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Skeletal muscle mitochondrial density, gene expression, and enzyme activities in human heart failure: minimal effects of the disease and resistance training

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Abstract

IMPAIRED SKELETAL MUSCLE ENERGETICS in heart failure (HF) patients ([32](#)) may contribute to physical disability and metabolic dysfunction. Decreased skeletal muscle oxidative capacity ([63](#)), secondary to reduced mitochondrial density ([12](#), [13](#)) and/or function ([10](#), [36](#), [52](#)), may contribute to exercise intolerance, the hallmark symptom of HF. Moreover, as mitochondrial dysfunction is associated with fiber atrophy ([7](#)), impaired energetics may reduce physical function by promoting muscle wasting and, in turn, weakness ([21](#)). Because mitochondrial dysfunction has been implicated in the pathophysiology of diabetes ([41](#)), alterations in mitochondrial content and/or function could also contribute to the high prevalence of insulin resistance in the HF population ([53](#)), which could provoke deleterious protein metabolic effects that predispose to atrophy ([56](#)). Knowledge of the unique effects of HF on mitochondrial biology is limited, because nearly all studies have failed to account for several factors that accompany the syndrome of HF and can alter mitochondrial structure and function, the most notable being muscle disuse ([55](#)) and hospitalization. Studies that have attempted to control for muscle disuse in the HF population by recruitment of sedentary controls have found minimal effects of HF on mitochondrial function ([8](#), [33](#), [68](#)). There are, however, deficiencies in mitochondrial biology that persist following control for muscle disuse [e.g., mitochondrial and cytosolic enzyme activity ([33](#))] and other aspects of the

mitochondrial myopathy of HF that have not been compared between patients and controls matched for activity level. Thus it is unclear whether deficits in mitochondrial content or aspects of mitochondrial function are related to the HF syndrome per se or are a consequence of muscle disuse and/or other disease-related factors (47).

If muscle disuse contributes to deficiencies in mitochondrial structure and function in HF (8, 33, 68), exercise interventions should improve muscle energetics. In support of this notion, aerobic exercise training improves physical function in HF patients (11), and some portion of its beneficial effect is likely related to increased skeletal muscle mitochondrial content and/or function (20, 71). Despite the many benefits of aerobic exercise training, it is generally not considered an effective intervention to increase muscle size or strength (24,26). Exercise that provokes muscle anabolism, such as resistance training, may need to be incorporated into standard, aerobic exercise-based rehabilitation regimens to more completely address the range of morphological and functional alterations in skeletal muscle in HF that limit physical function. Indeed, studies that have employed a combination of aerobic- and resistance-training programs in HF patients have shown improved aerobic fitness and muscle strength (4, 14, 67) accompanied by improved mitochondrial function (67). However, it is unclear whether the resistance-training component of the program contributes to improvements in mitochondrial biology or whether these improvements are related solely to the aerobic component.

Conventional wisdom, which has been gleaned largely from studies in younger individuals, holds that resistance training has minimal or deleterious effects on skeletal muscle mitochondrial content or function (22, 54). Recent studies, however, have challenged this notion (44, 62). In elderly individuals, in particular, beneficial effects of resistance training on mitochondrial content, enzyme activity, and the expression of genes regulating mitochondrial size and function (23, 31, 43), as well as improvements in aerobic fitness (15), have been found. In HF patients and controls, resistance exercise training improves physical function (1, 49), including activities that are believed to be dependent on aerobic metabolism, such as walking endurance (1, 42, 45, 49), suggesting that this training modality has salutary effects on muscle endurance. In HF patients, resistance training has been reported to increase aerobic fitness (14, 27), along with mitochondrial enzyme activity (45), suggesting that this training modality may potentiate muscle endurance through effects on mitochondrial biology. However, a systematic evaluation of whether resistance training has beneficial effects on mitochondrial structure, enzyme activity, and gene expression in HF patients has not been undertaken.

The goals of this study were twofold. First, we sought to evaluate the unique effect of HF on skeletal muscle mitochondrial content, gene expression, and enzyme activity. To accomplish this objective, we geared our recruitment strategies to mitigate the effects of chronic muscle disuse (55) and other aspects of the HF syndrome that could modify mitochondrial biology. Second, we sought to examine whether resistance training has beneficial effects on these mitochondrial properties. To accomplish this aim, these parameters were reassessed in the cohort after an 18-wk high-intensity, resistance exercise-training program.

METHODS

Subjects.

Thirteen patients (9 men and 4 women) with physician-diagnosed HF were recruited, enrolled, and completed baseline, pretraining testing. Ten of these patients (7 men and 3 women) completed the training program and posttraining testing. The etiology of HF was ischemic in four patients and nonischemic in nine patients. The average New York Heart Association (NYHA) function class of these patients was 2.2 ± 0.2 (mean $\pm$ SE), with one class I patient, eight class II patients, and four class III patients. Eight patients were characterized as having HF with reduced ejection fraction (left ventricular ejection fraction $<40\%$) and five patients as having HF with preserved ejection fraction ($>40\%$). Five patients had non-insulin-dependent type 2 diabetes mellitus (NIDDM). All patients were nonsmokers, were clinically stable, and had not been hospitalized for at least 6 mo prior to testing. None had evidence of hepatic, renal, or peripheral vascular disease or an active neoplastic process. Patients were receiving angiotensin-converting enzyme (ACE) inhibitors/receptor blockers ($n = 13$), β -blockers ($n = 12$), diuretics ($n = 6$), and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors ($n = 6$), and one female patient was receiving levothyroxine. Plasma creatine kinase (CK) levels were normal in all patients, and none were on sex steroid replacement therapy.

Fourteen controls (7 men and 7 women) were recruited, enrolled, and completed baseline, pretraining evaluations. Thirteen controls (7 men and 6 women) completed the training program and posttraining testing. Controls self-reported being sedentary to minimally active (≤ 2 sessions of ≥ 30 min of exercise per week) and not participating in exercise-training or weight loss programs. This recruitment criterion was included to obtain a control group with habitual activity levels that match the reduced physical activity level in the HF population (55), and this was verified using accelerometry measurements. Controls were nonsmokers, had no signs or symptoms of HF or coronary heart disease, had normal left ventricular ejection fraction ($>55\%$) and normal routine blood tests, and were not taking sex steroid replacement therapy. Seven controls had a history of hypertension: four were treated with diuretics and three with ACE inhibitors or angiotensin receptor blockers. All were normotensive at testing and showed no evidence of left ventricular hypertrophy or atrial enlargement by echocardiography. To control for the potential confounding effects of statins and thyroid replacement, four controls on stable doses of HMG-CoA reductase inhibitors and one female control on levothyroxine were included. Plasma CK levels were normal in all controls. Additionally, one control with NIDDM was included. Informed consent was obtained from each HF patient and control, and the protocol was approved by the Committees on Human Research at the University of Vermont.

The present study utilizes HF patients and controls recruited for a series of studies examining the effects of HF and resistance exercise training on skeletal muscle structure and function. The overall goal of this series of studies was to define the effects of the disease and training on muscle function at the whole body, whole muscle, cellular, and molecular levels. Data from baseline evaluations of skeletal muscle morphometry, protein expression, and function have been reported at the whole muscle, single muscle fiber, and myosin-actin cross-bridge level in these volunteers and others (34, 35, 59). Moreover, the effects of resistance training on whole body function, whole muscle strength determined by 1 repetition maximum (RM)

and dynamometry, and single muscle fiber myofilament structure and function and myosin-actin cross-bridge kinetics have been reported (49, 58). The present study focuses on the effects of HF and resistance exercise training on mitochondrial biology and restricts reporting of data to mitochondrial content/structure, enzyme activities, and gene expression, with the exception of data necessary to provide descriptive information regarding the population (e.g., clinical/physical characteristics) and the effects of the resistance-training program on muscle strength (i.e., 1 RM data).

Experimental protocol.

Eligibility was determined during screening visits, at which time medical history, physical examination, blood samples, whole muscle strength testing, a treadmill test, and echocardiography were performed. Throughout the testing, including screening evaluations, HF patients and controls did not alter their normal medication regimens. During an additional outpatient visit, eligible volunteers repeated whole muscle strength testing and were assessed for whole body physical function. At ≥ 1 wk later, during an inpatient visit, muscle tissue was obtained via percutaneous biopsy of the vastus lateralis, and body composition was assessed in the fasted state. Care was taken to standardize the anatomic location of the biopsy (proximodistally and anteroposteriorly) to minimize variation in muscle fiber types sampled during the biopsy (28). At ~ 1 wk following the biopsy, volunteers entered an 18-wk resistance-training program (3 times per week). At the completion of the training program, volunteers repeated baseline measurements (echocardiography on HF patients only). The posttraining muscle biopsy procedure was performed ≥ 5 days following the last bout of exercise to remove any residual effects of exercise on mitochondrial structure or function. There is precedent for chronic mitochondrial adaptations to exercise training persisting for this period of time (50). The biopsy was taken just proximal (~ 2 – 3 cm) to the pretraining site in an attempt to sample a similar location of the muscle while avoiding areas of tissue damage associated with the initial biopsy. For some measurements, there are fewer observations because of limitations in muscle tissue availability.

Body composition.

Total and regional body composition was assessed by dual-energy X-ray absorptiometry (GE Lunar, Madison, WI), as described previously (57). Body composition measurements were not performed on one congestive HF patient, because he exceeded the weight limit of the machine.

Peak O₂ consumption.

Peak O₂ consumption ($\dot{V}O_{2\text{peak}}$) was determined by treadmill test to volitional exhaustion using the protocol of Naughton et al. (39). $\dot{V}O_{2\text{peak}}$ was defined as the highest 30-s average O₂ consumption ($\dot{V}O_2$) during the final 2 min of the test.

Accelerometry.

Free-living physical activity energy expenditure was estimated using a single-plane accelerometer, as described elsewhere (59).

Ultrastructural measurements.

The fractional density, number, and average size of intermyofibrillar mitochondria were assessed using electron microscopy (EM), as described elsewhere (18), with minor modifications. Briefly, a small bundle of muscle fibers (~100 fibers) was tied to a glass rod, slightly stretched, and then fixed, stained, cut in cross section (~100 nm), and contrasted for transmission EM measurements, as described elsewhere (35). Three images (each ~280 μm^2 with no evidence of mechanical damage) were chosen and analyzed for each patient at $\times 5,000$ magnification. Intermyofibrillar mitochondria ($>1 \mu\text{m}$ from the plasma membrane and clearly integrated within the myofilaments) were highlighted manually (same observer for all micrographs), and the digitized image was thresholded and analyzed for mitochondrial fractional density (fractional area of fiber containing mitochondria), number of mitochondria per area (μm^2) of the fiber cross section, and average area per mitochondrion using ImageJ software (version 1.38X, National Institutes of Health, Bethesda, MD). Additionally, the z-band width was measured as a proxy of fiber type (51). To perform z-band measurements, the embedded muscle bundle was reoriented and realigned by viewing through $\times 10$ oculars of the ultramicrotome, so that the cutting blade was parallel to the longitudinal axis of the muscle fibers. Semithin (1- μm) sections were cut and stained with methylene blue and viewed at $\times 400$ magnification to ensure longitudinal orientation. Ultrathin (~100-nm) sections were cut and contrasted for transmission EM measurements. Eight randomly chosen z bands from each image were chosen, and measurements were conducted at five points along each band ($n = 120$ measurements per volunteer; same observer for all measurements). The utility of z-band width as a proxy of fiber type in the elderly in general and, more specifically, in groups of physically inactive elderly with and without chronic disease in the present study, is not certain. Nonetheless, it likely represents a rough estimate of the overall fiber type admixture of the EM samples.

Gene expression.

The expression of nuclear and mitochondrial genes important for regulation of mitochondrial reticulum size and function was assessed using quantitative RT-PCR, as generally described elsewhere (35), with minor modifications. Multiplexed amplification reactions were performed (iQ Supermix, Bio-Rad, Hercules, CA), and β_2 -microglobulin was used as an endogenous control, because it has been shown to be minimally affected by resistance exercise (29). Oligonucleotides for peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α , PGC-1 β , nuclear respiratory factor 1 (NRF-1), mitochondrial transcription factor A (TFAM), cytochrome *c* oxidase (COX) subunit 1 (COX-1), COX subunit 5b (COX-5b), and β_2 -microglobulin were purchased from Applied Biosystems (assay ID nos. Hs01016719_m1, Hs00991677_m1, Hs00192316_m1, Hs01082775_m1, Hs-02596864_g1, Hs00426948_m1, and Hs99999905_m1, respectively). All samples were run in duplicate, and fluorescence data were analyzed by Bio-Rad CFX Manager. Threshold cycle values were used to calculate gene expression relative to the average value for controls.

Enzyme assays.

Muscle tissue was homogenized in ice-cold buffer [5 mM HEPES, 1 mM EGTA, 1 mM DTT, 5 mM MgCl_2 , and 0.1% Triton X-100 (vol/vol), pH 8.7], incubated on ice for 60 min,

and then centrifuged for 20 min at 13,000 g and 4°C. The protein content of the supernatant was determined (Bio-Rad). All enzymatic assays were conducted in duplicate at 30°C. Citrate synthase (CS) was measured as described previously (5), with minor modifications. Briefly, sample was diluted in buffer consisting of 100 mM Tris, 100 µM 5,5'-dithiobis-(2-nitrobenzoic acid), and 50 µM acetyl-CoA at pH 8.0. The reaction was started with 25 mM oxaloacetic acid, and the increase in absorbance at 412 nm was measured. COX was measured on freshly homogenized sample according to the method of Wharton and Tzagoloff (66). Sample was added to 50 mM potassium phosphate buffer containing 0.3 mM bovine serum albumin, 5 mM MgCl₂, and 2 µM reduced cytochrome *c*. The change in absorbance over time was monitored at 550 nm. CK was measured as described previously (61). Briefly, homogenate was diluted in assay reagent consisting of 30 mM HEPES, 5 mM MgCl₂, 0.5 mM DTT, 1.2 mM ADP, 20 mM phosphocreatine, 20 mM glucose, 0.6 mM NADP, 0.01 mM P¹,P⁵-di(adenosine-5') pentaphosphate, 2 IU/ml glucose-6-phosphate dehydrogenase, and hexokinase at pH 7.1. The increase in absorbance at 340 nm was measured. For all enzyme assays, care was taken to ensure that the amount of homogenate added was within the linear response range of the assay.

Myosin heavy chain isoform distribution.

Isoform distribution of myosin heavy chain (MHC) was measured as described previously (35).

Resistance exercise-training program.

The resistance exercise-training program, which has been described in detail elsewhere (58), was designed to improve upper and lower body skeletal muscle strength using seven exercises of major muscle groups. The training intensity was set to 80% of 1 RM (2), and 1 RM was reassessed every 2 wk to account for improvements in strength. The progression of the program is described in detail elsewhere (58).

Statistics.

Differences between patients and controls at baseline were evaluated with unpaired *t*-tests. Repeated-measures analysis of variance was used, with group (HF patients vs. controls) as the between-subject factor and training (pre vs. post) as the within-subject factor, to evaluate training and group × training interaction effects (SPSS version 15, SPSS, Chicago, IL). For those variables in which multiple observations were performed within the same individual (e.g., EM data), a general linear mixed model (SAS version 9.2, SAS Institute, Cary, NC) was used. In this model, group and training effects are included, as described above, along with a random effect to account for the clustering of observations within individuals. For all repeated-measures analyses, if a significant main effect was noted (training or group × training), post hoc contrasts were performed to identify pair-wise differences. Relationships between variables were determined using Pearson's correlation coefficients. Values are means ± SE.

RESULTS

Cross-sectional comparisons.

Physical characteristics and functional measures of controls and HF patients are shown in [Table 1](#). No differences in age or body composition were found between groups. $\dot{V}O_{2peak}$ expressed relative to body mass was lower ($P < 0.01$) in HF patients than controls, as expected. Lower $\dot{V}O_{2peak}$ persisted when divided by ($P < 0.01$) or statistically adjusted for ($P < 0.01$) fat-free mass (not shown in [Table 1](#)). No difference in knee extensor 1 RM was found, although lower (22%) strength in HF patients was noted when data were statistically adjusted for variation in body mass (48.2 ± 3.3 vs. 37.4 ± 3.5 kg, $P < 0.05$). Importantly, our attempt to recruit controls matched to HF patients for habitual physical activity level was successful, as we found no difference in accelerometry-based average daily activity level between groups measured over 1 wk (7.8 ± 0.4 days).

Because groups had similar physical activity levels and because we controlled for other confounding factors in our recruitment criteria (e.g., time since last hospitalization), we were able to evaluate the unique effect of HF on mitochondrial content and morphometry, gene expression, and enzyme activities ([Table 2](#)). There was no effect of HF on mitochondrial fractional area, although there was a trend ($P \leq 0.10$) toward fewer mitochondria per fiber cross-sectional area in HF patients ($P = 0.10$). This trend toward fewer mitochondria was balanced out to yield no difference in mitochondrial fractional area, because average mitochondrion size was larger in HF patients ($P < 0.05$). These differences in the number and size of mitochondria are likely not due to group differences in fiber type proportions, since the average z -band width, an estimate of fiber type, did not differ between patients and controls (89.1 ± 5.1 and 85.9 ± 4.7 nm, respectively). These z -band widths are consistent with those reported previously from vastus lateralis biopsies obtained from healthy and diseased individuals ([18](#), [25](#)). z -Band width, although the best morphometric estimate of fiber type ([51](#)), should be viewed as a rough estimate, particularly in our case, because its utility has not been assessed in healthy and diseased physically inactive elderly individuals. Nonetheless, our z -band width data were buttressed by analysis of MHC isoform distribution in tissue homogenates, which showed no differences in MHC I, IIA, or IIX isoforms between groups ([Fig. 1](#)), further suggesting limited group variation in muscle fiber types. Finally, the expression of nuclear or mitochondrial genes that regulate mitochondrial size and function and the activity of COX, CS, and CK did not differ between controls and HF patients.

Because of the absence of differences in mitochondrial gene expression and enzyme activities between groups, we pooled data from controls and HF patients and evaluated their interrelationships ([Table 3](#)). There were correlations among the various transcriptional regulators (e.g., PGC-1 α/β , NRF-1, and TFAM), as well as between these transcriptional regulators and nuclear- and mitochondria-derived COX subunits. The level of mRNA for these transcriptional regulators and COX subunits, however, was generally not related to the activity of mitochondrial enzymes (CS and COX) or CK, with one exception: NRF-1 showed a positive relationship to total CK activity ($P < 0.01$). Finally, $\dot{V}O_{2peak}$ was not related to mRNA abundance of any gene or mitochondrial/cytosolic enzyme activities.

Training effects.

Although the original HF cohort consisted of 13 patients, three (2 men and 1 woman) did not complete the study: one was injured in a motor vehicle accident, another experienced acute

worsening of HF symptoms, and yet another withdrew from the study for personal reasons. Additionally, of the 14 controls who were included in baseline evaluations, one woman did not complete posttraining testing for personal reasons. Of the volunteers who completed the study, compliance with the training program was excellent (>90%) and was similar between HF patients and controls.

Resistance training did not alter body mass or composition or leg muscle mass, and no group $\times$ training effects were noted. Moreover, no training or group $\times$ training effects were found for daily physical activity level. Training did, however, increase knee extensor 1 RM by 47% ($P < 0.001$), with similar effects in controls and patients (i.e., no group $\times$ training effect). Increases in 1 RM for other exercises ranged from +22 to +80% (all $P < 0.001$) and were similar between controls and patients (data not shown). Despite these improvements in muscle strength, we found no effect of training on $\dot{V}O_{2\text{peak}}$ and no group $\times$ training interaction effects.

The effects of training on mitochondrial fractional density, number, and size are shown in [Fig. 2](#). Training did not alter any of the aforementioned variables. Moreover, no group $\times$ training effect was noted for any of these variables. There was no effect of training and no group $\times$ training interaction effect for z -band width, implying that lack of variation in ultrastructural measurements with training was not related to alterations in fiber type. The lack of effect of training on fiber type, as assessed by ultrastructural measurements, was reinforced with MHC isoform distribution measurements, which showed no changes in MHC I, IIA, or IIX with training and no group $\times$ training interaction effects ([Fig. 3](#)). Collectively, these data suggest that it is unlikely that the lack of variation in mitochondrial content/morphometry with training was related to bias introduced by alterations in fiber type distribution.

The effect of training on gene expression is shown in [Fig. 4](#). There was a significant training effect ($P < 0.01$) for TFAM expression. Post hoc contrasts showed significant increases in TFAM expression with training in controls ($P < 0.02$) and HF patients ($P < 0.01$). Additionally, there were training and group $\times$ training effects for COX I (both $P < 0.05$). Post hoc paired contrasts showed that these effects were due to a reduction in COX I expression in controls ($P < 0.01$), while COX I expression was unaltered in HF patients. There were no training or group $\times$ training effects for the expression of other mitochondrial/nuclear genes.

To further evaluate whether alterations in TFAM expression might be related to the resistance-training stimulus, we evaluated the relationship of the increase in TFAM with training to the improvement in knee extensor 1 RM measured over the 18-wk intervention. The increase in TFAM with training was related to improvements in knee extensor 1 RM ($r = 0.482$, $P < 0.05$, $n = 17$; [Fig. 5](#)). Parenthetically, there was no relationship between alterations in TFAM expression and the trend toward an increase in CS activity with training ($r = 0.406$, $P = 0.19$, $n = 12$).

The effect of training on mitochondrial enzyme activity is shown in [Fig. 6](#). Training had no effect on COX or CK activities. There was, however, a trend ($P = 0.095$) toward an increase

in CS activity with training. No group $\times$ training effects were observed for the activity of any enzyme.

DISCUSSION

Our study examined the effects of HF on skeletal muscle mitochondrial content, size, mRNA abundance, and oxidative enzyme activity and the effect of 18 wk of resistance exercise training on these parameters. A strength of our work is the experimental control for confounding factors that might influence mitochondrial biology, such as muscle disuse (55) and time since hospitalization, permitting evaluation of the unique effects of the HF syndrome. We found no effect of HF on mitochondrial fractional area but observed increased mitochondrion size and a trend toward fewer mitochondria per fiber area in HF patients. There was no effect of HF on the expression of transcriptional regulators of mitochondrial size and function or mitochondrial/cytosolic enzyme activities. Relationships among transcriptional regulators and with nuclear- and mitochondrial-encoded subunits of the COX complex suggest that these networks are intact. Resistance training increased TFAM expression in both groups, and this increase correlated with improved muscle strength. There were no changes noted, however, in mitochondria content/size, enzyme activities or expression of other transcriptional regulators, although CS activity showed a trend toward an increase with training. Collectively, our results suggest minimal effect of the HF syndrome on mitochondrial structure and function in humans when disease-related confounding factors (e.g., muscle disuse and hospitalization) are removed experimentally. Furthermore, resistance training has relatively minor effects on mitochondrial biology, implying that increased physical function with this training modality (49) is not likely related to mitochondrial adaptations.

Skeletal muscle oxidative metabolism is impaired in HF patients, and this is thought to be related, in part, to reductions in mitochondrial content (12). To our knowledge, only one study has assessed muscle fiber ultrastructure in human HF since this seminal work, confirming reductions in mitochondrial content (13). In contrast, our data argue against the notion of mitochondrial rarefaction in HF. Variation among studies is not explained by the characteristics of the populations, such as their disease severity (12) or medication use (70), considering that the more recent study (13) evaluated patients that were similar to our cohort for these factors. Instead, we posit that divergent findings relate to the fact that we controlled for muscle disuse in the HF population (55) by carefully selecting sedentary controls to match HF patients for weight-bearing activity (with verification of similar activity levels via accelerometry) and by testing HF patients ≥ 6 mo following their last hospitalization. The latter criterion removes the confounding effects of bed rest/muscle disuse associated with acute illness/hospitalization that could impact mitochondrial morphology, as well as other potentially confounding factors, such as malnutrition (46). In contrast, Drexler et al. (12) included HF patients with severe disease (NYHA class IV symptoms) who would likely have been studied during hospitalization. Considering that the reduction in mitochondrial content in their study was apparent only in these patients with severe disease, it is unclear whether these alterations are related to HF or some factor related to hospitalization. Esposito et al. (13) improved on these studies by screening patients and controls for activity level by

questionnaire to identify groups with similar self-reported activity levels. However, no objective assessment of activity status is provided, and there is no indication of when testing occurred relative to their last hospitalization. The latter point is particularly noteworthy, since the remediation of mitochondrial content following periods of acute disease exacerbation/hospitalization is likely slow (12). Accordingly, the reduced mitochondrial content noted by Esposito et al. may relate to acute and/or chronic muscle disuse or factors related to hospitalization. Thus we suggest that reduced skeletal muscle mitochondrial content observed in prior studies (12, 13) relates to muscle disuse and/or other disease-related confounding factors associated with acute disease exacerbation and hospitalization, rather than the unique effects of the HF syndrome.

Despite similar mitochondrial fractional areas, average area per mitochondrion was larger in HF patients but was balanced out to yield no net difference in fractional content by a trend toward a reduced number of mitochondria per unit area. Mitochondria are dynamic organelles that alter their morphology through fusion and fission (65), processes that have been shown to regulate myocyte viability (9, 48). It is tempting to speculate that differences in morphology reflect variation in mitochondrial structural dynamics between patients and controls, but prior reports show no effect of HF on the expression of proteins controlling fusion and fission (16). Thus the genesis of these differences and, more importantly, their relevance to mitochondrial function are unclear.

Variation in mitochondrial size and content may be accompanied by alterations in the expression of transcriptional regulators. However, we found no group differences in these variables, in agreement with prior studies (16, 60). Our work adds to this literature by showing patterns of relationships among these transcriptional regulators and with their downstream nuclear- and mitochondrial-encoded targets that are similar to those observed in healthy controls (16, 50), suggesting that these transcriptional regulatory networks are intact in sedentary elderly individuals with and without HF. Interestingly, unlike healthy controls (16), we did not observe relationships between transcriptional regulators and mitochondrial enzyme activities or $\dot{V}O_{2peak}$, suggesting dissociation between these transcriptional regulators and their phenotypic end points. The physiological significance of the absence of these relationships is not certain, since normal mitochondrial functional and biogenic responses have been noted in HF patients following aerobic exercise training (20,37), adaptations that are thought to rely on these transcriptional regulators.

In keeping with similar mitochondrial densities and no differences in expression of respiratory chain components, we found no effect of HF on mitochondrial CS or COX activities. Additionally, we found no effect of HF on total CK activity. Our results differ from prior work showing reductions in these enzymes in HF patients (33, 40, 52). Here again, the majority of studies did not account for confounding factors, such as muscle disuse and time since last hospitalization, that might diminish enzyme activity in patients. One study that did attempt to control for muscle disuse by recruiting sedentary controls based on their maximal $\dot{V}O_2$ ($\leq 110\%$ of predicted values) (33) reported reduced CS and CK activities in HF patients. Differences in results between our study and the work of Mettauer et al. (33) may relate to our more rigorous evaluation of (and therefore control for) physical activity

levels in our control population, since maximal $\dot{V}O_2$ may not be a sufficient metric to identify controls with weight-bearing activity similar to that of HF patients. Alternatively, reduced enzyme activity noted by Mettauer et al. may be explained by the fact that they studied end-stage patients prior to heart transplantation (i.e., all NYHA III patients), whereas we assessed clinically stable patients with mild to moderately severe HF. Indeed, Williams et al. (68), who recruited sedentary controls (criteria for sedentary status not defined) and patients with mild to moderately severe HF, found no differences in CS activity. Caution is urged, however, in interpreting differences in disease severity as evidence that enzyme activity is regulated by a unique feature of the disease process (e.g., neurohumoral activation), since patients typically undergo extensive, inpatient clinical management (i.e., hospitalization) before they are scheduled for transplant. Thus it is plausible that factors associated with hospitalization (e.g., muscle disuse and malnutrition) could explain the reduced enzyme activities observed by Mettauer et al.

Results from our case-control comparisons add to a growing body of literature suggesting minimal effects of HF on mitochondrial function when the confounding effect of muscle disuse is removed (8, 33, 68). Our work advances these prior studies to further demonstrate no effect of HF on mitochondrial content (12) and enzymes important for energy homeostasis (33). Additionally, our results (Fig. 1) and those of others (33, 45) argue against the notion that HF is associated with a switch in muscle fiber type toward a more fast-twitch, glycolytic phenotype, a presumed hallmark feature of the skeletal muscle myopathy of HF that would affect the overall oxidative character of the muscle. Collectively, these findings suggest that many of the alterations in skeletal muscle mitochondrial biology/oxidative phenotype observed in HF patients may not be due to the unique effect of the disease process per se but, instead, may relate to accompanying muscle disuse or other disease-related confounding factors (47). From a clinical perspective, this would be advantageous, since these muscle changes may then be more amenable to clinical/therapeutic intervention. In particular, considering the effect of even modest amounts of exercise/activity on mitochondrial structure and function (6, 33), our findings would suggest that exercise interventions offer one therapeutic approach to correct skeletal muscle abnormalities and improve physical functional capacity.

The beneficial effects of aerobic-type exercise training on mitochondrial content and function in the HF population are well characterized (20, 37), but there are no data, to our knowledge, on the effect of resistance training on mitochondrial biology in HF patients, despite numerous studies showing that resistance training has beneficial effects on walking endurance (45, 49) and $\dot{V}O_{2peak}$ (14, 27). Our data, however, suggest minimal effects of resistance training on mitochondrial biology. We did observe increased TFAM expression, and this was graded to strength improvements, as evidenced by the relationship to gains in knee extensor 1 RM (Fig. 5). Our observation of changes in TFAM, but not other transcriptional regulators, may relate to the fact that TFAM is the most robust respondent of this transcriptional network to training (50). Considering the important role played by TFAM in mitochondrial adaptations to contractile activity (17), our results would suggest some effect of resistance training on mitochondrial metabolism. Indeed, we noted a trend toward an increase in CS activity with training. On balance, however, the magnitude and range of

adaptations in mitochondrial structure, gene expression, and enzyme activity with resistance training were minimal compared with aerobic training (50), and we observed no improvement in $\dot{V}O_{2\text{peak}}$. Thus, although resistance training improves muscle strength and, consequently, the capacity to perform activities of daily living (49), our data suggest that resistance training is not an effective intervention to improve skeletal muscle mitochondrial biology in sedentary elderly individuals with and without HF.

Several caveats to our study should be acknowledged. 1) Inclusion of volunteers with NIDDM (5 HF patients and 1 control) and the fact that most HF patients are insulin-resistant (53) may have confounded case-control comparisons and/or diminished the responsiveness of mitochondrial parameters to training. The likelihood of an influence on case-control comparisons, however, is minimal, considering group differences were not found. Similarly, diabetes is unlikely to explain the absence of a training response, since recent studies suggest greater responsiveness of mitochondrial function to aerobic training in diabetics (30). 2) Although we measured mitochondrial structure, gene expression, and enzymatic activity, we did not directly assess the integrated function of mitochondria, because no differences in mitochondrial function in HF patients vs. sedentary controls were shown in prior studies using in vitro and in vivo approaches (8, 33, 68). Although our study more rigorously controlled for the physical activity status of our controls, it is unlikely that such measurements would have added significantly to the literature (8, 33, 68). Instead, we sought to advance prior work in this field by evaluating those parameters of mitochondrial structure and/or function that have not been evaluated (mitochondrial content/size and expression of transcriptional regulators) or have been shown to persist in comparisons with sedentary controls (e.g., CS and CK activity) (13, 33). In this context, our findings add to the literature by clarifying a minimal effect of HF, per se, on mitochondrial biology. 3) We cannot discount the effects of medications, such as ACE inhibitors (70), on variability in results among studies, particularly those conducted prior to the advent of ACE inhibitor/receptor blocker and β -blocker use in HF patients (12). Generally speaking, ACE inhibitors/angiotensin receptor blockers would be expected to diminish group differences in mitochondrial parameters (70). However, because an effect of HF on mitochondrial biology has been observed in studies in which the majority of patients were taking these medications (13, 33), it is unlikely that medication use alone explains variable findings among studies. The effect of these medications on the adaptation to training is unclear. Whereas some studies suggest that ACE activity may modify the adaptive response to training (69), more recent studies suggest that this is not related to modification of mitochondrial biology (19). Moreover, we should note that whether these medications affect mitochondrial structure/function is controversial (38, 70), and there are no data regarding their effects on the mitochondrial adaptations to resistance training. Parenthetically, considering the clinical utility of these medications, their widespread use throughout the disease spectrum, and ethical issues related to withholding these medications prior to study, the skeletal muscle mitochondrial phenotype of HF is inextricably linked to these medications. Therefore, from a practical perspective, the mitochondrial phenotype in human HF will include the effects of these medications. 4) Our findings are limited to patients with mild to moderately severe HF, because patients with severe HF (e.g., NYHA class IV), by definition, have refractory disease requiring hospitalization. We specifically designed our recruitment criteria to exclude

the confounding effects of factors associated with hospitalization [e.g., malnutrition ([64](#)), bed rest/muscle disuse, and intensive therapeutic intervention], particularly the effects of muscle disuse ([8](#), [33](#), [68](#)). Because of the disease characteristics of our population, we have refrained from referring to much of the data from animal models, which are most reflective of severe HF in humans, because their findings are likely not germane to the nature of the mitochondrial phenotype in our study.

In summary, our data suggest that the HF syndrome has minimal effects on mitochondrial structure, enzyme activity, and gene expression when the confounding effects of muscle disuse and other disease-related factors are removed experimentally. Accordingly, we propose that many of the skeletal muscle mitochondrial/energetic abnormalities described in prior studies ([12](#), [13](#), [33](#), [40](#), [52](#)) are related to other factors, such as muscle disuse and hospitalization. If this is the case, exercise-training regimens may be effective at correcting skeletal muscle energetic abnormalities in HF patients. Indeed, aerobic training and a combination of aerobic- and resistance-training regimens have supported this notion ([20](#), [37](#), [67](#)). Our data suggest, however, limited utility of a resistance-training-only regimen to improve mitochondrial biology in sedentary elderly individuals with and without HF. This does not discount the substantial functional benefits achieved with resistance training ([49](#)) or the possibility that it may potentiate the beneficial effects of aerobic exercise when used in combination ([4](#)), but it suggests that functional benefits from resistance exercise training are likely not related to alterations in mitochondrial biology. Considering current recommendations for cardiac rehabilitation programs to encompass both aerobic and resistance components ([3](#)), our results would suggest that the effect of resistance training to enhance physical functional capacity ([49](#)) is primarily explained through modulation of skeletal muscle contractile function, rather than mitochondrial structure/function.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or National Institutes of Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.J.T. and P.A.A. are responsible for conception and design of the research; M.J.T., M.S.M., K.A.W., and P.A.A. performed the experiments; M.J.T. and K.A.W. analyzed the data; M.J.T. and M.S.M. interpreted the results of the experiments; M.J.T. prepared the figures; M.J.T. drafted the manuscript; M.J.T., M.S.M., K.A.W., and P.A.A. edited and revised the

manuscript; M.J.T., M.S.M., K.A.W., and P.A.A. approved the final version of the manuscript.

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Figures and Tables

Table 1.

Body composition, aerobic capacity, and muscle strength in HF patients and controls

	Controls	HF Patients
Age, yr	71.7 ± 1.7	71.8 ± 3.4
Body mass, kg	79.1 ± 5.7	93.3 ± 7.9
Fat mass, kg	28.8 ± 2.2	34.2 ± 4.1
Fat-free mass, kg	47.9 ± 3.9	52.9 ± 4.5
Leg fat-free mass, kg	14.8 ± 1.2	15.6 ± 1.4
$\dot{V}_{O_{2peak}}$, ml · kg ⁻¹ · min ⁻¹	23.9 ± 1.2	14.3 ± 1.1 *
Knee extensor 1 RM, kg	44.2 ± 3.8	41.8 ± 6.4

	Controls	HF Patients
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Physical activity level, kcal/day	281 ± 34	240 ± 37
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Values are means ± SE; $n = 14$ controls and 13 heart failure (HF) patients, except for body composition measurements, where $n = 14$ and $n = 12$, respectively. $\dot{V}O_{2peak}$, peak O_2 consumption; RM, repetition maximum.

* $P < 0.01$. Note that knee extensor 1 RM was lower in HF patients when adjusted for body size ($P < 0.05$).

Table 2.

Mitochondrial content, number, and average size, gene expression of regulators of mitochondrial size and function, and mitochondrial enzyme activities

	Controls	HF Patients
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Mitochondrial content and size

Fractional area, % total fiber area	2.65 ± 0.29 (8)	2.54 ± 0.32 (7)
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Number, μm^2 fiber area ⁻¹	0.444 ± 0.056 (8)	0.301 ± 0.062 (7)
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Average size, μm^2	0.069 ± 0.007 (8)	0.092 ± 0.007* (7)
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Gene expression

	Controls	HF Patients
PGC-1 α , % of control	1.00 $\pm$ 0.14 (11)	0.93 $\pm$ 0.21 (10)
PGC-1 β , % of control	1.00 $\pm$ 0.12 (10)	0.78 $\pm$ 0.08 (10)
NRF-1, % of control	1.00 $\pm$ 0.09 (11)	0.94 $\pm$ 0.08 (10)
TFAM, % of control	1.00 $\pm$ 0.08 (11)	0.86 $\pm$ 0.10 (10)
COX-1, % of control	1.00 $\pm$ 0.13 (10)	0.76 $\pm$ 0.11 (10)
COX-5b, % of control	1.00 $\pm$ 0.08 (11)	0.89 $\pm$ 0.11 (10)

Enzyme activity

CS, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$	3.61 $\pm$ 0.48 (8)	3.74 $\pm$ 0.39 (11)
COX, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$	0.29 $\pm$ 0.16 (8)	0.24 $\pm$ 0.08 (11)
CK, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$	810 $\pm$ 74 (8)	970 $\pm$ 76 (11)

Values are means $\pm$ SE of number of volunteers in parentheses. PGC, peroxisome proliferator-activated receptor- γ coactivator; NRF-1, nuclear respiratory factor 1; TFAM, mitochondrial transcription factor A; COX, cytochrome *c* oxidase; CS, citrate synthase; CK, creatine kinase. Mitochondrial content and size were measured by electron microscopy and gene expression by RT-PCR.

* $P < 0.05$. Note trend ($P < 0.10$) toward fewer mitochondria per fiber cross-sectional area in HF patients.

Fig. 1.

Skeletal muscle myosin heavy chain (MHC) isoform distribution in tissue homogenates from controls and patients with heart failure (HF). Values are means $\pm$ SE; $n = 14$ controls and 13 HF patients.

Table 3.

Correlations among mRNA abundance of genes regulating mitochondrial size and function and enzyme activities

	PGC-1 α	PGC-1 β	NRF-1	TFAM	COX-1	COX-5b	$\dot{V}O_{2peak}$
PGC-1 α							0.055 (20)
PGC-1 β	0.051 (20)						0.198 (20)
NRF-1	0.222 (21)	0.567 [†] (20)					0.105 (20)
TFAM	0.661 [†] (21)	0.456 [*] (20)	0.495 [*] (21)				0.118 (20)
COX-1	0.116 (21)	0.185 (20)	-0.068 (21)	0.499 [*] (21)			0.232 (20)
COX-5b	0.341 (21)	0.628 [†] (20)	0.722 [†] (21)	0.771 [†] (21)	0.254 (21)		0.225 (20)
COX activity	-0.138 (18)	-0.139 (17)	-0.226 (18)	-0.368 (18)	-0.009 (17)	-0.088 (18)	0.237 (19)
CS activity	0.080 (18)	-0.182 (17)	0.054 (18)	-0.259 (18)	-0.222 (17)	-0.083 (18)	-0.005 (19)
CK activity	0.071 (18)	0.206 (17)	0.613 [†] (18)	0.089 (18)	0.205 (17)	0.432 (18)	-0.166 (19)

Values are Pearson correlation coefficients; sample sizes are in parentheses. Units for data are shown in [Tables 1](#) and [and22](#).

* $P < 0.05$.

[†] $P < 0.01$.

Fig. 2.

■ = pre □ = post

Skeletal muscle fiber mitochondrial fractional area, number, and size in controls and HF patients before (pre) and after (post) resistance exercise training. Values are means $\pm$ SE; $n = 5$ controls and 4 HF patients.

Fig. 3.

■ = pre □ = post

Skeletal muscle MHC isoform distribution in controls and HF patients before and after resistance exercise training. Values are means $\pm$ SE; $n = 13$ controls and 10 HF patients.

Fig. 4.

Skeletal muscle mRNA abundance for nuclear and mitochondrial genes that control mitochondrial size and function in controls and HF patients before and after resistance exercise training. Values are means $\pm$ SE; $n = 9$ controls and 8 HF patients, except for PGC-1 β , where $n = 7$ each for controls and HF patients because of limited availability of muscle tissue. * $P < 0.05$ for training and group $\times$ training effects. ** $P < 0.01$ for training effect.

Fig. 5.

Relationship of training-induced changes in mitochondrial transcription factor A (TFAM) mRNA abundance to changes in knee extensor 1 repetition maximum (RM) in controls ($\square$) and HF patients ($\blacksquare$). AU, arbitrary units. Values are means $\pm$ SE; $n = 9$ controls and 8 HF patients.

Fig. 6.

Activity of citrate synthase (CS), cytochrome oxidase (COX), and creatine kinase (CK) in skeletal muscle tissue homogenates in controls and HF patients before and after resistance exercise training. Values are means $\pm$ SE; $n = 7$ controls and 9 HF patients. There was a trend toward a training effect for CS activity ($P = 0.095$).

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