Published in final edited form as:

J Card Fail. 2016 May ; 22(5): 347–355. doi:10.1016/j.cardfail.2016.02.002.

Analysis of Skeletal Muscle Torque Capacity and Circulating Ceramides in Patients with Advanced Heart Failure

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Abstract

Background—Heart failure (HF)-related exercise intolerance is thought to be perpetuated by peripheral skeletal muscle functional, structural, and metabolic abnormalities. We analyzed specific dynamics of muscle contraction in patients with HF compared with healthy, sedentary controls.

Methods—Isometric and isokinetic muscle parameters were measured in the dominant upper and lower limbs of 45 HF patients and 15 healthy age-matched controls. Measurements included peak torque normalized to body weight, work normalized to body weight, power, time to peak torque, and acceleration and deceleration to maximum strength times. Body morphometry (dual energy X-ray absorptiometry scan) and circulating fatty acids and ceramides (lipodomics) were analyzed in a subset of subjects (18 HF and 9 controls).

Results—Extension and flexion time-to-peak torque was longer in the lower limbs of HF patients. Furthermore, acceleration and deceleration times in the lower limbs were also prolonged in HF subjects. HF subjects had increased adiposity and decreased lean muscle mass compared

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Supplementary Data

Supplementary data related to this article can be found at doi:10.1016/j.cardfail.2016.02.002.

with controls. Decreased circulating unsaturated fatty acids and increased ceramides were found in subjects with HF.

Conclusions—Delayed torque development suggests skeletal muscle impairments that may reflect abnormal neuromuscular functional coupling. These impairments may be further compounded by increased adiposity and inflammation associated with increased ceramides.

Keywords

Heart failure; skeletal muscle function; time to peak torque

Patients with heart failure (HF) develop exercise intolerance, fatigue, and dyspnea.¹ These symptoms are perpetuated primarily by impaired skeletal muscle function and only in part by central hemodynamic dysfunction.^{2–6} HF is associated with metabolic changes within muscle fibers that relate to exercise intolerance.⁷ An overall shift in skeletal muscle fiber type from the slow-twitch type I to the fast-twitch type II reflects deconditioning of the muscle as well as a specific muscle myopathy.^{8,9} A shift from slow-twitch to fast-twitch muscle fibers should result in decreased endurance, but potentially faster muscle contraction in HF patients. Unfortunately, there are few data focusing on the speed and dynamics of muscle contraction in HF patients.

Exercise intolerance associated with HF is further worsened through an enhanced ergoreceptor reflex activity in which receptors of exercise metabolites induce an increase in blood pressure and breathlessness.^{10,11} As a result, HF patients progressively lose the ability to generate and maintain force with a concurrent reduction in ventilatory drive.³ Reduced strength and fatigability has been demonstrated in HF patients through multiple studies of endurance and isometric and isokinetic torque.^{12–16} Although it is well-known that exercise training can improve mortality and morbidity in HF patients,¹⁷ more information is needed regarding the musculoskeletal impairments associated with HF. Furthermore, other characteristics such as muscle workload and power as well as an analysis of muscle function specifically in the upper extremities needs to be characterized.

The aim of the present study was to determine the dynamics of muscle contraction in systolic HF patients compared with healthy, sedentary controls in both the upper and lower extremities. Because of the possible role of fat mass and mediators of inflammation on muscle function, we studied body composition and circulating lipids such as fatty acids and ceramides in a subset of study subjects. We hypothesized that the HF patient would have impaired skeletal muscle function in both the upper and lower limbs, with an increase in muscle contraction time parameters. Furthermore, we hypothesized that the substudy would show increased adiposity and elevated levels of fatty acids and ceramides.

Methods

Study Cohort

A total of 45 patients with systolic dysfunction HF (age 57 ± 1 years) and 15 age-matched control subjects (age 56 ± 2 years) participated in the study. All HF subjects had a left ventricular ejection fraction of $\geq 35\%$, were stable on standard HF medications according to

American Heart Association/American College of Cardiology and Heart Failure Society of America HF guidelines¹⁸ for at least 3 months, and had no major cardiac event in the previous 6 months. All controls were free of established cardiovascular disease as assessed by medical history and fasting blood chemistries. Subjects were allowed to have controlled cardiovascular risk factors such as isolated hyperlipidemia or pharmacologically treated hypertension. All subjects provided written informed consent. This study was approved by the Institutional Review Board of Columbia University Medical Center (CUMC).

Cardiovascular Fitness Testing

Cardiopulmonary exercise testing was used to assess overall aerobic fitness. Subjects performed a ramping exercise protocol on an electronically braked cycle ergometer (Ergometrics 800, SensorMedics Inc., Yorba Linda, CA), following the American Thoracic Society guidelines.¹⁹ Briefly, following baseline measurements, subjects were asked to maintain 50–60 rpm as wheel resistance increased each minute. Once the subject was unable to maintain the 50–60 rpm or requested to stop, the test was terminated and subjects performed 3 minutes of active recovery. Peak oxygen consumption ($\text{VO}_{2\text{peak}}$) was measured using computer-assisted open-circuit spirometry. Rate of perceived exertion, blood pressure, and heart rate were measured throughout exercise. The cardiopulmonary exercise testing was performed before all other exercise protocols. Additionally, all subjects completed a 6-minute walk test, as described previously.²⁰

Muscle Strength Testing

Isometric and isokinetic measurements were completed on the dominant extremities using the Biodex System 4 Pro (Biodex Medical Systems, Shirley, NY). For isometric measurements, subjects contracted for 6 seconds at 60° for the knee and 30° for the elbow. The subjects alternated between extension and flexion for a total of 6 repetitions with a rest time of 30 seconds between each repetition.

For isokinetic measurements, subjects quickly performed repetitive extension and flexion movements in 2 protocols. The first isokinetic test completed 5 repetitions at a speed of 60° per second followed by a rest of 2.5 minutes. For the second isokinetic test, subjects completed 25 repetitions at a speed of 180° per second.

The subjects completed all 3 protocols on the dominant knee before testing the dominant elbow. Subjects were encouraged during every testing protocol. As outlined in the Biodex System 4 manual, isometric data were collected to measure peak torque (greatest muscular force) to body weight (PKTQ/BW). Isokinetic data were collected to measure PKTQ/BW, work (total muscle force within a repetition) to BW, average power (amount of work divided by time), fatigability, time to peak torque, acceleration time (time to reach isokinetic speed), and deceleration time (time to go from isokinetic speed to 0). Acceleration and deceleration represent a muscle neurological capability. The normalization to BW allows for a more relative and pertinent assessment of function activity.¹³

All subjects also completed a handgrip strength test for the dominant hand. Maximal handgrip strength was the average measurement of 3 attempts on the dominant hand and was normalized for total body weight.²¹

Dual Energy X-Ray Absorptiometry

A subset of 9 control and 18 HF subjects, who agreed to this additional test, completed a full dual energy x-ray absorptiometry (DXA; Hologic QDR4500W, Discovery Model with 4.5.3 software) scan to assess body composition. Total lean mass, fat mass, and trunk/limb fat mass ratios were analyzed. Additionally, lean and fat mass measurements in the dominant leg were also determined.

Metabolic Measurements

Blood samples were drawn on the day of Biodex muscle testing from 5 controls and 17 HF subjects for ceramides and free fatty acid (FFA) analysis. The samples were spun for 20 minutes at 2500 rpm at 4°C. Aliquots of 500 µl serum or plasma were put into cryovials and frozen at -80°C. Blood laboratory values were collected from the CUMC medical record of 6 control subjects and all 45 HF patients.

Ceramide Analysis

Ceramide levels were assessed using liquid chromatography/mass spectrometry (LC/MS) lipidomics and samples were extracted using chloroform:methanol extraction. Briefly, 3 ml of the chloroform:methanol (2/1, v/v) containing 20 µl of a 2 µM internal standard mixture (Avanti LM-6002, containing C12 and C25 ceramides) were added to 100 µl aqueous of serum in a clean glass tube. The mixture was vortexed well. One-half milliliter of water was added to the mixture to allow for phase separation. The mixture was vortexed again and centrifuged at 3000 *g* for 10 minutes. The lower organic phase was transferred to a second clean glass tube using a Pasteur pipette. Two milliliters of chloroform were added to the residual aqueous phase, followed by vortex mixing and centrifugation again at 3000 *g* for 10 minutes to extract the remaining lipids. The lower organic phases were pooled and evaporated under nitrogen. The extracted lipids were reconstituted in 30 µl of methanol and transferred to LC/MS autosampler vials (Waters, P/N 600000670CV) for injection.

All experiments were carried out on a Waters Xevo TQ MS ACQUITY ultraperformance liquid chromatography [UPLC] system (Waters). The system was controlled by Mass Lynx Software, version 4. 1. The sample was maintained at 4°C in the autosampler and 5 µl was loaded onto a Waters ACQUITY UPLC BEH Phenyl column (3 mm inner diameter × 100 mm with 1.7-µm particles), preceded by a 2.1 × 5 mm guard column containing the same packing. The column was maintained at 40°C throughout analysis. The UPLC flow rate was continuously 300 µl/min in a binary gradient mode with the following mobile phase: initial flow conditions were 20% solvent A (H₂O, containing 0.2% formic acid and 10 mM ammonium formate) and 80% solvent B (methanol, containing 0.2% formic acid and 10 mM ammonium formate). Solvent B was increased linearly to 95% over a 2-minute period and to 98% in the subsequent 6 minutes. This was followed by a reduction of solvent B to 80% starting at 8.2 minutes and continuing through 9 minutes. Ceramides of interest eluted between 4.0 and 7.5 minutes. Positive electrospray ionization (ESI) tandem mass spectrometry was performed using the following parameters: capillary voltage, 3.8 kV; source temperature, 150°C; desolvation temperature, 500°C; desolvation gas flow, 1000 L/h; and collision gas flow, 0.15 mL/min. The optimized cone voltage was 22 V, collision energy for multiple reactions monitoring mode was 26 eV. For multiple reactions monitoring mode

analysis, the following transitions were used: C12 ceramide 482.5→264.2, C14 ceramide 510.5→264.2, C16 ceramide 538.5→264.2, C18:1 ceramide 564.5→264.2, C18 ceramide 566.5→264.2, C20:1 ceramide 592.6→264.2, C20 ceramide 594.5→264.2, C22:1 ceramide 620.6→264.2, C22 ceramide 622.6→264.2, C24:1 ceramide 648.6→264.2, C24 ceramide 650.6→264.2, C25 ceramide 664.7→264.2, C26:1 ceramide 676.8→264.2, and C26 ceramide 678.8→264.2.

FFA Analysis (LC/MS)

FFA levels were assessed using LC/MS lipidomics and samples were extracted with a chloroform:methanol extraction. Briefly, 3 ml of chloroform:methanol (2:1 v/v) containing 1 nmol deuterated palmitic acid as internal standard were added to 20 µl of serum in a clean glass tube. After the addition of 0.5 ml water, the mixture was vortexed and spun at 3000 *g* for 10 minutes. The lower organic phase was transferred to another clean glass tube with a Pasteur pipette. Two milliliters of chloroform was added to the aqueous phase followed by vortex and spin. The lower organic phases were pooled and removed under nitrogen. Lipid extracts were reconstituted in 50 µl of methanol and transferred to LC/MS vial (Waters, P/N 600000670CV) for injection.

All experiments were carried out on a Waters Xevo TQ MS ACQUITY UPLC system (Waters). The system was controlled by MassLynx Software, version 4.1. The sample was maintained at 4°C in the auto-sampler and a volume of 5 µl was loaded onto a Waters ACQUITY UPLC BEH C18 column (2.1-mm inner diameter × 100 mm with 1.7-µm particles), and a 2.1 × 5 mm guard column with the same packing material. The column was maintained at 40°C. The flow rate was 300 µl/min in a binary gradient mode with the following mobile phase gradient: initiated with 15% phase A (H₂O containing 10 mM ammonium acetate) and 85% mobile phase B (methanol containing 10 mM ammonium acetate). Gradient of methanol was increased linearly to 100% over 8 minutes and maintained until 10 minutes. The column was reconditioned with the initial gradients for 2 minutes and the next sample was injected. Negative ESI-tandem mass spectrometry with selected ion recording mode was performed using the following parameters: capillary voltage −3.8 kV, source temperature 150°C, desolvation temperature 500°C, desolvation gas flow 1000 L/h. Different species were identified by comparing the retention times of experimental compounds with those of authentic standards. Concentrations of FFAs in the serum were quantitated by comparing integrated peak areas for those of each species against those of known amounts of purified standards. Loss during extraction was accounted for by adjusting for the recovery of the internal standard added before extraction.

Statistical Analysis

Descriptive characteristics, muscle assessment parameters, DXA scan parameters, FFA, and ceramide levels in each group were determined by unpaired 2-tailed *t* test. Nonparametric, Kruskal-Wallis tests were run to confirm the *t* test results. Categorical characteristics were tested for group differences using Fisher exact test. All data were measured for equality of variance and tests of normality. Data are expressed as means ± standard deviation. A Pearson matrix was used to determine correlations between the selected variables. Statistical

significance was set at $P < .05$ and effect sizes (Cohen's d) for all variables were between 0.5 and 0.8. The statistical analysis was repeated for the substudy subjects.

One subject was unable to perform the full testing protocol and was removed from part of the analysis. Furthermore, one-third of the HF patients had diabetes and a separate analysis was performed to examine changes in skeletal muscle assessment between the HF patients with and without diabetes. There were no differences shown, so data were presented as all HF subjects combined. Similar results were shown in the subanalysis of subjects that underwent a full body scan and blood draw for ceramides and FFA measurements.

Ethics Statement

All HF subjects were undergoing evaluation for HF at CUMC. We obtained permission from their primary providers to approach subjects, and all subjects signed an informed consent before further inclusion criteria were met. Minorities were included in this study, but no vulnerable or disadvantaged populations were involved. Subjects were recruited during the clinical evaluation process after permission to approach the subject has been obtained from their personal physician. Subjects were asked to sign consent by a member of the study team. Participation was entirely voluntary and in no way affected their care.

All efforts were made to minimize risk to the subject. The cardiopulmonary test and Biodex muscle function testing occurred in the outpatient clinical area. Emergency equipment and medical personnel were in the immediate vicinity. Blood samples were taken during the outpatient visits at low risk. Serious adverse events were to be reported to the institutional review board within 24 hours. All adverse events were reviewed on a biweekly basis by a cardiologist otherwise not affiliated with this proposal. All patient information was kept absolutely confidential in full compliance with Health Insurance Portability and Accountability Act rules.

Results

Baseline demographics are presented in Tables 1 and 2. All subjects completed the Biodex protocol for the dominant knee and elbow. As shown in Table 1, the control and HF subjects were similar in age and resting hemodynamic measurements. The HF subjects had a slightly higher body mass index than the controls. As expected, the HF subjects had a lower VO_{2peak} and 6-minute walk scores compared with healthy controls. Dominant hand grip strength was not significantly different between the 2 groups. The HF subjects had lower hematocrit, platelet count, and potassium and cholesterol levels compared with healthy controls. All other laboratory parameters were not significantly different (Table 1). The New York Heart Association functional class, comorbidities, HF etiology, and lists of medications are shown in Table 2.

Lower Limb Skeletal Muscle Assessment

Skeletal muscle function parameters on the dominant lower limb are shown in Table 3. HF patients had lower isometric peak torque/BW flexion compared with control subjects. The dominant knee in HF patients showed lower torque in both extension and flexion total strength as measured by PKTQ/BW for isokinetic exertion at $60^{\circ}/se$ (Fig. 1) and $180^{\circ}/s$.

Both, work during extension and flexion normalized to BW were lower in the HF patients at 60°/s and 180°/s compared with the controls. Total power for flexion at 60°/s and 180°/s was also lower in the HF patients. There were no correlations between HF class and the skeletal muscle function parameters.

As shown in Fig. 2, extension and flexion time-to-peak torque in the dominant lower limb at 60°/s (529 ± 137 vs 437 ± 116 and 900 ± 367 vs 641 ± 288 msec, $P = .02$ and $P = .02$; respectively) and 180°/second (241 ± 113 vs 166 ± 40 and 528 ± 89 vs 419 ± 171 msec, $P = .02$ and $P = .002$; respectively) were longer in the HF patients compared with controls. Furthermore, acceleration during extension in the lower limb at 180°/s (81 ± 32 vs 61 ± 18 msec, $P = .03$) was prolonged in HF subjects compared with controls. Deceleration during flexion of the lower limb was prolonged in the HF group during 180°/s (180 ± 46 vs 149 ± 42 msec, $P = .03$). Strong trends were seen in the remaining acceleration and deceleration parameters of the dominant lower limb (see Table S1). There were no correlations between HF class and the skeletal muscle time parameters. Furthermore, there was no correlation between the skeletal muscle time parameters and $\text{VO}_{2\text{peak}}$ except for a minor correlation at 180°/s for leg extension in the HF group ($R^2 = 0.14$, $P = .03$).

Upper Limb Skeletal Muscle Assessment

Although there were similar trends to values of the lower limb, there were no significant differences between the HF patients and control subjects for skeletal muscle functional parameters in the dominant upper limb (Table 4). Furthermore, we found strong trends of slower times in the HF subjects, but there were no significant differences in extension or flexion for time-to-peak torque, acceleration, or deceleration in the dominant upper limb between HF and control subjects (Table S1).

DXA Scan

Within the subset of study subject, body mass index (28.8 ± 5.4 vs 25.2 ± 2.7 kg/m², respectively) and total lean mass (54.5 ± 11.6 vs 49.6 ± 9.4 kg, respectively) were not significantly different between the HF and control subjects, but total fat mass was higher in the HF group compared with control subjects (25.8 ± 8.7 vs 16.9 ± 4.6 kg; $P = .029$). Furthermore, the trunk/limb fat mass ratio was higher in the HF compared with control subjects (1.2 ± 0.3 vs 0.8 ± 0.2 ; $P = .001$). Of note, compartmental lean mass and fat mass of the dominant leg were not significantly different between the HF and control subjects.

Dominant leg lean mass positively correlated with peak torque/BW in extension and flexion in the HF subjects ($R^2 = 0.21$, $P = .05$ and $R^2 = 0.23$, $P = .03$, respectively) but not in control subjects ($R^2 = 0.01$, $P = .83$; $R^2 = 0.06$, $P = .54$, respectively) (Fig. 3A and 3B). Similarly, the trunk/limb fat mass ratio was positively correlated to extension peak torque/BW in the HF subjects ($R^2 = 0.35$, $P = .01$) but not to flexion peak torque/BW ($R^2 = 0.11$, $P = .16$). The trunk/limb fat mass ratio was not significantly correlated to peak torque/BW in extension or flexion in the control subjects ($R^2 = 0.03$, $P = .65$; $R^2 = 0.01$, $P = .84$, respectively) (Fig. 3C and 3D).

Lipidomic Analysis of Circulating Fatty Acids and Ceramides

Using LC/MS lipidomics, levels of circulating fatty acids and ceramides, known markers of abnormal metabolism and lipotoxicity, were analyzed. Total fatty acid levels were not different between HF subjects and controls. However, lower levels of circulating unsaturated fatty acids were found in HF subjects compared with controls (243 ± 181 vs 353 ± 328 μM). Circulating ceramides were higher in HF subjects compared with controls (5.8 ± 1.8 vs 4.4 ± 0.6 μM). Of note, in particular the very long chain ceramides, C22:1 and C24:1, were higher in the HF group compared with controls (0.08 ± 0.04 vs 0.02 ± 0.01 and 1.34 ± 0.41 vs 0.73 ± 0.08 μM , respectively) (Fig. 4 and Table S2). There was no correlation between ceramide levels and muscle function testing or body composition in the HF patients or controls.

Discussion

Our current study shows that patients with HF have prolonged muscle contractility dynamics as shown by increased time-to-peak torque intervals as well as slower acceleration and deceleration times compared with healthy controls. Further, muscle function and dynamics in the upper limb of HF patients is not significantly different from healthy controls. Body morphometry revealed increased adiposity and lower lean muscle mass in HF subjects as well as decreased circulating unsaturated fatty acids contrasted by increased circulating ceramide levels.

The results of the present study extend prior findings of reduced peak torque and power in leg extension in HF compared with controls.¹² These studies reported a trend toward slower time to target velocity seen in the HF patients.¹² Our study has expanded on this finding using a specified protocol for the assessment of muscle contraction dynamics and shows significantly slower time-to-peak torque for both extension and flexion in the lower dominant leg. Furthermore, we found that HF patients had slower acceleration and deceleration times during extension and flexion of the lower dominant leg. These impaired time parameters are unexpected because HF individuals have been shown to develop an increase in the relative amount of glycolytic, fast-twitch type II muscle fibers, which theoretically should result in faster contractile velocities.^{8,22} This discrepancy suggests that the relative increase in type II muscle fibers in HF patients may be accompanied by impaired contractile function.^{8,12,23} Because of the minor correlation between VO_2peak and leg extension in HF patients, we cannot rule out deconditioning effects on contractile velocities in HF patients.

Our findings of slower muscle contractile velocities may be explained by previous data showing impaired muscle fiber recruitment at submaximal contraction through electromyographic assessment suggesting impaired neuromuscular functional coupling.²⁴ Excitation-contraction (E-C) coupling initiates the contraction of the myocardial muscle following the release of Ca^{2+} from ryanodine receptors in the sarcoplasmic reticulum in response to voltage-gated Ca^{2+} channels in the transverse tubules.^{25,26} In HF, myocardial E-C coupling is impaired due to a variety of mechanisms such as a sarcoplasmic Ca^{2+} leak, elevated cellular Na^+ levels, and structural remodeling.²⁷⁻²⁹ At least in part, similar E-C coupling impairments including reduced sarcoplasmic Ca^{2+} ATPase expression are seen in

the skeletal muscle of HF patients,³⁰ but further research is needed to specifically define the abnormalities in skeletal muscle in HF.³¹ Another possible mechanism was suggested by Harridge et al., who used twitch interpolation in HF patients and reported that poor resistance to fatigue was not due to poor muscle activation but from intrinsic neuromuscular abnormalities.¹⁴

The present study also demonstrates that HF patients have reduced musculoskeletal function and time parameters primarily in the larger, lower limb muscle groups, whereas the smaller muscle groups remain unaffected. This may be due to a decrease in daily activities, such as prolonged walking, because of dyspnea stimulated by enhanced ergoreceptor stimulation in HF.³² Ergoreceptors are receptors that bind metabolites produced from exercise that signal the nervous system to increase ventilation, presenting as breathlessness. Although these receptors have been found in both the upper body¹⁰ and the lower body muscles,¹¹ activity patterns of the lower and upper extremities are different, which might in part explain this difference in skeletal muscle functional impairment. This difference between the upper and lower limbs of HF patients found in the present study contradicts previous findings from Gosker et al., who demonstrated reduced musculoskeletal function occurred in both the lower and upper body of HF patients.³³ However, HF patients in the Gosker et al. study had a lower average $\text{VO}_{2\text{peak}}$, compared with our current study, which has been shown to correlate with increased mortality risk.^{33–36} Although we saw trends of impaired skeletal muscle function in the upper limb, the lack of significant differences in the dominant upper arm may be due to our HF subjects being healthier than shown in previous studies with 58% of our HF subjects being in New York Heart Association class I or II. Further, larger muscle groups might be affected first and smaller muscle group dysfunction may be indicative of progressively worse and advanced HF.

The detailed morphometric analysis in a subset of patients in our study revealed a decrease in lean muscle mass consistent with a progressive loss of muscle in the setting of muscle wasting and cardiac cachexia.^{23,37} Further, we found increased adiposity of HF subjects compared with controls reflected by higher total adipose mass and adipose mass normalized to body weight as well as an increased trunk/limb adipose mass ratio reflecting increased central obesity in HF subjects. These findings might reflect a distinct metabolic characteristic that develops in HF subjects and is associated with progressive muscle wasting and catabolism.

Circulating metabolic molecules can serve both as markers and mediators of advanced HF-related mechanisms. Profiling of circulating fatty acids did not reveal differences in total or individual fatty acids except lower levels of unsaturated fatty acids. This findings might mechanistically support the supplementation of polyunsaturated omega-3 fatty acids in subjects with HF. Circulating ceramides, known mediators of inflammation and lipotoxicity, were found to be increased in HF subjects compared with controls. In particular, very long chain ceramides (C22:1 and C24:1) were higher in plasma of HF patients compared with controls. Although these findings are consistent with abnormal profiles of lipid intermediates and ceramides associated with the progressive, pro-inflammatory state of HF, there was no significant correlation to muscle function or body composition in our subset of HF patients. Further analysis may be required to examine these correlations.

Our study has several limitations. First, as a result of the lack of muscle biopsies to determine skeletal muscle fiber type, we are unable to conclude that our HF patients fully developed the previously reported shift from type I to type II muscle fibers. However, numerous studies have confirmed this shift in muscle fiber type in various stages of HF.^{8,9} Second, although not significantly different, our control group had a greater percentage of female participants than the HF group. It is possible that women may demonstrate a different skeletal muscle phenotype with respect to muscle function and time parameters. Third, the HF subjects were taking a variety of medications, which could affect the parameters measured in this study, specifically beta-blockers and statins that have been shown to cause muscle impairments.^{38,39} Fourth, given the smaller sample size of this study the use of a multivariate analysis was not appropriate for controlling for all relevant confounders. Furthermore, because of the limited sample size, this study was underpowered to detect all but large effect sizes (power < 64% for effect size < 0.7), indicating a clear possibility for type II errors to occur, especially given the number of tests. As such, null findings should be interpreted cautiously. Finally, given our cross-sectional study design, we cannot discount the possibility of differences in genotypes or lifestyle behavior patterns within the groups. To minimize the influence of lifestyle behavior patterns, we selected subjects with sedentary lifestyle for our control group.

The results of our study demonstrate that HF patients have slower time-to-peak torque as well as slower acceleration and deceleration times compared with healthy, sedentary controls. Our findings point toward an impairment of intrinsic muscle fiber contractile patterns in conjunction with impaired excitation-contraction coupling. Further studies are needed to elucidate the exact role of circulating molecules for the progressive impairment of skeletal muscle function in HF. Better understanding of musculoskeletal changes associated with HF is crucial to potentially reverse exercise intolerance and improve quality of life in this patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the National Heart, Lung, and Blood Institute (K23 HL095742-01, P30 HL101272-01, UL1 RR 024156, HL073029) and the Herbert and Florence Irving Scholar Award (UL1 TR000040) to both Dr. Schulze and Dr. Brunjes. Grant support was also awarded to Dr. Brunjes from the National Institutes of Health (2 T32 HL 7343-36).

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Fig. 1. Representative leg extension curves. Average values taken every 10 msec from 45 HF (black) and 15 control (gray) subjects during the third leg extension (of 5 repetitions) at 60°/s. Vertical line shows time to peak torque. Values expressed as means $\pm$ standard error of the mean (dashed lines). HF, heart failure.

Fig. 2.

Time-to-peak torque during extension and flexion. Extension (A) and flexion (B) moving at 60°/s, and extension (C) and flexion (D) moving at 180°/s in the dominant leg of control (circles) and HF (squares) subjects. Values are means $\pm$ standard deviation. HF, heart failure.

Fig. 3.

Correlation between dual energy X-ray absorptiometry and muscle function parameters.

Peak torque/BW for extension (A) and flexion (B) moving at 60°/s correlated with dominant leg lean mass, and peak torque/BW for extension (C) and flexion (D) moving at 60°/s with trunk/limb mass ratio in control (dashed) and heart failure (solid) subjects. Significant correlation for HF subjects in figure B ($P = .039$, $R^2 = 0.227$) and C ($P = .007$, $R^2 = 0.354$). BW, body weight.

Fig. 4.

Heat map of ceramide levels. A graphical representation of the ceramide raw data compared with the average control values for each ceramide. Green represents values >1 , whereas red represents values <1 . The darker the color, the closer the value is to 1. There appears to be a greater amount of ceramide levels in the heart failure group compared with controls.

Table 1**Subject Characteristics**

	Control (n = 15)	Heart Failure (n = 45)	P Value
Gender (M/F)	9/6	37/8	.092
Age (y)	56 ± 8	57 ± 9	.482
Body mass index (kg/m ²)	25.1 ± 4.0	27.9 ± 4.5	.033
Resting heart rate (bpm)	78 ± 17	71 ± 10	.060
Resting SBP (mmHg)	126 ± 14	117 ± 17	.083
Resting DBP (mmHg)	78 ± 11	75 ± 11	.388
Functional assessment			
Hand grip strength (kg)	34.5 ± 11.2	35.9 ± 10.4	.687
Hand grip/body weight (%)	46.9 ± 10.2	44.6 ± 13.1	.545
VO ₂ max (ml·kg ⁻¹ ·min ⁻¹)	30.4 ± 8.0	18.3 ± 8.0	<.001
6-min walk (m)	470 ± 79	370 ± 77	<.001
Laboratory parameters (n = 6)*			
WBC (× 10 ³ /μl)	6.6 ± 3.3	6.6 ± 2.2	.975
Hematocrit (%)	43.1 ± 1.4	39.2 ± 4.6	.049
Platelet count (× 10 ³ /μl)	271 ± 106	208 ± 51	.017
Sodium (mEq/l)	138 ± 1	139 ± 3	.571
Potassium (mEq/l)	4.7 ± 0.5	4.3 ± 0.4	.044
Calcium (mg/dl)	9.2 ± 0.6	9.3 ± 0.5	.736
Blood urea nitrogen (mg/dl)	14.4 ± 4.5	19.2 ± 7.3	.160
Creatinine (mg/dl)	0.97 ± 0.12	1.16 ± 0.42	.272
Albumin (mg/dl)	4.4 ± 0.2	4.3 ± 0.4	.556
Total bilirubin (mg/dl)	0.55 ± 0.21	0.78 ± 0.50	.283
Direct bilirubin (mg/dl)	0.10 ± 0.01	0.18 ± 0.23	.427
Aspartate aminotransferase (U/l)	18.7 ± 5.5	22.2 ± 7.0	.241
Alanine transaminase (U/l)	22.3 ± 8.6	20.1 ± 7.8	.521
Alkaline phosphatase (U/l)	57.3 ± 10.2	92.4 ± 110.4	.444
Cholesterol (mg/dl)	223 ± 21	169 ± 46	.027
HDL (mg/dl)	57 ± 10	43 ± 13	.047
LDL (mg/dl)	104 ± 39	95 ± 34	.605
Triglycerides (mg/dl)	145 ± 13	175 ± 159	.714
Glucose (mg/dl)	114 ± 56	107 ± 41	.789

Values expressed as mean ± standard deviation. DBP, diastolic blood pressure; F, female; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; SBP, systolic blood pressure; VO₂max, maximum oxygen consumption; WBC, white blood cell.

* Laboratory values were available only from 6 controls; *P* < .05 vs control.

Table 2**Disease Etiology and Medication**

	Controls (n = 15)	Heart Failure (n = 45)
NYHA class, no. (%)		
I	—	10 (22)
II	—	16 (36)
III	—	17 (38)
IV	—	2 (4)
Etiology of HF, no. (%)		
Dilated cardiomyopathy	—	26 (58)
Ischemic cardiomyopathy	—	17 (38)
Hypertrophy	—	1 (2)
Other	—	1 (2)
Comorbidities, no. (%)		
Diabetes	—	15 (33)
Hypertension	1 (7)	30 (67)
Hypercholesterolemia	2 (13)	27 (60)
Kidney disease	—	5 (11)
Medications, no. (%)		
β-blocker	1 (7)	42 (93)
Aspirin	1 (7)	38 (84)
ACE/ARBs	—	36 (80)
Statins	—	32 (71)
Diuretics	—	31 (69)
Digoxin	—	17 (38)
Coumadin	—	9 (20)
Insulin	—	9 (20)
Oral antidiabetic agent	—	9 (20)
Spironolactone	—	8 (18)

ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; NYHA, New York Heart Association; other abbreviation as in Table 1.

Table 3

BioDex Results for the Dominant Leg

	Control (n = 15)	Heart Failure (n = 45)	P Value
Isometric			
Peak torque/BW—extension (%)	197 ± 59	181 ± 49	.303
Peak torque/BW—flexion (%)	97 ± 29	79 ± 22	.015
Isokinetic (60°/s)			
Peak torque/BW, extension (%)	178 ± 51	145 ± 44	.019
Peak torque/BW, flexion (%)	91 ± 27	64 ± 19	<.001
Work/BW, extension (%)	159 ± 40	132 ± 37	.018
Work/BW, flexion (%)	91 ± 28	63 ± 24	.001
Total work, extension (J)	535 ± 224	487 ± 184	.414
Total work, flexion (J)	293 ± 131	222 ± 120	.054
Average power, extension (watts)	75 ± 35	66 ± 27	.320
Average power, flexion (watts)	38 ± 19	28 ± 16	.049
Isokinetic, 180°/s			
Peak torque/BW, extension (%)	123 ± 37	103 ± 29	.032
Peak torque/BW, flexion (%)	77 ± 21	62 ± 19	.010
Work/BW, extension (%)	120 ± 36	99 ± 31	.032
Work/BW, flexion (%)	66 ± 25	44 ± 21	.002
Total work, extension (J)	1808 ± 728	1589 ± 601	.251
Total work, flexion (J)	878 ± 368	638 ± 418	.052
Average power, extension (watts)	131 ± 57	104 ± 45	.067
Average power, flexion (watts)	60 ± 29	38 ± 28	.012

Values expressed as mean ± standard deviation. BW, body weight.

Table 4**BioDex Results for the Dominant Arm**

	Control (n = 15)	Heart Failure (n = 45)	P Value
Isometric			
Peak torque/BW, extension (%)	58 ± 25	56 ± 17	.643
Peak torque/BW, flexion (%)	77 ± 23	68 ± 18	.120
Isokinetic, 60°/s			
Peak torque/BW, extension (%)	44 ± 16	43 ± 13	.768
Peak torque/BW, flexion (%)	61 ± 18	55 ± 14	.221
Work/BW, extension (%)	47 ± 19	48 ± 15	.833
Work/BW, flexion (%)	71 ± 23	66 ± 17	.361
Total work, extension (J)	170 ± 84	178 ± 62	.695
Total work, flexion (J)	244 ± 109	246 ± 77	.913
Average power, extension (watts)	22 ± 12	23 ± 9	.866
Average power, flexion (watts)	32 ± 15	31 ± 10	.746
Isokinetic—180°/s			
Peak torque/BW, extension (%)	36 ± 10	35 ± 8	.762
Peak torque/BW, flexion (%)	53 ± 18	50 ± 12	.590
Work/BW, extension (%)	36 ± 14	36 ± 11	.991
Work/BW, flexion (%)	56 ± 23	55 ± 15	.844
Total work, extension (J)	536 ± 281	555 ± 222	.787
Total work, flexion (J)	878 ± 467	907 ± 327	.794
Average power, extension (watts)	35 ± 20	34 ± 16	.811
Average power, flexion (watts)	56 ± 34	53 ± 21	.767

Values expressed as mean ± standard deviation. Abbreviation as in Table 3.