analyses was a positive relationship between phospho-Akt/Akt levels and MHC protein content. The physiological basis for these correlations is suggested from studies showing that variation in the expression or activation of Akt in skeletal muscle alters both muscle size and contractility (6, 29, 45), as well as work in preclinical models of HF, where enhanced activation of Akt via transgenic overexpression of IGF-1 or growth hormone application can remediate muscle atrophy and contractile dysfunction (10, 47, 50). MHC protein content serves as a molecular marker of muscle size (i.e., myofibrillar protein mass) and function considering that it comprises 25% of total muscle protein (60) and that its content within the muscle is related to muscle performance (11, 17, 56). Together with results from more mechanistic studies in animal models (47, 50), we interpret these relationships as evidence that diminished phosphorylation of Akt in HF patients may contribute to the erosion of skeletal muscle size and function. More specifically, as we and others have shown that HF causes a selective depletion of MHC protein content and that this may contribute to reduced strength and endurance (39, 56, 58), these alterations in Akt phosphorylation may have relevance to the development of physical disability in HF patients.

We also observed a relationship between phospho-mTOR/mTOR and knee extensor isometric torque after adjusting for variation in muscle size, suggesting an effect of mTOR activation on the intrinsic contractile function of the muscle. This relationship agrees generally with recent findings suggesting a role for mTOR in the regulation of normal muscle contractile function (5) and, more specifically, with preclinical studies showing that interventions that activate the IGF-1/Akt/mTOR pathway improve muscle contractile function in HF (10). Considering that there was a tendency for phospho-mTOR/mTOR to be related to MHC protein content (P = 0.07), this relationship may reflect a role for mTOR activation in the regulation of myofilament protein expression. Although we did not observe a reduction in phospho-mTOR/mTOR with heart failure, there is some evidence from animal models of HF-related muscle atrophy for reduced mTOR activation (50).

Several limitations to the present study deserve discussion. Our attempt to carefully control for confounding factors inherent to the HF population (i.e., muscle disuse, hospitalization, etc.) limited our sample size and, in turn, statistical power. In some cases, we observed differences in signaling molecule phosphorylation that did not reach significance (e.g., p70 S6K), which complicates the interpretation of our results. For those that do reach significance (e.g., Akt phosphorylation), however, we have very high confidence in the fidelity of these differences and, more importantly, their relationship to the heart failure syndrome. In all cases, the directionality of group differences in gene expression/signaling molecule phosphorylation favor the notion that HF is associated with reduced anabolic and/or elevated catabolic tone in skeletal muscle. An additional limitation is the possibility that medications taken by HF patients influenced our results. Although this may be the case, we do not believe that the medications promoted group differences in gene expression/signaling molecule phosphorylation for several reasons. First, the two main classes of medications—ACE inhibitors/angiotensin receptor blockers and β blockers—function to counter the HF-related hyperactivity of the renin-angiotensin and sympathetic nervous systems, respectively. Because experimental evidence has shown that high angiotensin levels downregulate Akt activation and its downstream targets (50) and that excessive sympathetic activation promotes muscle catabolism (24), we would predict that, if anything, these medications would diminish differences in signaling molecule activation between HF patients and controls, not promote them. Second, for those medications with a reputation for altering skeletal muscle, such as statins, we sought to frequency-match groups, recruiting control volunteers taking these medications. Third, preclinical models of HF, in which no medications are administered, show a similar pattern of signaling molecule activation (47) as the present study, further supporting the notion that HF medications did not promote alterations in signaling molecule activation. One final caveat is the fact that we studied HF patients not characterized by muscle atrophy relative to controls. This could be viewed as a limitation presuming that alterations in signaling molecule phosphorylation are only present in atrophic patients. On the contrary, because atrophy is associated with paradoxical activation (i.e., phosphorylation) of these signaling molecules in some chronic diseases [i.e., chronic obstructive pulmonary disease (COPD); (12)], we are confident that differences observed in our study are not related to prior muscle atrophy. In fact, because our patients are relatively early in the course of the disease, these alterations in phosphorylation status may reflect early defects that presage muscle loss and dysfunction.

In summary, our results suggest that HF diminishes Akt activation, as indicated by reduced phosphorylation of S473. A novel finding of our study is that diminished phosphorylation of Akt was associated with reduced MHC protein content, a molecular marker of muscle size and functionality. Importantly, these findings were made in patients and controls matched for age and physical activity levels and who displayed similar muscle size. Moreover, HF patients were characterized by mild to moderate disease and were evaluated distal to any bout of acute disease exacerbation or hospitalization. Thus we are confident that our results reflect the unique effects of the HF syndrome, rather than muscle disuse, atrophy, end-stage disease, or acute illness. In this context, our results highlight signaling molecules/pathways that may be altered by the HF syndrome to promote muscle atrophy and dysfunction via modulation of myofilament protein metabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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