Title: Exercise Therapy to Reduce Heart Failure Symptoms; Sorting Mechanisms of Benefit
Prime PI; Pittsburgh site: Daniel Forman, MD; Boston Site-PI Daniel Gottlieb, MD

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BACKGROUND AND SIGNIFICANCE

Scope of the problem; high prevalence of HF and associated functional decline

Prevalence of Heart Failure (HF) is rising, particularly due to the high incidence of HF among today's expanding population of older adults (1). Whereas mortality from coronary artery disease, sudden death, and other cardiovascular illnesses has declined, more adults are surviving these diseases but then developing HF in their senior years (5). Exercise intolerance is among the most common complaints expressed by the burgeoning elderly HF population. Moreover, functional decline compounds overall HF risks with increased mortality as well as increased propensity to frailty, dependency, and diminished quality of life (6).

Contemporary HF therapy tends to target central cardiac pathophysiology, and metrics of treatment efficacy (e.g., improved ventricular pumping, reduced ventricular remodeling, reduced arrhythmia, and improved cardiac loading) often have little bearing on/reflection of physical function (7). In contrast, exercise training and other therapies primarily oriented to peripheral physiology (blood perfusion and skeletal muscle health) specifically target functional gains (3,4,8). Nonetheless, it is not clear which exercise modalities, intensities, and/or adjunctive therapies yield the greatest (and most sustained) benefits and/or why this is the case. These differences are crucial, both as a means to clarify pathophysiology and therapy, and also as a means to achieve sustainable health behaviors (9). It remains an irony that many adults (older and younger) do not adhere to healthful exercise behaviors (4,9). A better understanding of the mechanistic benefits of exercise therapy may help foster exercise as a vital aspect of therapy, by augmenting efficiency and providing an unambiguous rationale.

Heart failure and skeletal muscle changes

Whereas HF pathophysiology had originally been conceptualized as a predominantly cardiac pumping abnormality, pathophysiological insights have evolved extensively in the last 20 years. Neurohormonal, inflammatory and molecular mechanisms have all become recognized as related components of the disease, with complex systemic ramifications (7). An injurious cascade underlies heart disease, and also links it to vascular endothelium, kidney function, pulmonary function, and other physiological manifestations.

Changes in skeletal muscle have been described as part of systemic HF pathophysiology, presumably mediated by intrinsic cellular mechanisms (e.g., inflammation, oxidative stress, and cytokines), but also likely affected by differences in tissue perfusion, autonomic function, and deconditioning (10). These complex biologic mechanisms and physiological interrelationships contribute to increased fatigability, dyspnea and functional declines (3,4).

Both intrinsic skeletal muscle cellular weakening as well as atrophy (reduced total muscle mass) contribute to functional deterioration (11-15). Respiratory symptoms stem from similar physiological consequences. When respiratory and limb muscles in a HF patient are compared, respiratory muscle impairment is relatively greater than muscle impairment in the limbs. Decreased diaphragmatic strength correlates to decreased tidal volume, end-tidal carbon dioxide, peak oxygen consumption (VO_2), tidal volume to minute ventilation ratio (VT/VE) and increased respiratory rate, VE, peak dead space ventilation to tidal volume ratio (VD/VT), ventilation to oxygen consumption (VE/VO_2) ratio and ventilation to carbon dioxide (VE/VCO_2) ratio/slope. In addition, increased ergoreflexes have been associated with abnormal ventilatory responses such that afferent fibers in skeletal muscle (15) trigger a respiratory muscle metaboreflex. This is a sympathetic autonomic stimulus that increases respiratory rate, diaphragmatic blood flow, and vasoconstriction in resting and exercising limbs, and which thereby decreases exercise tolerance (16).

Phenotype and Metabolism: Phenotypic and metabolic changes in peripheral skeletal muscle of HF patients are well described (3,4,17-19). Phenotypic changes in fiber composition include reduced type I oxidative fibers (slow twitch) with increases in anaerobic type II (fast twitch) or hybrid fibers. Histomorphometric analyses also demonstrate decreased fiber sizes as well as reduced number and altered structure of mitochondria. Even though there is an increase in skeletal muscle fibers with anaerobic, fast twitch characteristics, fiber size and cross-sectional area are dramatically reduced, leading to a significant reduction in muscle force production in HF (20).

Related studies indicate metabolism of the skeletal muscle is altered in HF with shifts in aerobic/anaerobic enzyme distribution from slow oxidative towards fast oxidative and fast glycolytic isoforms (19), decreased levels of mitochondrial creatine kinase, as well as downregulation of the sarcoplasmic reticulum Ca^{2+}-ATPase 1 (SERCA-1) (21). While some have attributed HF skeletal muscle weakening and atrophy primarily to disuse, others assert the complex skeletal muscle changes in HF constitute a disease-related myopathy (22,23) that is then compounded by the effects of muscle disuse and deconditioning (24).

HF-related changes in the diaphragm strongly support the concept of intrinsic HF myopathy. HF is associated with diaphragmatic muscle changes consistent with increased activity (25), i.e., a shift from fast to

slow myosin isoforms, an increase in oxidative capacity and decreased glycolytic activity. Nonetheless, intrinsic performance remains deficient.

HF stimulates an imbalance of catabolic over anabolic molecules (26), insulin resistance (27), and oxidative stress (28). In the course of the disease, a variety of organ systems are affected, with predictable central cardiac as well as peripheral abnormalities (29). TNFα induces catabolic metabolism, reduced skeletal muscle contractility, and ultimately, muscle atrophy (30). NF-κB is a transcription factor that regulates the expression of proinflammatory cytokines, e.g. TNFα and IL-1β as well as iNOS (31-33), and likely plays a key role in skeletal muscle functional derangement. Intracellular accumulation of nitric oxide generated by iNOS may produce levels high enough to inhibit key enzymes of the oxidative phosphorylation either directly through post-translational protein modification by NO (-*S*-nitrosylation) or indirectly through formation of reactive NO metabolites (3,4). Therefore, elevated iNOS is linked to diminished citrate synthase and other indices of oxidative metabolism.

Activation of Muscle Protein Breakdown: Muscle protein breakdown is regulated by several cellular systems that include lysosomal proteases, the Ca^{2+}-dependent calpain system, and most significantly, the ATP-dependent ubiquitin (Ub)-proteasome pathway (UPP). The UPP has been implicated in the enhanced protein breakdown of atrophying skeletal muscle in a number of disease models (34,35) including diabetes mellitus, cancer, renal failure, starvation, sepsis, and HF (36-39). Through transcriptional profiling, two E3-ligases, atrogin-1 (also called MAFbx-1) and MuRF-1 (muscle ring finger protein-1), key components of the Ub-proteasome system in muscle have been shown to be highly induced in processes of muscle atrophy of different origin.

Transcriptional changes and activation of the ubiquitin-proteasome pathway in atrophying muscle in animal: In seminal studies, Dr. Lecker and coworkers performed microarrays on a variety of atrophying muscles from animals due to different causes (fasting, tumors, uremia and uncontrolled diabetes), and defined a set of approximately 120 genes, which they termed “atrogenes,” that are induced and suppressed similarly in all these states of muscle atrophy (35). A representative group of these atrogenes is shown below (**Figure 1**).

Clone	Unigene	Primary Sequence Name		F T U D
Degradation-Related Atrogenes				
3137251	Hs.183842	UBB	ubiquitin B	
1723142	Hs.61661	FBXO32	Atrogin-1/MAFbx	
2195309	Hs.82159	PSMA1	proteasome 20S subunit, alpha 1	
315082	Mm.930	Ctsl	cathepsin L	
Growth Factor-Signaling-Related Atrogenes				
671661	Mm.578	Igfbp5	insulin-like growth factor binding protein 5	
334077	Mm.29891	Foxo1	forkhead box O1	
1555545	Hs.142827	P311	P311	
Structural-Related Atrogenes				
782235	Hs.172928	COL1A1	collagen, type I, alpha 1	
Metabolism-Related Atrogenes				
422907	Mm.38786	Pdc-E2	pyruvate dehydrogenase	
1335140	Hs.2795	LDHA	lactate dehydrogenase A	
440344	Mm.2338	Gins	glutamine synthetase	
480068	Mm.192991	Mt1	metallothionein 1	

Figure 1: Examples of commonly regulated genes (atogenes) in different conditions of muscle atrophy. Fold-change atrophy/control presented as heatmaps with increasing upregulation in red, and

Many atrogenes were linked to protein degradation, including polyubiquitin, Ub-fusion proteins, the Ub ligases atrogin-1/MAFbx which Dr. Lecker discovered and characterized (39) as well as MuRF-1, multiple subunits of the 20S proteasome (and its 19S regulator) and cathepsin L. Many atrogenes were also down-regulated, including genes required for ATP production and late steps in glycolysis as well as transcripts for extracellular matrix proteins. A number of genes not previously implicated in muscle atrophy were dramatically up-regulated (lipin, metallothionein, AMP deaminase, RNA helicase related protein, TG-interacting factor) and several growth-related mRNAs were down-regulated (P311, JUN, IGF-1-BP5). Corroborating literature points to the importance of myostatin as a key gene regulating signal (40).

Our pilot data is similarly significant in demonstrating increased FoxO expression in human HF patients (see our pilot data). We also show key correlations between functional indices and FoxO, evidence which supports the premise that atrophy-associated transcriptional signaling patterns may be modified through exercise. Peak VO_2 ($mlO_2 \bullet kg^{-1} \bullet min^{-1}$) correlates with FoxO ($r=-0.52$, $p<0.01$), MuRF-1 ($r=-0.42$, $p<0.05$), GLUL ($r=-0.36$, $p<0.5$), PGC-1α ($r=0.49$, $p<0.05$), and IGFBP-5 ($r=0.60$, $p<0.01$). Additionally, GLUL correlates with strength and power ($r=-0.37$, $p<0.05$ and $r=-0.36$, $p<0.05$, respectively) and IGFBP-5 correlates with VE/VCO_2 slope ($r=-0.44$, $p<0.05$).

Growth regulating effects of IGF-1: Skeletal muscle studies in patients with advanced HF and cardiac cachexia have revealed involvement of IGF-1 in muscle catabolism/hypertrophy. Catabolic syndromes in chronic inflammation, sepsis or cancer show an altered state of the GH/IGF-1 axis most probably due to a peripheral IGF-1 deficiency, attributable in part to impaired IGF-1 response to GH (41,42) and possibly exacerbated by typical declines in IGF-1 with age. Proinflammatory cytokines such as TNFα uncouple the GH/IGF-1 axis through suppression of the GH receptor. In response, a state of GH resistance develops, commonly characterized by high GH and normal-to-low IGF-1, attributable in part to increased serum and local expression of proinflammatory cytokines such as IL-1β and TNFα.

Dr. Lecker and his colleagues demonstrated (in an animal model) that at the cellular level, decreased IGF-1 signaling triggers muscle atrophy in large part by activating (through dephosphorylation) downstream transcriptional activators of the FoxO family (43). In a related animal study, Dr. Schulze showed reduced local expression of IGF-1 in the quadriceps muscle of animals with HF as compared to controls (0.47±0.7 versus 0.77 ±0.09, p<.01), even while serum levels were normal (44). Muscle expression of IGF-1 receptors was increased, as was expression of both IL-1β and TNFα. Also, cross-sectional area (CSA) was diminished.

Schulze et al.'s study indicates that despite normal serum levels of IGF-1 and proinflammatory cytokines, local skeletal muscle expression of IGF-I is substantially reduced in HF, indicating a reduction in local anabolic function. Since decreased IGF-1 levels and a reduced muscle fiber CSA correlate with increased levels of IL-1β, these results also suggest a cytokine-mediated local catabolic process. A subsequent animal study by Dr. Schulze demonstrated a close link between skeletal muscle atrophy associated with ubiquitin proteolysis in chronic left ventricular dysfunction and the regulatory role of local IGF-1 (45) (see preliminary data). Similar work by Heinke et al. recently demonstrated that upregulation of myostatin is associated with muscle wasting in humans as part of HF (46).

Exercise training augmentation of PGC-1α expression in animal models: PGC-1α is a transcriptional coactivator that promotes mitochondrial biogenesis and a genetic program of oxidative metabolism. PGC-1α coactivators play a critical role in the maintenance of glucose, lipid, and energy homeostasis and has been shown to be downregulated in HF. Work by Sandri et al. (47) showed that injecting PGC-1α into fibers reduced the capacity of FoxO3 to bind to and transcribe from the atrogin-1 promoter and to thereby trigger fiber atrophy. Several reports demonstrate that PGC-1α signaling and associated anabolic benefits increase in response to exercise, even in the face of circulatory restriction (48-50).

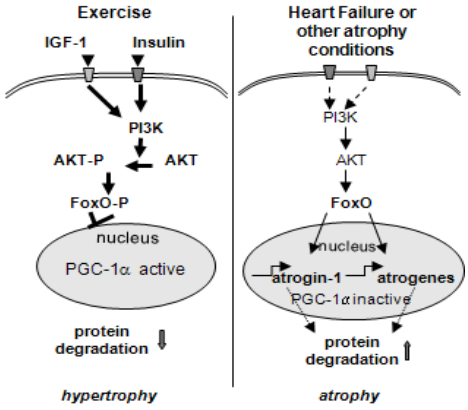

Figure 2: Hypothesized roles of IGF-1 signaling and PGC-1α activity in regulating muscle mass: While there is no evidence directly linking IGF-1 signaling with PGC-1α activation, both promote muscle growth. With exercise, IGF-1 inhibits FoxO action, primarily through its phosphorylation by AKT. PGC-1α is also strongly activated with exercise. In contrast, in states of muscle disuse and atrophy, FoxO is dephosphorylated and activated, leading to induction of ubiquitin ligases. PGC-1α expression is markedly suppressed under these conditions as well.

Suppression of proteolytic gene expression, induction of IGF-1 and PGC-1α, and related benefits of exercise training: Animal models have created a rationale to consider exercise training as a means to modify proteolytic gene expression and activity in skeletal muscle. Healthy rats showed diminished ubiquitin expression after 5 days of exercise (51). Konopka et al. showed decreased FoxO3 and myostatin expression in older women who completed a 12 week aerobic training regimen (52). Other studies suggest similar benefits (53) with different training regimens and subject ages. Adams et al, showed similar benefit in younger HF patients with decreased MuRF-1 expression in HF patients who completed 4 weeks of aerobic training (54). Still, the data regarding muscle proteolytic gene expression in relation to function and the benefits of exercise remain relatively sparse. Furthermore, the complexity of the field is compounded by variable responses relative to acute exercise versus chronic exercise: there are data indicating that proteolytic gene expression initially increases in response to acute exercise but over time (and with chronic exercise) can be blunted (55).

A key correlate relates to the utility of exercise to reduce the inflammatory and catabolic milieu that may induce differences in gene expression patterns. Adamopoulos et al. showed that 12 weeks of aerobic exercise training (5 days a week, 60-80% max HR) significantly reduced plasma levels of TNF-α and IL-6, and showed

that changes in the inflammatory peptide correlated closely with increases in the peak VO_2 (56). An even more interesting relationship was demonstrated by Gielen et al., who showed that exercise induced changes in cytokine expression in humans are relatively greater at the level of the skeletal muscle tissue than they are by serum assays, *providing strong rationale to study effects of exercise on a local tissue level rather than serum level, to ascertain true exercise training effects* (23).

Data showing benefits of exercise training to increase IGF-1 in humans are also relatively sparse. Schulze and coworkers completed a seminal study that showed increased local skeletal muscle IGF-1 expression among stable HF patients after 6 months of aerobic exercise training (57). Another study by Fielding and his coworkers focused on healthy older adults, exploring the benefits of a combined nutritional supplementation and resistance training regimen on local skeletal muscle IGF-1 expression. After 10 weeks, the investigators showed a 6-fold increase in local IGF-1 expression (58).

A series of recent investigations have focused on exercise benefits to stimulate PGC-1α. Kuhl et al. showed benefit of a combined aerobic-strength regimen to increase PGC-1α in healthy adults (59), while a low-intensity endurance regimen in coronary artery patients did not induce similar PGC-1α changes (60). Other literature reflects similar inconsistencies: Pilegaard et al showed robust PGC-1α effects after 4 weeks of leg extensor exercise (50), while Konopka et al. showed no similar PGC-1α responses (52). Furthermore, as part of their study on a knee extension stimulus, Norrbom et al. postulated that the type of exercise may be relevant, with strength training much more likely to stimulate PGC-1α than aerobic type exercise (61).

<u>Exercise Training: Multiple training modalities, each with physiological rationale</u>

- *Aerobic training:* Originally, exercise training for HF was predominantly oriented toward aerobic training modalities, building on the rationale that aerobic exercise stimulates healthful central cardiac responses (3,4) in adults without heart disease. Aerobic training has the potential to induce increased stroke volume and inotropy in healthy adults, as well as increased peripheral oxygen utilization. While it was generally assumed that exercise training in systolic HF patients would provide similar benefits, it was ultimately demonstrated that peripheral training effects surpass central benefits. Aerobic exercise is associated with increased skeletal muscle blood perfusion, endothelial responsiveness, healthful shifts in skeletal muscle histomorphometry, and improved mitochondrial function (oxidative capacity). Underlying these changes, improved autonomic, neurohormonal, and anti-inflammatory benefits have also been demonstrated. While it was once assumed that the potential for adverse cardiac remodeling was a risk associated with aerobic training, in fact, just the opposite occurs, probably due to decreased inflammation (62).

Overall, aerobic training provides particular benefit in relation to neurohormonal and inflammatory milieu, with improved autonomic responses, peripheral perfusion, and even remodeling benefits.

- *Strength training:* HF patients typically display substantial skeletal muscle atrophy, changes in fiber type, and associated decreases in overall strength, often presented as dyspnea and exercise intolerance (20). Reduced flexibility and balance are also associated with skeletal muscle physiological changes in HF patients. Strength training has been studied as a means to allay some of this predictable atrophic risk. In addition, increased skeletal muscle mass is a potential means to increase aerobic capacity since VO_2 becomes more efficient. Increasing strength parameters in HF patients facilitates increased functional capacity, thereby prolongs independence and improves quality of life.

The early work from Dr. Forman (see the preliminary data section) showed HF patients derived increase muscle strength from training affects. Likewise, in a small trial Magnusson et al. (63) 11 HF patients were randomized into two groups, knee extensor strength training and/or endurance training, each 3 days/week for 8 weeks. After training, overall exercise tolerance increased from 99 to 114 watts ($p<0.05$). Peak dynamic knee extensor work rate showed the greatest increase in association with endurance training (40%, $p<0.01$). Capillary per fiber ratio of the vastus lateralis was also greater in the endurance trained legs ($p<0.05$). However, the dynamic and isometric strength and cross-sectional area of the quadriceps femoris were relatively greater with strength training. The study is most relevant in demonstrating that exercise effects vary depending on whether the training is strength or aerobic-based.

Selig et al. (64) demonstrated that moderate-intensity strength training positively effects muscular strength and endurance, peak VO_2, forearm blood flow (FBF), and heart rate variability (HRV) in 39 HF patients. Patients underwent tests for strength and endurance of the knee and elbow extensors and flexors, peak VO_2, HRV, FBF at rest, and FBF activated by forearm exercise or limb ischemia. Patients were then randomized to 3 months of resistance training vs. endurance training vs. usual care, after which they underwent repeat assessments. Strength increased in the resistance training group by 30% ($p< .01$), vs. 21% in the endurance group ($p<.01$) vs. almost unchanged in the usual care (strength $p<.005$, endurance $p<.003$ in the strength

training group vs. the usual care). Peak VO_2 improved in the resistance training group by 15% (p<.01), but decreased by 18% (p <.05) in the usual care group (p <.001 strength training vs. usual care).

Overall, these studies indicate that strength training increases skeletal muscle hypertrophy and force production, but also translates into aerobic benefits, as increased strength essentially enables patients to sustain aerobic activity for longer periods before fatiguing (65).

Combined Aerobic and Strength Training: Understanding the benefits of aerobic training versus strength training leads many to believe that a combination of the two may be the most beneficial form of exercise for HF patients. The overall rationale is to incorporate benefits of each, i.e., endothelial, autonomic, neurohormonal, and anti-inflammatory effects, in combination with direct stimulus for cell growth and anti-atrophic benefit. In the study mentioned above, Magnussen et al (63) demonstrated synergy when endurance and strength exercise training were combined. Likewise, Maiorana et al. (66) utilized an 8 week exercise intervention consisting of circuit weight training (CWT) for 13 male HF patients. The CWT sessions were held 3 days per week for one hour, involving a combination of treadmill walking, cycle ergometry, and resistance weight training. Results of the study demonstrated that CWT improved cardiorespiratory fitness and muscular strength in patients with HF. Significant improvements were seen in muscle strength (392 to 462 kg P= 0.001), peak VO_2 (19.5 ± 1.2 vs. 22.0 ± 1.5 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$, p< 0.01), RPP, and submaximal heart rate after training (121 ± 3 vs. 134 ± 5 beats/min, P < 0.01). The study concluded that resistance training combined with aerobic training lead to beneficial improvements in strength and functional capacity for HF patients.

In addition to physiological benefits, Radzewitz et al. (67) demonstrated that combined aerobic and strength training lead to significant improvements in quality of life for male and female HF patients. The training program lasted 4 weeks and consisted of strength training (2-3 times per week), cycle ergometry (3 times per week), and a 6MWT as a training mode (2 times per week). The study results showed significant improvements in cardiopulmonary exercise testing (CPX)-based assessments as well as in quality of life.

The strength-aerobic combination has also been demonstrated to effectively modify inflammation. Conraads et al. (68) demonstrates decreased markers of inflammation, cytokines and cytokine receptors in HF patients after exercise training. They examined the effects of a 4 month training program (N=23) where the HF patients exercised 3 times per week for one hour. Training involved 30 minutes of resistive weight training and 20 minutes of cycling or jogging. Exercise training reduced circulating cytokines and improved peak VO_2. Significant improvements were also seen in NYHA functional class, submaximal and maximal work rate, and work efficiency.

- *Inspiratory Muscle Training (IMT):* HF patients have also been demonstrated to have reduced inspiratory muscle strength and endurance which exacerbates dyspnea (69) and exercise intolerance. Inspiratory muscle strength correlates to VO_2 in HF and is an independent predictor of survival, i.e., comparable to peak VO_2 as a predictor of survival (70,71).

IMT has the potential to improve the respiratory muscle metaboreflex as well as many of pathophysiologic manifestations of HF. Quality of life, heart rate, respiratory rate, peak VO_2, 6MWT distance, VE, the VE/VCO_2 slope and oxygen uptake efficiency slope (OUES), and recovery oxygen kinetics have all been shown to significantly improve after IMT (11-16) in HF patients.

Dall'Ago et al studied the benefits of IMT in 32 HF patients in a randomized, controlled 12 week trial. Maximal inspiratory pressure increased 115% in the IMT group with 17% improvement in peak VO_2 and 19% improvement in 6MWT distance (12). Even more compelling, Chiappa et al. (13) showed that IMT improved blood flow to resting and exercise limbs in the HF population. Specifically, a 4 week IMT regimen reduced calf vasoconstriction and improved forearm blood flow, i.e., systemic benefits were derived from the inspiratory training regimen as well as diaphragmatic hypertrophy. This account suggests IMT is analogous to strength training, such that high intensity IMT stimulates cell growth with peripheral adaptations.

A randomized controlled IMT trial in HF patients by Laoutaris et al. also showed that increased aerobic effects from IMT (72). IMT led to increased maximal inspiratory pressure (Pimax), (111±6.8 versus 83±5.7 cmH_2O, p<0.001), and sustained maximal inspiratory pressure (SMIP) (527822±51358 versus 367360±41111 cmH_2O/sec x 10(-1), p<0.001), and also increased peak VO_2 (17.8±1.2 versus 15.4±0.9 m $mlO_2 \bullet kg^{-1} \bullet min^{-1}$, p<0.005), and 6MWT distance (433±16 versus 367±22 meters, p<0.001). Moreover, perceived dyspnea, assessed using the Borg scale, was reduced for both on the treadmill (12.7±0.57 versus 14.2±0.48, p<0.005) and walking (9±0.48 versus 10.5±0.67, p<0.005) evaluations. Quality of life score was also improved (21.1±3.5 versus 25.2±4, p<0.01).

There are two commonly used devices for IMT, the threshold IMT and the Test of Incremental Respiratory Endurance (TIRE) IMT. Both devices involve inhaling against a resistance to stimulate diaphragmatic strengthening. Threshold IMT device is the most widely used technique (12,13,73). TIRE IMT provides a

complementary training effect because it entails different strengthening mechanisms (72,74). Threshold IMT is referred to as inspiratory pressure threshold loading (IPTL) which prohibits the subject from inhaling until a set negative pressure is achieved and overcome. This is accomplished through the use of weighted plungers or spring loaded valves (12,13,73). TIRE is referred to as inspiratory flow resistive loading (IFRL) and creates resistance to the inspiratory muscles by manipulating airflow. Subjects inhale through a 2 mm leak within a mouthpiece that limits airflow and measures pressure with resistance being dependent on the velocity or flow of inspiration. A faster airflow correlates with greater inspiratory muscle power which has been corrected for and adjusted with specific software within the TIRE RT2 device (72,74). In contrast to Threshold IMT, TIRE IMT sustains workload of the inspiratory muscles throughout all of inspiration, essentially creating an isokinetic training stimulus that has been associated with an accelerated and relatively more potent training effect (72,74). TIRE IMT also provides several readily available outcome measures that are not available with Threshold IMT. The outcome measures with potential for the greatest diagnostic and rehabilitative value in persons with HF include SMIP, accumulated SMIP, inspiratory muscle time of contraction, and inspiratory vital capacity (IVC) (72,74). Whereas threshold IMT affords convenience and copious preliminary data, TIRE IMT has inherent advantages since it is an isokinetic IMT stimulus and it is more visually engaging (as the generated pressure can be watched on a screen). Therefore, combination of the two techniques provides a synergistic training benefit.

Overall, IMT improves multiple physiologic variables in HF patients. Decreased diaphragmatic blood flow and fatigue, increased peripheral blood flow, attenuated respiratory metaboreflexes, and improved respiratory muscle fiber type (38% and 21% respective improvements in Type I and II fibers have been demonstrated) (12-16,69-76), with related improvements in aerobic and strength capacities. Thus, IMT elicits favorable central and peripheral adaptations with improved respiratory efficiency, exercise tolerance, and physical function (12-16,69-76).

In summary, our proposal responds to the clinical challenge of treating HF, a disease that is already common and that will increase in prevalence as the population of older adults expands. HF-related decrements in physical function correspond to specific skeletal muscle gene expression patterns and there is compelling rationale that exercise training can improve function by inducing different molecular skeletal muscle signaling patterns. Using the excellent infrastructure and methodology we established in the course of our pilot work, we plan to implement and compare the effects of 4 different training regimens, delineating functional changes after training as well as skeletal muscle molecular signaling patterns underlying these differences.

PRELIMINARY DATA

1. Pilot Project

The pilot project *Ubiquitin Proteolysis and PGC-1α in Skeletal Muscle in Heart Failure* closed enrollment on October 1, 2011. Analysis is ongoing, but multiple reports have already been generated that substantiate the premise for this investigation. Moreover, the body of work and productivity attest to Dr. Forman's capacity to work effectively with Merit funding. This activity also corresponds to an evolution of methods and sophistication in the molecular assessments that are certain to enhance efficacy as our investigations accelerate and broaden.

The initial phase of work (before transcriptional data were available from muscle biopsies) was particularly oriented to functional assessments in relation to body composition in HF vs. controls. We also embarked on the novel assessment of CPX in terms of recovery dynamics after exercise.

A second phase of analysis (now underway) is focusing on gene expression patterns in relation to functional attributes and body composition. This work substantiates the hypotheses of the pilot investigation, i.e., that specific gene expression profiles underlie different performance attributes. These insights also support the rationale of the proposed trial. A third phase of work is anticipated shortly, i.e., integration of serologic markers for inflammation and metabolism to the assessments of genes, muscle function, and body composition, and to thereby expand the breadth and sophistication of our analyses.

An implicit aspect of this work is that it attests to the first-class research infrastructure we have been able to organize in the last 3 years. We have developed a highly successful program and research facility at the VA Boston Healthcare System (VABHS), facilitating recruitment, functional assessments, muscle biopsies, dual-emission X-ray absorptiometry **(**DXA), exercise training, and echocardiography, as well as tissue storage, data storage, and data analysis. We have become a substantial resource within the VABHS research program, growing our own program and work, but also enriching other investigators whose work entails exercise assessment, exercise training, and exercise science.

- *Phase I:* We compared functional capacity between HF and controls, evaluating CPX indices, strength testing, body composition, and basic clinical measures. We showed that key aerobic and strength indices were significantly decreased in HF patients (peak VO_2 [15.4±4.2 vs. 23.4±6.6,mlO_2•kg^{-1}•min^{-1} p<0.0001], VAT [10.9±2.1 vs. 14.4±4.0 mlO_2•kg^{-1}•min^{-1}, p<0.0001], VE/VCO_2 slope [35.7±10.6 vs. 29.1±4.6, p<0.0001], 1RM [154.8±52.0 vs. 195.3±56.8kg, p<0.01], power [226.4±99.2 vs. 313.3±130.6 watts, p<0.01]), and the 6MWT distance (388.8±114.9 vs. 536.5±182.7 meters, p<0.001) reflecting the pathophysiological-associated functional decrements in an HF population.

Table 1

Phase I. Evolving scientific insights regarding functional measures and body composition, in HF vs. controls.

Title	Summary	Venue
Comparing physical and functional measures in HF patients and healthy controls	While age correlated inversely to all functional variables in HF and controls, differences between groups were evident in relation to peak VO_2, leg endurance, 6MWT, and the Duke Activity Status Index (DASI). While Peak VO_2 correlated with leg endurance, DASI did not correlate with physical function measures.	*American College of Sports Medicine, 2011*
The Influence of Functional Parameters on Quality of Life in Systolic HF Patients	The Kansas City Cardiomyopathy Questionnaire (KCCQ) correlated with aerobic indices in HF patients but not strength parameters. Aerobic capacity may be a key determinant in quality of life in HF patients.	*Society of Behavioral Medicine, 2011*
Strength and Aerobic Capacities Both Contribute to Functional Decline in HF	HF patients had a significantly lower aerobic and strength indices than controls. Peak VO_2 correlated to strength (1RM) in the HF group. Peak HR correlated to peak VO_2 in HF and controls. Peak heart rate correlated to strength decrements in HF only. Lower peak HR affects aerobic and strength capacity in HF patients.	*HF Society of America, 2011*
Lean Mass as an Indicator of Muscle Strength in Patients with HF	Lean body mass is a better determinate of strength measures in HF participants than aerobic outcomes. Aerobic and strength measures were decreased in HF patients. Lean body mass correlated only with the strength parameters.	*HF Society of America, 2011*
Fat Measurement and Functional Characteristics in HF Patients	Adiposity may stimulate increased strength capacities in HF patients, an effect that helps explain the paradoxical mortality benefits with which adiposity is also associated. Fat mass correlated with increased strength capacity in HF patients.	*HF Society of America, 2011*
Handgrip Strength Reflects Decreased Lean Tissue and Function in Elderly HF Patients	Strength and aerobic parameters were significantly decreased in HF patients. Hand grip assessment correlated with diminished strength capacity and reduced lean body mass in HF patients.	*Gerontological Society of America, 2011*
Relationship Between Body Composition and Oxygen Uptake Efficiency Slope in HF Patients	The oxygen uptake efficiency slope (OUES) may better reflect peripheral contributors to functional capacity than standard CPX measures. While peak VO_2 and the VE/VCO_2 slope showed no associations with body composition, the OUES showed significant correlations	*Submitted: Am Coll of Sports Med, 2012*
The 6MWT as an Indicator of Functional Capacity in HF patients	The 6MWT may provide superior assessment of comprehensive functional status of elderly HF patients than other functional measures. Functional indices (aerobic and strength) were reduced in HF patients. The 6MWT showed the most significant correlations with strength indices, possibly providing a better assessment of certain key aspects of function compared to CPX.	*Submitted: Am College of Cardiology, 2012*

We also analyzed recovery kinetics in HF patients, a novel application of CPX which had not been assessed in great detail to this point. While prior studies showed that recovery dynamics were prolonged in HF patients (i.e. VO_2 and heart rate during recovery remained elevated in HF patients, reflecting disease-related central and peripheral abnormalities), we broadened this work to include assessment of ventilatory dynamics during recovery and to also investigate the interrelationship with strength capacity.

A related manuscript is now under review: *Dynamic assessment of ventilatory efficiency during recovery from exercise to enhance CPX*. Ventilatory efficiency assessed during recovery may provide added clinical benefit. Absolute and change scores during recovery were significantly decreased in HF patients. Differences in absolute values at 1 minute of recovery were more significant as compared to change scores assessed at 1 minute of recovery. Strength correlated with recovery kinetics better than aerobic exercise parameters.

- *Phase II:* Exploring the relationship between functional capacity and skeletal muscle gene expression patterns in older HF patients: Overall, several changes in gene expression associated with atrophy in animal models are also seen in HF patients: UBB (5.0±2.4 vs. 3.7±1.7, p<0.05), PSMA1 (4.4±2.0 vs. 3.5±1.6, p<0.05), FoxO3 (6.5±4.3 vs. 4.3±2.8, p<0.05), and GLUL (19.7±20.3 vs. 10.0±7.4, p<0.05). FoxO1 also approached significance (7.9±6.2 vs. 5.0±3.5, p=0.055).

Analyses of gene expression in relation to functional capacity also show several key correlations between generally suppressed in atrophying muscle and generally suppressed aerobic capacity in HF patients. PGC-1α correlated with peak VO_2 (r=0.49, p<0.05) and $P_{ET}CO_2$ (r=0.43, p<0.05). IGFBP5 correlated with peak VO_2 (r=0.60, p<0.01), the VE/VCO_2 slope (r=-0.44, p<0.05), and $P_{ET}CO_2$ (r=0.52, p<0.01), and approached significance with peak VE/VO_2 (r=-0.40, p=0.053). There were significant correlations between expression of genes induced in atrophying muscle and function. Peak VO_2 correlated with MuRF-1 (r=-0.42, p<0.05), FoxO1 (r=-0.50, p<0.01), FoxO3 (r=-0.52, p<0.01), and GLUL (r=-0.36, p<0.05). $P_{ET}CO_2$ correlated with atrogin-1 (r=-0.38, p<0.05) and MuRF-1 (r=-0.38, p<0.05). GLUL correlated with 1RM (r=-0.37, p<0.05) and sub-maximal power (r=-0.36, p<0.05). These data provide strong rationale for further analysis of atrophy-associated gene expression in skeletal muscle following a tailored exercise intervention.

Table 2: Abstracts submitted to the Am College of Cardiology, 2012 reflecting phase II analysis:

PGC-1α and IGFBP-5 Expression in Skeletal Muscle of HF patients	PGC-1α and IGFBP-5 expression in skeletal muscle correlate with CPX and strength indices of physical function.
Gene Expression of the Ubiquitin-Proteasome Pathway is reflected by Ventilatory Expired Gas Analysis Indices at Peak Exercise in Heart Failure	FoxO1 and FoxO3 expression correlates with reduced aerobic and strength parameters
Correlations between Recovery Kinetics and Skeletal Muscle Gene Expression in Systolic Heart Failure	Skeletal muscle atrophy-associated gene expression patterns (Atrogin-1, MuRF-1, FoxO1 and FoxO3) in controls correlate with delayed VO_2 recovery kinetics, while anabolic PGC-1α, IgF1 and IgF-binding protein-5 were associated with increased VO_2.

2. Prior work in exercise training and exercise testing for heart failure patients

Dr. Forman brings related expertise in cellular metabolism and functional capacity in older HF patients. In one of his earliest training studies, he investigated older female HF patients participating in a resistance training trial; patients randomized to a 10-week training program had significantly increased muscle strength (increased 43.4 ±8.8% in resistance trainers vs. -1.7 ± 2.8% in controls, p= 0.001), muscle endurance (increased by 299 ±66% vs. 1 ±3%, p= 0.001), and 6MWT distance (increased by 49 ±14 m (13%) vs. -3 ± 19 m (-3%) in controls, p = 0.03). Most importantly, increased citrate synthase activity (35 ± 21%) was independently predictive of improved 6MWT distance (p = 0.0024) (8).

In a subsequent study (16 week program), HF patients (ages 67-82, mean 73 years old) in a strength training arm demonstrated increased skeletal muscle mitochondrial size, (23.4% increased size, p< 0.015) and associated increases in muscle strength and endurance (knee extensor and flexion), as well as increased functional capacity (peak VO_2, VAT, and 6MWT distance) (17).

While on faculty at Boston University, Boston Medical Center was selected by the NHLBI as one of HF-ACTION's (A CHF Trial Investigating Outcomes of Exercise TraiNing) 9 U-site hubs, with Dr. Forman as the U-site principal investigator (85). Dr. Forman created the infrastructure for recruitment and training into that landmark HF training trial. During the course of the 5 year trial, Dr. Forman changed professional affiliations to Brigham and Women's Hospital. Therefore, his role as principal investigator transitioned into his new affiliation, i.e., Dr. Forman had to formulate an infrastructure for this complex trial a second time. During this academic transition, Dr. Forman was commended by the HF-ACTION steering committee for excellent start-up and recruitment at the new institution.

Dr. Forman not only led the exercise testing, training, and organization for HF-ACTION, but was then selected as lead writer on the HF-ACTION baseline data paper focused on older HF patients, showing important age-related covariates contributing (but not entirely explaining) age-related changes in CPX performance. Age was the strongest predictor of peak VO_2 and the VE/VCO_2 slope in this population of HF patients (accounting for 13% of the variability in peak VO_2 and 4% of the VE/VCO_2 slope after controlling for other variables in the model). BMI, sex, race, and NYHA classification were also independent predictors of peak VO_2; when added to age they accounted for 39% of the variability. Furthermore, adding peak HR increased R^2 from 0.392 to 0.494. Age-related effects were nonlinear and showed splines at age 40 years for peak VO_2 and age 70 years for the VE/VCO_2 slope. These effects do not appear to be mediated via age-related increases in comorbidities (86).

Dr. Forman also recently completed a study (presented at the AHA, 2011; manuscript in submission) of the 6MWT completed by enrollees in HF-ACTION in comparison to the CPX data, assessing their relative utility as prognostic gauges. 6MWT distance correlated significantly with peak VO_2 and the VE/VCO_2 slope: unadjusted r = 0.537, -0.259 and adjusted r = 0.333, -0.176 (p<.0001 in all). Moreover, using C-indices 6MWT distance predicted hospitalization/death and death (unadjusted and adjusted) with similar efficacy to peak VO_2 and the VE/VCO_2 slope (Table 3).

Table 3: C-indices to Compare Risk Prediction between 6MWD and CPX

	All-cause Hospitalization/Death		All-cause Death	
	Unadjusted	Adjusted	Unadjusted	Adjusted
6MW	0.581	0.620	0.650	0.722
Peak VO_2	0.606	0.630	0.682	0.733
VE/VCO_2 slope	0.561	0.606	0.647	0.708

While still working on HF-ACTION, Dr. Forman collaborated with Gloria Yeh and Russell Phillips in the NCCAM Trial Tai Chi Mind-Body Movement Therapy for Patients with Chronic HF. Dr. Forman completed all CPX for that study, which showed that tai chi (TC) improved quality of life, mood, and exercise self-efficacy in patients with HF as compared to a matched attention control population (87).

Dr. Forman also spearheaded a related study comparing TC to aerobic training (AT) in a HF-PEF population. After 12 weeks of training, change in peak VO_2 was similar between groups, but 6MWT distance increased more after TC (+69 ±46 m vs. +10±31 m, p=0.02). Furthermore, while TC and AT were similar with respect to Minnesota Living with Heart Failure score and self-efficacy, POMS-depression scores improved more in TC (-1.7±2.8 vs. +1.6±3, p=0.05). Cardiorespiratory parameters during training showed lower oxygen uptake (4.3 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ vs. 9.4 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$, p<0.01), respiratory rate and heart rate with TC relative to AT. Overall, the utility of TC to achieve similar or better performance benefits with a lower aerobic stimulus reinforces Dr. Forman's contention that *skeletal muscle may be a key determinant of function*. TC may provide particular benefit with its focused skeletal muscle emphasis. That study is now submitted for publication.

Working with Lynne Stevenson, Dr. Forman has also completed a series of analyses on CPX for HF patients. One project focused on chronotropic incompetence among systolic HF patients who were pacemaker-dependent for their exercise HR responses (88). Patients with pacemakers received higher doses of beta-blockers, had worse chronotropic incompetence (95 vs. 71%, p=0.001) and worse aerobic functional performance (12.2±3.4 vs. 14.2±4.1 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$; p=0.004); the study indicates that function could likely be improved if pacemaker settings were reevaluated.

In another analysis with Drs. Stevenson and Forman focused on differences in functional capacity in the time after a HF patient reached his/her VAT (89). Patients who ultimately stopped exercising due to leg fatigue demonstrated much greater capacity to exercise beyond their VAT than patients who stopped exercising due to dyspnea. *This study suggests that differences in the time after someone reaches the VAT may be an important consideration in HF management. It seems likely that differences in skeletal muscle may underlie these functional differences, such that primary focus on skeletal muscle may improve outcomes.*

3. Muscle proteolysis by the ubiquitin-proteasome system (UPS) in HF and regulatory role of IGF-1 demonstrated in an animal model

In an animal study (45), Dr. Schulze demonstrated the key role of proteolysis through the ubiquitin proteasome system (UPS) in HF and the key regulatory role of IGF-1 in skeletal muscle tissue. Muscle fiber cross sectional area (CSA) in the thigh was significantly reduced 12 weeks after myocardial infarction and related development of chronic left ventricular dysfunction (CLVD). Furthermore, increased amounts of ubiquitinated protein conjugates were demonstrated in extracts from atrophying skeletal muscle along with enhanced activity of the 20S proteasome, i.e., findings that indicate an activation of the ubiquitin-proteasome pathway in the setting of CLVD **(Figure 3**).

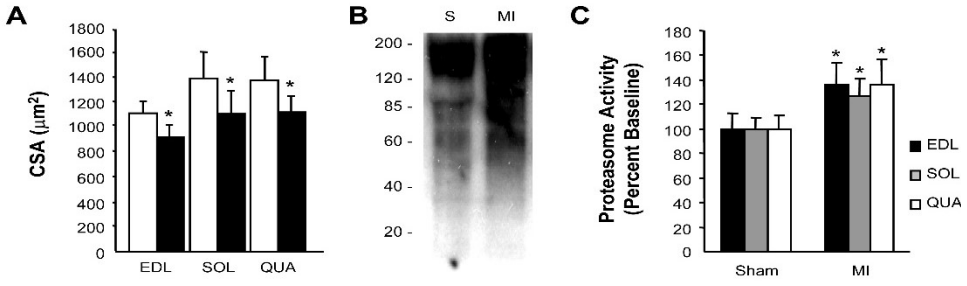

Figure 3. Muscle Atrophy and Increased Activity of the Proteasome in Mice with Left Ventricular Dysfunction (CLVD). **A,** Skeletal muscle atrophy develops with CLVD. **B,** Increased protein ubiquitination in skeletal muscle with CLVD. **C,** Activity of the 20S proteasome increases in several atrophying muscles in chronic CLVD (all *P*<0.05 vs. WT sham) (EDL – M. extensor digitorum longus; SOL – M. soleus; QUA – M. quadriceps; n=6 animals per datapoint).

Not only did Schulze's analysis demonstrate that ubiquitin conjugates and the UPS was strongly induced in several different hindlimb muscles, it also showed that one particular ubiquitin protein ligase (discovered by Dr. Lecker) was dramatically induced in these muscles (**Figure 4**).

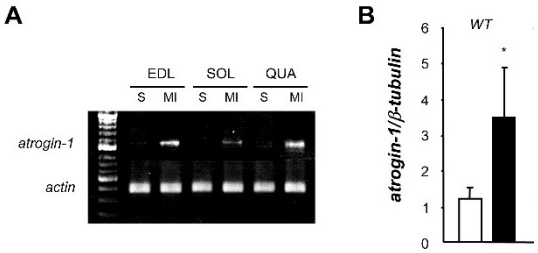

Figure 4. Expression of *atrogin-1/MAFbx*. A, Expression of *atrogin-1/MAFbx* increases in several muscles of animals with CLVD. B, Quantitative real-time PCR revealed an increase of *atrogin-1/MAFbx* expression in the atrophying quadriceps muscle of animals with CLVD (*P<0.05 vs. WT sham; n=6 animals per datapoint) which is prevented

Dr. Schulze has also completed novel analyses of IGF-1 in HF patients, reporting reduced expression of IGF-1 in skeletal muscle of humans with HF (57) (**Figure 5)**, and correlated those changes to markers of impaired muscle function and morphology. In particular, local expression of IGF-1 correlated significantly with thigh muscle cross sectional area assessed by computed tomography. Notably, patients in a 6-month aerobic exercise program corrected their local expression of IGF-1 in skeletal muscle. This fits with the signaling paradigm known to lead to atrogin-1 and UPS activation in atrophying muscle (44), namely that reduced IGF-1 levels promote FoxO dephosphorylation, nuclear translocation and transcription of atrogin-1 and other UPS genes.

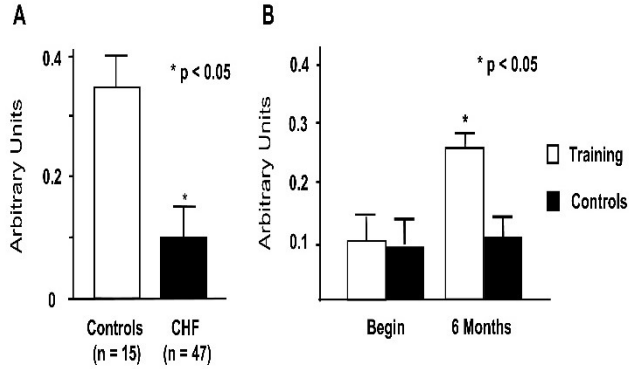

Figure 5. Skeletal muscle expression of IGF-1. A) local expression of IGF-1 is significantly reduced in skeletal muscle of patients with CHF. B) 6 months of supervised exercise training increases the local expression of IGF-1 in skeletal muscle of patients with CHF while patients in the sedentary life style group did not show any changes in local expression of IGF-1 (n= 9 per group)

Beyond Dr. Schulze's seminal work in this literature, his efforts now continue at Columbia University where he has assumed a key clinical/research leadership role in the transplant service. Drs. Schulze and Forman continue to collaborate in a complementary manner. Dr. Forman has directly contributed to 3 of Dr. Schulze's projects which were presented at the 2011 Heart Failure Society of America:

Table 4: Collaborations with Dr. Schulze

Mechanical unloading via left ventricular assist device implantation ameliorates adipose tissue inflammation and increases adipocyte size in patients with advanced HF
Ventricular Assist Device Implantation Corrects Skeletal Muscle Function, Oxidative Capacity and Local Expression of Insulin-like Growth Factor-1 in Patients with Advanced HF
Impairment of muscle fiber recruitment and decreased total muscle work capacity during isokinetic exercise in patients with advanced HF

4. Suppression of IGF-1 mediated signaling and PGC-1α expression promote muscle atrophy

Experiments by Dr. Lecker have demonstrated PGC-1α is another component of the transcriptional profile of atrophying muscle. He demonstrated that PGC-1α is suppressed at the transcriptional level in all kinds of atrophying muscle studied (**Figure 6)** (47).

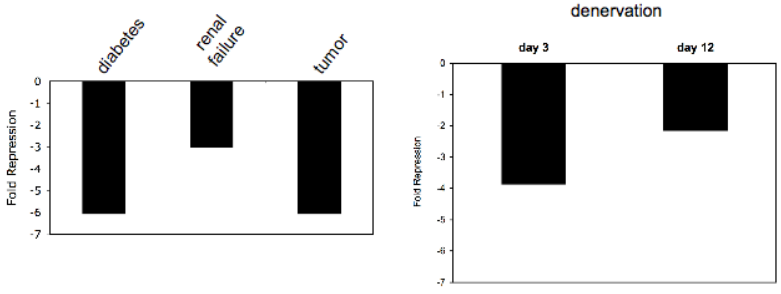

Figure 6. PGC-1α is suppressed in atrophying skeletal muscle. mRNA for PGC-1α was measured by rt-PCR in skeletal muscle from rats with streptozotocin-induced diabetes, 4/5 nephrectomy, and Yoshida ascities hepatoma, and mice following section of the sciatic nerve.

The integrated role of PGC-1α and IGF-1 suppression, as well as ubiquitin-mediated proteolysis will provide critical insights into the molecular mechanisms underlying skeletal muscle atrophy.

5. Application of gene expression analyses to age-related issues of sarcopenia

As a leading investigator in the area of aging and sarcopenia, Dr. Fielding brings a synergistic experience to this research investigation. In a prior study of 26 men and women aged 72-98, muscle biopsies of the vastus lateralis were completed before and after 10 weeks of strength training (58). Strength increased by 257±62% (P=0.0001) and Type II fiber area by 10.1± 9.0 % (P=0.033). Exercise was associated with a 2.5-fold increase in neonatal myosin expression (P=0.0009) which was highly related to the change in Type II fiber area (r= -0.934, P=0.0002) and an increase of 491±137% (P<0.0001) in IGF-1 expression. Z band damage increased by 141±59% after exercise training (P=0.034). These processes of damage and regeneration were closely correlated. Strength increases were largest in those with the greatest adaptations of myosin, IGF-1, and ultrastructural damage, as well as the greatest increases in energy intake during the trial. In particular, this study highlights Dr. Fielding's expertise with microscopy and the sophistication regarding skeletal muscle physiology that he imparts to the proposed study.

6. Prior work in Inspiratory Muscle Training

Dr. Cahalin brings a key expertise as a leader in the field of IMT and will guide this critical component of the study. Working with Drs. Dec and Semigran, he completed an IMT study on advanced HF patients (NYHA class III and IV, LVEF 23±13%) and found improvements in maximal inspiratory and expiratory pressures as well as symptoms as early as two weeks after initiating an IMT regimen that was far more convenient and efficient than others which had been studied at that time (73). A subsequent study by Drs. Cahalin, Semigran, and Dec also examined the effects of IMT in advanced HF patients, but employed a cross-over design and compared IMT to cycle ergometry (CE) (75). The results of that study demonstrated the effects of "specificity of training" with IMT significantly improving inspiratory muscle strength and endurance and CE significantly improving cycling performance. Both IMT and CE significantly improved 6MWT distance, estimated peak VO_2, and quality of life, with IMT eliciting relatively greater improvements (75). Dr. Cahalin and his colleagues also performed a comprehensive review of the effects of diaphragmatic breathing in patients with chronic obstructive pulmonary disease (COPD) (74) and identified similarities in several respiratory indices of HF and COPD patients.

MATERIALS AND METHOD

In a 4-year randomized, controlled trial, the efficacy of 3 exercise training regimens to modify symptoms of exercise intolerance and dyspnea in older systolic HF patients will be compared. Since aerobic training, combined aerobic and strength training, and IMT each constitute different training stimuli, we expect to find differences in how HF patients undergoing each regimen are affected with respect to functional attributes as well as in terms of constitutive skeletal muscle features (phenotype and gene signal patterns). We anticipate insights from this work will facilitate clinical implementation of exercise training that more efficiently yields improvements in highly relevant parameters (improved symptoms and functional capacity) and which also better moderate peripheral pathophysiological aspects of the disease (such as skeletal muscle and tissue perfusion). Patients will be randomized between 4 different exercise training regimens, and endpoints will focus on functional differences, in skeletal muscle phenotypes, gene expression, as well as related differences in skeletal muscle vascular perfusion and serological differences in metabolism and inflammation.

Overall Study Protocol: 150 systolic HF patients will each undergo an initial clinical and mechanism-oriented assessment battery (involving 2-3 visits, depending on the site where they are enrolled) before and after being enrolled into one of 3 exercise training arms. The pre-/post- evaluations will include 1. a battery of physical function (aerobic and strength) evaluations, 2. serology, 3. muscle biopsy, and 4. DXA scan. The study population will be randomized into one of three 12-week exercise training arms (aerobic, combined aerobic-strength, and inspiratory muscle training [IMT]).

A SAS program will be written to randomly allocate patients to the exercise training arms (see statistics below), also balancing gender and statin-use (90) among these 3 exercise training groups. Enrollment into the exercise training groups will be on a rolling admission.

Notably, we used a 2-3 visit comprehensive assessment protocol as part of our pilot study. It was well tolerated by subjects with no complaints of excessive fatigue or inconvenience. We provided a gap of at least 15 minutes between each of the subparts, with leeway to allow extra time if that is requested by the subject or if it seemed indicated to the study staff. Furthermore, visits were arranged such that assessments requiring physical exertion were alternated with those which are physically non-taxing (e.g., questionnaires done at rest) to help

ensure that the overall protocol was well-tolerated. Heart rate and relative perceived exertion were also checked prior to each subpart of the assessment to confirm that the subject's baseline parameters had been restored before testing proceeded.

Exercise training will be 3X/week, for 60 minutes, over 12 weeks for each of the 3 exercise regimens through a combination of on-site and home-based exercise sessions. Phone calls will be completed weekly by trained exercise based research staff to check in with study patients, work to overcome barriers and modify exercise regimens. Each participant will exercise for a maximum of 12 weeks. If subjects miss training sessions due to intercurrent illness, travel or other reasons, the duration will not be extended as the proposed trial is based on an 'intention to treat' model.

The comprehensive 2-3-visit evaluation (including physical function plus muscle biopsy, and DXA) will be repeated at the end of the exercise training period.

Expansion into a multi-site protocol: In its first year, the study experienced initial delays as the software necessary for IMT was upgraded to be compatible with newer versions of Windows as well as the VA system. Moreover, even after enrollment began, recruitment of systolic HF patients was more difficult than projected. In response to this challenge, the study will be expanded to multiple sites as well as home based exercise session, each with enhanced recruitment and adherence strategies. This change in study design coincides with Dr. Forman's plans to transition to the VA Pittsburgh Healthcare System (VAPHS) which will be used to the advantage of the protocol design.

1. Dr. Forman will continue as PI of the overall trial as well as site PI at the (VAPHS). Four training arms (Aerobic vs. Strength vs. Combined aerobic-strength, vs. IMT) will be completed at VAPHS, along with pre- and post- skeletal muscle biopsies, bloodwork, DXA and functional assessments. 100 HF patients will be enrolled at that campus, drawing from its robust HF program as well as its extensive HF telehealth database (see recruitment strategies below).
2. The study will also continue at the VABHS where enrollment goals will be 50 HF patients. Daniel Gottlieb, MD will assume responsibility as the Boston site PI. Exercise randomization at the VABHS will be limited to aerobic vs. IMT, both to increase efficiencies for the reduced staffing available once Dr. Forman and members of his staff have departed for Pittsburgh, and also to ensure adequate enrollment to a RR&D SPIRE-funded 1 I21 RX001423-01A1 substudy (Exercise Training in HF: Structural and Functional Cardiac Remodeling [PI-Jayashri Aragam]) which will explore correlative effects of IMT vs. aerobic training on cardiac remodeling. Also, given Dr. Aragam's role as principal investigator in the ancillary SPIRE study of exercise effects on cardiac remodeling as well as the distinctive skillset required for the echo-based analysis of skeletal muscle perfusion (that is part of the original protocol), both the perfusion and remodeling ultrasound assessments will be studied exclusively at the VABHS. This too is a decision to help maximize local enthusiasm for the protocol, as it is becomes a distinctive VABHS goal and responsibility.

Table 4: Outline of protocol (by visit)

Initial Assessment	Visit 1	Informed consent and physical exam, physical functional assessment battery (aerobic, strength, 6MWT, Hand Grip, gait speed, Sit to Stand, and questionnaires)
	Visit 2 (within one week of visit 1)	Skeletal muscle biopsy; Serology and DXA scan Randomization
	Visit 3 (2-3 days after visit 2) (VABHS only)	Reassessment of biopsy site
Exercise Training	Visits 3/4-38/39	Exercise training sessions (3X/weekly; 60 minutes; 12 weeks)
Post assessment	Visit 39/40-41/43	Post-exercise assessment battery and additional group of assessments completed at visits 1 to 2/3

Table 5: Expansion to a Multi-Site Trial

	Recruitment goals	Exercise arms
VAMC Pittsburgh	100	Aerobic (35), Combined aerobic-strength (30 enrollees), IMT (35 enrollees)
VAMC Boston	50	Aerobic (25 enrollees); IMT (25 enrollees)

Total: 150 HF patients: Aerobic—60; IMT—60; Combined—30;

Study Population

- *Study Population:* 150 male and female systolic HF patients with mild-moderate symptoms while on optimal medical therapy will be enrolled. This target will allow for adequate power to address the study objectives even after accounting for a 20% drop-out rate due to attrition and/or suboptimal tissue samples for genetic analysis. Given the expansion to two campuses, and the variation in randomization at each site, the overall proportions of patients enrolled will vary from the original study design but still provide a sound statistical foundation for the anticipated analyses (see statistics section below).
- HF diagnosis will be contingent on a previous hospitalization for HF or physician assessment of HF associated. Each candidate will be examined at the time of enrollment by a cardiologist to ensure that he/she is clinically stable.
- *Inclusion Criteria:*

- NYHA class II or III for the previous three months despite a minimum of 6 weeks of optimal treatment
- Age ≥50 years
- Left ventricular EF (LVEF) (by echocardiogram or radionucleotide imaging study within 12 months of enrollment).
- Optimal therapy according to AHA/ACC and HFSA HF guidelines, including treatment with ACEI and beta-blocker therapy (for at least 6 weeks), or have documented reason for variation, including medication intolerance, contraindication, patient preference, or personal physician's judgment.
  - ✓Patients using aspirin (ASA) will be eligible, but asked to hold the medication for 48 hours prior to biopsy. This technique has previously been used with consistent safety. Patients will also be asked to avoid non-steroidal anti-inflammatory medications (NSAIDs) for 48 hours prior to the biopsy.
  - ✓Patients using warfarin for atrial fibrillation will be enrolled if their cardiologist approves holding warfarin for 72 hours prior to the procedure (usually those with Chads score of 2 or lower), or if they are willing to undergo bridging therapy with enoxaparin. The investigators have established a muscle biopsy-related protocol regarding patients who are using warfarin (See *Recruitment standards for patients using warfarin* in the appendix 12).
  - ✓Patients who are using clopidogrel (Plavix) or an equivalent medication will only be enrolled if their cardiologist indicates that it is permissible for them to be off the platelet medication for 5 days prior to the biopsy. Furthermore, we supply all cardiologists with guidelines to help with this decision based on criteria developed originally by the interventional cardiologists at Brigham and Women's Hospital. (See *Recruitment standards for patients on anti-platelet therapy* in the appendix 13). Essentially, decisions regarding eligibility for patients using thienopyridines is individualized, and based on the type of stent that had been deployed, the date of the original procedure, and the complexity of the vascular lesions.
  - ✓Patients using statins will be enrolled; in the pilot study it was evident that most patients eligible for this study were on statins since prevalence of hypercholesterolemia is high and most clinicians are attentive to prescribing statins as part of their standard of care. While this is potentially a confounding issue, we will ensure that statin use is balanced in each arm.

- *Exclusion Criteria:*

- Major cardiovascular event or procedure within the prior 6 weeks.
- HF secondary to significant uncorrected primary valvular disease (except mitral regurgitation secondary to left ventricular dysfunction). If valve replacement has been performed, patient may not be enrolled for 12 months after this procedure.
- Allergy to lidocaine
- Dementia
- Severe COPD (FEV1<50%), PVD, and/or Anemia
- End-stage malignancy
- Severe valvular heart disease
- Mechanical valve replacement requiring warfarin
- Orthopedic exercise limitation
- Currently taking clopidogrel and have a recent stent placement and/or a complex atheroscrotic lesion such that holding clopidogrel creates disproportionate risk.
- Any bleeding disorder that would contraindicate biopsy such as history of clinically significant bleeding diathesis(e.g., Hemophilia A or B, Von Willebrand's Disease or congenital Factor VII deficiency).

- Women who are pregnant, breastfeeding, or likely to become pregnant within the next 6 months
- Psychiatric hospitialization within the last 3 months
- ICD device with heart rate limits that prohibit exercise assessments or exercise training. Referring physicians will be provided with an opportunity to reprogram devices so that patients can participate.
- Chronic use of oral corticosteroids or medications that affect muscle function. Notably, patients using statins will be eligible, and this will be factored into the randomization and analysis.
- Chronic ETOH or drug dependency.

Recruitment Strategies

VAPHS: Director of the VAPHS HF Program will join the study staff as co-investigator, and will work with Dr. Forman in achieving the study recruitment goals. The HF program not only administers care to over 400 suitable HF patients, but has established a regional orientation through its robust tele-health program that will be used to expand recruitment potential. It is anticipated that study physiologists will set up satellite exercise programs in local VA programs in Clarksberg, West Virginia and Butler, PA where there are high local concentrations of eligible HF patients.

VABHS: Recruitment in VABHS will primarily draw from the West Roxbury (WX) campus) where study investigators Drs. Gottlieb, Aragam, Joseph, and Brown are on staff. Dr. Joseph is Director of the VABHS HF program, a highly successful clinical program with over 500 HF patients, and a strong track record of recruitment into clinical trials. Dr. Joseph works with two physician, two nurse practitioners, and two fellows to manage patients in this program, and they have established a track record of working together to identify HF patients who can be invited to participate in trials (consistent with HIPAA guidelines). Moreover, as part of their collaboration on the pilot grant, Drs. Forman and Joseph not only recruited from the HF clinic sessions, but also organized a comprehensive HF list of patients who have been attached to the HF program over the past 8 years; this has helped grow recruitment efficacy, and is a recruitment asset that will be used for the proposed trial.

The WX VABHS is also where the Division of Cardiology's echocardiography suite is located. Dr. Forman and Aragam have established a successful recruitment mechanism using the clinical echocardiography service during the pilot study. All patients with LVEFs ≤5% are now systematically identified as potential candidates for HF trials. Working with the institutional review board (IRB) to comply with HIPAA regulations, we established this robust recruitment strategy that we expect to continue in relation to this new protocol. Dr. Aragam is Director of the Echocardiography Laboratory. She has now joined this study as key personnel, and is particular invested as she has also received complementary funding (as a VA SPIRE award 1 I21 RX001423-01A1) to complete a substudy focused on remodeling differences in relation to aerobic vs. IMT.

In addition to the 500 patients followed directly by the WX HF program, there are over 500 patients in the VABHS with a chart diagnosis of HF that are followed through primary care and preventive care clinics. Dr. Lazzari works in the Primary Care Division at the Jamaica Plain (JP) campus and over sees the residents' clinic. Dr. Lazzari will be instrumental in recruitment with in primary care. We expect that the comprehensive HF list culled by Drs. Forman and Joseph will help identify HF patients managed by primary care providers; we also expect the echocardiography database will help identify eligible patients in this group. Dr. Forman has also established a successful track record of recruiting HF patients directly from community-based outreach facilities such as the Causeway Street and Dorchester Clinics. These efforts have been particularly useful in expanding minority representation in the study population of the pilot trial.

VABHS also has the potential to expand recruitment to Brigham and Women's hospital (BWH) and the Boston Medical Center (BMC). All assessment visits and intervention visits will take place at JP VABHS. The BWH and BMC HF groups meets regularly, and trials are announced on a regular basis for which recruitment is a goal. Overall, both programs have a strong track record of recruitment into exercise trials. Dr. Forman has successfully recruited BWH female and male HF patients into the NHLBI HF-ACTION which was conducted at BWH, and he also recruited patients into the NCCAM Tai Chi Mind-Body Movement Therapy for Patients with Chronic Heart Failure trial which was conducted at Beth Israel Deaconess Hospital. It is anticipated that the clinical support with this trial will be even stronger with the allied interests of Drs. Forman, Stevenson, and Joseph all increasing attention and priority to the investigation. Moreover, as an exercise trial, it has appeal to patients that stands out in comparison to trials which are otherwise predominantly oriented to pharmacological or device interventions. The VA-based study coordinator will respond to patient phone calls that are generated from BWH announcements related to questions or desire to enroll.

Drs. Joseph and Stevenson are on staff at BWH. Dr. Stevenson is Director of the BWH HF Program. Based on Dr. Forman's successful recruitment into 2 previous exercise-training trials at BWH and BMC, it is anticipated

that enrollment of minorities (particularly Hispanic and African American) and women will suceed at BWH and BMC.

Demographics: VAPHS: treats 85% Caucasian and 15% African American, with 5% women. VABHS: treats 92% Caucasian, 7% African American, 1.6% Hispanic, and 5% Asian, Pacific Islander, American Indian or Alaska Native HF patients, and the majority of patients are men. The BWH HF program is nationally and internationally acclaimed with over 1500 patients, including 30% women fitting the criteria for this protocol, and 30% minorities. BMC HF includes 40% minorities. Overall recruitment for the 3 sites is expected to reach a broad range of ethnicities, and include approximately 20% women.

Functional Assessments and Exercise Training:
VAPHS: All study-related patient assessments and training sessions will be completed at the VAPHS (University Drive campus) where Dr. Forman and his staff will have office space, ample computer facilities, and a superior infrastructure for exercise training investigations. Exercise training will take place at the University Drive Campus as well as in satellite locations anticipated in Clarksburg, West Virginia and Butler, Pennsylvania.

VABHS: All study-related patient assessments and training sessions will be completed at the JP VABHS where Dr. Gottlieb and his study staff have office space, ample computer facilities, and a superior infrastructure for exercise training investigations within the Clinical Studies unit (CSU). To increase patient recruitment, the exercise training will expand to the Brockton campus of the VABHS where a density of eligible HF patients have expressed willingness to enroll, but only if the training can be located at that campus.

Pantel Vokonas, MD is assuming role as interim Director of the VA Boston Cardiac Rehabilitation (CR) Program at the JP VABHS, a state-of-the-art program and infrastructure (including a1600 sq foot exercise training space complete with extensive, first-rate strength and aerobic training equipment), which is also based at the JP VABHS. As Director of CR, Dr. Vokonas has discretion to organize the training arms required for this protocol in coordination with the CR clinical program. Moreover, Dr. Vokonas' office sits immediately adjacent to the CR training space and the contiguous CSU where all functional assessments and muscle biopsies for this study will be performed, facilitating requisite supervision and safety for all components of this investigation.

All assessment visits and training sessions will be conducted by a team of ACLS credentialed staff (cardiologist, nurses, and exercise physiologist). A defibrillator will be maintained in the immediate vicinity in each study campus. Consistently, study staff will be required to participate in regular mock codes to maintain expertise with emergency management. Furthermore, visits will only be completed during the time that appropriate emergency support services are available, including hospital code teams or the equivalent (depending on each site). All these decisions will be made by the local site PIs with the final approval of Dr. Forman.

- *Cardiopulmonary Exercise Testing:* Treadmill exercise testing in association with air-gas-exchange, generally considered to be an optimal gauge of aerobic capacity (92), will be supervised by Dr. Forman at VAPHS, and Drs Gottlieb and Vokonas in VABHS. All these physicians have supervised large numbers of studies over many years. Furthermore, Dr. Forman has employed the technique in multiple research investigations (HF-ACTION, Tai Chi Mind Body Exercise for HF, and most recently for the VA based pilot study for this proposal) and brings his perspective and sophistication to the broader study team. Similar expertise exists on each study campus.

A motor-driven treadmill will be used to generate the exercise stimulus. A modified Balke protocol will be utilized. A lightweight disposable pneumotach device will be positioned in the enrollee's mouth during the exercise for gas exchange assessments (MedGraphics Ultima or equivalent system). Peak VO_2, VAT, the VE/VCO_2 slope, and respiratory exchange ratio (RER) will be measured, as well as hemodynamics (max heart rate [HR] and blood pressure [BP]), time, and ECG waveforms). Any abnormalities will be reported to the patient's cardiologist and primary care physician; continued participation in the study will require physician clearance.

Both the Borg Rate of Perceived Exertion (RPE) (93) and the Modified Borg Scale for Perceived Dyspnea (Shortness of Breath) will be completed during the CPX and the 6MWT to quantify exercise associated symptoms before and after the test.

All CPX test data will be analyzed and validated by Drs. Arena and Forman and a CPX core lab will be organized at the VAPHS.

- *Six Minute Walk Test:* The 6MWT is also a commonly used assessment of aerobic capacity and submaximal endurance in HF patients. As a measure of everyday walking activity, it is often felt to complement the insights

afforded by CPX indices. Using standard methodology described by the American Thoracic Society (94), patients will be asked to walk back and forth along a 30 meter course as quickly as possible for 6 minutes. A supervising exercise physiologist will encourage patients to walk as quickly as possible. The test will be scored in meters walked in 6 minutes, rounding to the nearest meter. As noted, the Borg Rate of Perceived Exertion (RPE) and the Modified Borg Scale for Perceived Dyspnea (Shortness of Breath) will be completed during the 6 minute walking test (and CPX).

- *Gait speed*: The 5-meter gait speed test has been advocated as a key measure of frailty (95) Patients will be asked to walk a distance of 5 meters at a comfortable walking pace; the time to complete it will be recorded.
- *Muscle Strength and Fatigability:* Muscular strength and fatigability will be measured using a Keiser pneumatic strength apparatus with computerized data acquisition which allows for the collection of weight, power, force, and fatigability of movement.

To assess muscle strength patients will complete a one repetition maximum (1RM) using both legs. Patients will be instructed to push out as fast as they can then to slowly release in a standardized 4-stage process:

1) Warm up with 6-8 repetitions at a very light weight, familiarizing the patient with the machine.
2) Two minutes of rest, then three sets of reps (resistance at approximately 50% of the patient's maximum) providing the patient with reps associated with resistance.
3) Two more minutes of rest, then 1 repetition at weight approximating the 1RM.
4) If the patient can lift that weight, one more minute of rest will follow, with the weight then increased, and the patient trying again. The patient will continue to perform single repetitions at increasing resistances until he/she is no longer able to push the weight, cannot maintain proper form, or does not feel comfortable continuing.

After the 1RM testing, patients will have 4 minutes to recover and have an opportunity to relax. Power assessments will then be performed with the patient pushing against a resistance of 40% of the 1RM. The patient will perform 5 repetitions, pushing out as rapidly as possible, then releasing slowly.

After 4 minutes of rest muscle fatigability will be tested. The patient will push against a resistance of 60% of the 1 RM. He/she will be instructed to complete as many repetitions as they can without stopping, each time fully extending and releasing the leg press until exhaustion, proper form is no longer maintained, or until 1 minute of time has elapsed. The patient will be asked to complete as many repetitions as they are able to/comfortable with. The Borg Rate of Perceived Exertion (RPE) and the Modified Borg Scale for Perceived Dyspnea will be assessed as part of the strength/fatigability evaluation.

- *Hand Grip Strength:* Hand grip strength is commonly used as an assessment of upper body strength, and is also an important factor in activities of daily living. Patients will be asked to keep their arm at a right angle with the elbow next to their side while performing the test. They will then be asked to squeeze a dynamometer with a maximum effort isometric contraction that will be maintained for 5 seconds. No other body parts will be allowed to move. The test will be repeated 3 times with a 60 second rest in between repetitions. The test will be scored in pounds of pressure squeezed. The maximum force attained will be used as the performance measure.
- *Sit to Stand Test (STS):* STS (96) is commonly used in an older population as a functional assessment of lower body strength as well as factors associated with balance and mobility. Using the standard 30 STS protocol, patients will be asked to start seated in a straight back chair without armrests. The evaluator will ask patients to cross their arms in front of their chest and then instruct them to sit and stand all the way up as many times as they can in 30 seconds without using their arms. The test is scored by the number of stands over 30 seconds.
- *Test of Incremental Respiratory Endurance:* TIRE is a measure of respiratory performance. It is achieved by using a specialized pneumotach-type device (RT2; DeVilbiss Healthcare Ltd) connected in series with a laptop or desktop computer (72,74). Inspiratory muscle strength is measured as the maximum inspiratory pressure (MIP) at residual volume (RV). Single breath inspiratory work capacity will be measured as SMIP and will be measured from RV to total lung capacity (TLC). Total inspiratory muscle work achieved during a testing or training session will be measured as accumulated SMIP (ΣSMIP) at 40% of SMIP, and will be the primary measurement of inspiratory muscle endurance.
- Quality of Life and Functional Assessment Questionnaires:
  - ✓ The Duke Activity Status Index (DASI) (97) is a 12-item scale that has been validated in cardiac patients against peak VO_2 , and has been demonstrated to be a reliable and responsive tool to quantify physical activity in daily living.
  - ✓ The Kansas City Cardiomyopathy Questionnaire (98) will be used to measure disease-specific health-related quality of life (QOL). This 24-item instrument produces the following scores: Physical Limitation, Symptoms, Symptom Stability, Social Limitation, Self-efficacy, Quality of Life, and two summary

measures, and is widely used as a standard by which quality of life, self-efficacy, and other personal perspectives related to functional capacity at baseline and over time .

✓ Dyspnea will be assessed using both the Modified Borg Scale for Perceived Dyspnea (93) during CPX as well as the Chronic HF Questionnaire—dyspnea Subscale (99) which was developed to specifically assess HF in five specified activities over the preceding two weeks. While dyspnea questionnaires are often criticized as an imperfect science, these two questionnaires provide reasonable assessment of this critical symptom of this evaluation (100). Moreover, dyspnea assessment in HF has recently been shown to portend both diagnostic and prognostic information in patients with HF (101).

Based on the pilot protocol, it is expected that all questionnaires will be finished in under an hour, and that they will not constitute an excessive burden to any subjects. All questionnaires will be collected and scored using Teleform technology.

- *Effects of Confounders:* Potential confounders will be considered in all functional assessments. Age, height, weight, medications, and other pertinent clinical parameters will be assessed. DXA data facilitates normalization of data to lean body mass. Effects of sleep, physical activity, diet will also be considered and assessed by questionnaires.
  - ✓ Sleep will be assessed using the Pittsburgh sleep index (102)
  - ✓ Exercise self-efficacy will be assessed by using the Exercise Confidence Survey (110)
  - ✓ Diet will be assessed using a 3 day food record (103)
  - ✓ Physical activity will be assessed using the CHAMPS questionnaire (104)
  - ✓ Medication will be assessed (using the VA electronic medical record/current medication list, with different medications within each class [ACE inhibitors, Angiotensin Receptor Blockers, Beta Blockers, Diuretics, etc.] grouped into low and high dosage categories). Statins, benzodiazepines, and narcotics will also be factored in analysis.

Exercise Training:

A set of standardized features will be integrated into 3 distinctive training regimens:

i. Study participants will be randomized into one of 3 training regimens (see below). Study staff will then develop a personalized regimen consistent with the training mode for that patient, using the preceding study-based CPX, walking assessments, IMT or strength evaluations to help guide initial exercise programs.

ii. Exercise prescription will be initiated in on-site individualized training sessions, during which each subject will work directly with study personnel to refine their home-based program that corresponds to the training regimen to which they were randomized.

iii. For patients who may not be able to tolerate 60 minutes of exercise, duration will start in each exercise group with smaller increments that are then extended progressively as tolerated. Each regimen will entail approximately 50 minutes of exercise (preceded by 5 minutes of warm-up and followed by 5 minutes of cool-down), scheduled 3x weekly for 12 weeks. A day of rest will be recommended between each scheduled exercise session. This volume of exercise has consistently been shown to significantly improve functional performance and physiologic measures of interest in patients with systolic HF (3,20,75).

iv. Exercise in each regimen will be balanced with the training volume of the others.

v. Prior to and immediately following exercise training in all 3 regimens, a 5-minute warm-up and a 5-minute cool-down period will occur. All participants will undergo the same the warm-up and cool-down sequences that consist of slow aerobic (less than 30% of their maximum) and stretching.

vi. Participants in each group will meet at least twice onsite with study staff at which time participant will go through an onsite exercise training session and reassessment. Based on these reassessments, exercise prescription for each subject may be adjusted as needed. Additionally, when possible once per month patients will be mailed an activity monitor to wear for one week after which patients will mail back so that the study staff can capture an assessment of the work being completed at home.

vii. Should a subject become ill or unable to exercise for 3 or more sessions, the regimen will be reassessed and exercise training resumed with aerobic, combined, or IMT modalities adjusted to best facilitate stability, with duration and intensity then advanced as tolerated.

- *Regimen #1: Aerobic Training:* Aerobic endurance exercise entails rhythmic motion of large muscle groups in aerobic activities such as walking, jogging, and cycling, employing a general principle that exercise should exceed baseline capacities and thereby induce progressive physiological adaptations.

Initially, aerobic training intensity will be guided by the VAT delineated by CPX, which has been validated as an optimal training stimulus. The Borg scale (rate of perceived exertion index) from 6 to 20 will be used to help guide exercise from moderate to high training intensity (12 to 16) over the course of the trial. The aerobic exercise period will total 50 minutes (preceded and followed by warm-up and cool-down respectively); if the

patient cannot initially sustain exercise for this duration, shorter intervals of exercise will be used (e.g., exercise then rest then exercise using a 2/1 work to rest ratio with aims of 10 minute bouts of continuous exercise), that progressively elongate over weeks towards a 50 minute continuous exercise maximum

Walking is the preferred mode of aerobic exercise, using higher walking speed used to achieve greater intensity as needed. However, exercises utilizing other equipment that involve major muscle groups of the lower extremities (e.g. exercise bicycle) may be incorporated into the endurance training to supplement walking. Exercise will be monitored through the use of an activity monitor with accelerometer functions.

- *Training Regimen #3: Combined Aerobic-Strength Exercise Training:* The combined strength and aerobic regimen will be designed such that subjects begin exercise at a low intensity and then progress as tolerated. Strength training will focus on the major groups of muscles in the upper and lower extremities. The aerobic training will focus on increasing the subject's aerobic stamina.

Exercise sessions will last 50 minutes and start with a 5 minute low intensity warm up and end a 5 minute cool down. All subjects will progress towards 25 minutes of continuous aerobic exercise followed by 25 minutes of strength training. Strength training will consist of dynamic concentric and eccentric contractions of the upper and lower extremities using the same techniques as described above.

Strength training will build towards 2 sets of 10-12 repetitions during which the movement of the exercise will be at a speed of 2-3 seconds per contraction and extension phases and 2-3 seconds between repetitions. Exercises will include a sit to stand squat targeting improvements in the gluteus maximus, adductor, hamstrings, and quadriceps muscles. The standing leg curl will be used to reinforce hamstrings strength. The seated dumbbell row will be used to strengthen back muscles including the trapezius and rhomboids. The arm curl will be used to increase bicep strength. The calf raise will also be used to increase calf strength.

Relatively lower weights and higher speeds will be used in the strength-aerobic protocol this variation facilitates strength training integrated with an aerobic training stimulus. The weights should reflect RPE's of 11-13 on a 20 point Borg scale. The weight for the exercise will be increased if the RPE falls below an 11 on the Borg scale. Exercise will be monitored through the use of an activity monitor.

- *Training Regimen #4: Inspiratory Muscle Training:* IMT will be administered 3x/week for 50 minutes (preceded and followed by warm-up and cool-down respectively); each session will include two different methods, TIRE and Threshold IMT. For each, subjects will wear a noseclip. TIRE IMT provides both power and endurance IMT and will be performed with the PrO2 IMT device. Threshold IMT provides strength and endurance IMT and will be performed with the Threshold IMT device.

TIRE IMT will be administered at 50% for four weeks, 65% for four weeks, and the remaining four weeks at 80% of the Maximal Inspiratory Pressure (MIP) and Sustained MIP (SMIP) using a visual target that the patient watches on a monitor during the training. The visual target has been observed to facilitate inspiratory performance via biofeedback while the IMT progresses in respect to the work to rest ratio, with rest periods decreasing from 60 seconds at level A to 45, 30, 15, 10 and 10 seconds at levels B through F, respectively. TIRE IMT is characterized by through-range IMT with the need for patients to match or exceed 90% of the desired percentage of the on-screen target throughout the entire inspiratory effort (from RV to TLC). Thus**,** the training session continues until three task failures indicated by an inability to match 90% of the on-screen target, or until a maximum of 36 resisted breaths have been performed with an average duration of 25 minutes (72,74). Adherence and performance during TIRE training is captured automatically via TIRE computer software and each session is automatically performed at the desired workload (50-80% of MIP).

Skeletal Muscle Biopsies: At least one day after the CPX, 6MWT, and strength/fatigability tests are completed, a skeletal muscle biopsy will be performed. These biopsies will be performed by Dr. Forman at VAPHS, Drs. Gottlieb or Martin at VABHS. Each respective investigator has had experience in the technique is monitored by the local institution for procedural safety and success of these procedures. The inclusion criteria (above) lists details regarding patient eligibility in the context of ASA, NSAID, and thienopyridine use.

Patients will lie comfortably in the supine position for biopsy of the vastus lateralis muscle in the left leg. Biopsy sites will be prepped with betadine and 2% xylocaine (without epinephrine), with a small stab cut, then completed using a #11 blade scalpel. A 5 mm Bergstrom muscle biopsy needle will be inserted through the skin incision and advanced into the muscle. Suction will be generated using a syringe attached to the outside portion of the needle, to thereby suck skeletal muscle into a hole on the side of the needle positioned in the muscle tissue; this draws a small piece of muscle tissue (usually 100-200 mg of muscle tissue) into the hollow shaft of the needle, which is then cut with a cutting trochar that slides through the shaft to cut the tissue drawn within its core. After harvesting the first sample, the needle will be rotated 90 degrees, and a second sample extracted to

maximize yield for analysis. The wound site will be closed with steri-strips and a sterile pressure bandage. All patients will return within 2-3 days for reassessment of the wound and to confirm normal healing.

Muscle specimens will be divided into 2 portions, immediately processed, and stored. One fragment will be placed in sterile cryotubes containing RNA preservation solution (RNAlater) and immediately frozen in liquid nitrogen and set for transport to Dr. Brown's laboratory at the VABHS. The second fragment of the biopsied muscle will have the muscle fibers aligned for microscopy, placed in OCT, then cooled in a thawing isopentane slurry and placed in a cryotube and frozen in liquid nitrogen for morphological analysis (this will occur under the supervision of Dr. Brown in coordination with the insights provided by Dr. Fielding) at the VABHS. All samples will initially be stored at the respective sites where they were collected then shipped in batches to the JP VABHS campus (using ample pre-existing research storage facilities available for this investigation) where they will be analyzed by Dr. Brown (PCR transcriptional and morphological analyses). Samples for morphological analysis will be assessed at the VA laboratory of Dr. Brown and a study consultant using an eclipse microscope purchased for this study.

Skeletal Muscle Analyses: Since the study's original funding, the VA Boston established its own laboratory facility for skeletal muscle research. Dr. Brown, a VA investigator, and his laboratory staff will complete all skeletal muscle gene expression analyses. Dr. Lecker, an investigator from Beth Israel Deaconess Hospital, will no longer conduct PCR analyses but will remain on the study staff for his analytic insights. The analyses will center on genes previously defined as induced or suppressed in atrophying skeletal muscle (atrogenes) (35).

- *Atrogenes:* We will analyze gene sequences that code for skeletal muscle atrophy in the muscle biopsy specimens from HF patients and controls. As discussed above, these genes belong to a specific set of atrophy genes, aka atrogenes, which we previously characterized to be regulated in several models of skeletal muscle atrophy (35). We will particularly focus on FoxO, PSMA1 and GLUL, PGC1 and IGF-1 because our pilot studies defined significant differences in expression of these genes and correlations with muscle function in HF patients, but genes within all the following functional groups will also be analyzed:
  1. Protein degradation atrogenes: atrogin-1/MAFbx, MuRF-1, Polyubiquitin (UbB UbC), proteasome subunits (PSMA1), cathepsin L.
  2. Growth factor pathways (molecular signaling related to growth): PGC-1α, FNDC5 (irisin), IGF-1, IGFBP5, FoxO1, FoxO3, myostatin, follistatin.
  3. Metabolic atrogenes, lactate dehydrogenase, glutamine synthase.
- mRNA extraction and rtPCR: Total skeletal muscle mRNA will be extracted from muscle biopsies using Trizol reagent and well established protocols. Typically 5-10 μg of total RNA is obtained from approximately half of a biopsy specimen. Samples will be stored in nuclease-free water at -80C. Total RNA will be converted to cDNA by reverse transcription using SuperScriptII reverse transcriptase (Stratagene) in standard reactions according to the manufacturer's recommendations.

  mRNA levels will be determined by real-time PCR using the Applied Biosystems® StepOne real-time PCR analyzer. Multiplexed amplification reactions will be performed using GAPDH as an endogenous control (GAPDH primers/VIC-labeled) using the TaqMan One Step PCR Master Mix reagents Kit (#4309169, Applied Biosystems). Probes/primers for the specific genes to be analyzed will be purchased directly from Applied Biosystems. The following amplification settings will be used: Stage 1 (reverse transcription): 48°C for 30 min; Stage 2 (denaturation): 95°C for 10 min and Stage 3 (PCR): 95°C for 15 sec and 60°C for 60 sec for 40 cycles. The Ct (Threshold cycle) values for each reaction will be transferred to a Microsoft Excel spreadsheet and calculation of relative gene expression will be performed from this data according to published algorithms (TaqMan Cytokine Gene Expression Plate 1 protocol, Applied Biosystems). All RNA samples will be analyzed in triplicate, with the mean value used in subsequent analyses.

  Of note, in our pilot studies, biopsy data was initially evaluated on different 96-well plates to allow biopsy processing before the completion of the study; however, this was associated with high variability and poor precision. Analyses were then repeated with all of the biopsy samples on the same plate. This technique strengthened our analysis and revealed key differences and correlations between gene expression and function. In this proposed study, we shall prepare and store cDNA from the muscle biopsies in small groups as the biopsies are performed, and then perform rtPCR analyses batched by exercise arm in order to minimize plate-to-plate variation in the rtPCR data.

- *Microscopic Analyses:* Dr. Brown and a consulting microscopy technician will complete all light microscopic analyses at the Boston VA. Dr. Fielding has an extensive track record of skeletal muscle phenotypic analysis. Both he and his laboratory staff will work directly with Dr. Brown to ensure the phenotypic assessments are completed in the most useful fashion. Given the controversial perspectives regarding the effects of disease vs.

deconditioning on skeletal muscle physiology and function, the attention to phenotypic assessments will be prioritized as a vital perspective relative to gene expression patterns and serologic assessments of inflammation.

- Muscle Tissue Histology and Morphometric Assessments

  Standard histological techniques will be employed to analyze cross-sectional area of muscle fibers, as well as damage, inflammation, and atrophy. Histochemistry will be performed to differentiate type I from type II fibers (cytochrome C oxidase activity). Additionally immunohistochemistry using monoclonal antibodies specific to the MHC isoforms will be used to assess the hybrid skeletal muscle types. These histomorphometric and enzymatic parameters will be correlated to atrogene expression to delineate whether specific gene expression patterns translate into pertinent physiological changes, as well as changes in exercise performance.

  In addition, standard measures of aerobic enzymes (citrate synthase) will be completed to establish correlations to functional parameters and associated signaling patterns. Citrate synthase will be assayed spectophotometrically from skeletal muscle biopsies.

Serum analyses: To be completed at the same time of the muscle biopsy. A core of study nurses are available at the CSU to complete the phlebotomy required. Serum will be obtained from all individuals in this study. Standard phlebotomy technique in sterile fashion will be utilized. Whole venous blood samples will be centrifuged at the CSU for 10 min at 4C. Samples will be put into dry ice and transported to freezer storage at the JP campus. Serum will be stored at -80C until batch analyses are completed. Serum analyses will be completed at the VABHS laboratories.

Assays will be completed that are pertinent to the secondary analyses of this investigation. Insulin, CRP, IL-6, IL-1, IL-1 receptor, TNF-α, TNFα receptor, Cortisol, Adiponectin (high molecular weight and total), Glucose, electrolyes, and liver function serology will all be assessed

Dual energy x-ray absorptiometry (DXA): DXA will be completed by Dr. Lazzari. A GE Healthcare Lunar iDXA Bone Densitometer 5th Generation instrument with encore software version 13.6 is available for these assessments. A total body scan will be used to determine total body fat free mass and regional body composition (107). Subjects will lay flat on their back on an open table for 15 minutes while a bar passes over them. Using these DXA-based measurements, CPX performance indices will be normalized to fat free mass, thereby eliminating confounding effects of body fat. This will modify the tendency to underestimate performance among those who are overweight.

Subject Retention: Utility of exercise will be emphasized to each exercise block throughout the course of the trial. If patients miss sessions, they will be immediately telephoned. A letter and email (if available) will be used to reach those who do not show for two or more sessions. As noted, exercise will be modified upon a patient's return from any missed sessions, but since exercise training is being administered on an intention to treat basis, the sessions will not be "made-up." Every effort will be made to foster a mutually supportive group dynamic, with the hope and intention to build expectations among the participants to support and encourage one another.

Project Time Line

Year 1: Organization of the grant, overcoming delays associated with development of the inspiratory muscle training equipment and staffing. Overcoming recruitment obstacles in Boston

Year 2: Launching programs in Pittsburgh and Chicago during first 6 months of the year. Recruitment/functional assessment/exercise training will accelerate in year 2 as Pittsburgh and Chicago campuses gear up.

Year 3: Recruitment/functional assessment/exercise training will continue during year 3.

Year 4: Recruitment/functional assessment/exercise training will continue during year 4. It is assumed that preparations of abstracts and presentations will begin in year 4. While it will be important to achieve our total recruitment N for appropriate power in final papers, it is expected that preliminary data will facilitate opportunities to contribute to dialog and advancements within the scientific community. Additionally all the exercise training sessions (and related assessments) will be achieved midway through year 4. Likewise, all skeletal muscle gene expression and microscopy analyses will be completed. The second half of year 4 will be devoted to analysis of the data, focusing on the relationship between gene expression, metabolism, and functional correlates, as well as related phenotypic assessments. Manuscript preparation will prioritized as well as goals to submit proposals for related analyses (i.e., the next steps to fundamentally modify HF therapeutic strategies based upon the outcomes anticipated by this trial).

Study Team: Throughout the trial, Dr. Forman will meet regularly (by phone) with co-investigators on a monthly basis in year 2, then quarterly in year 3, and then increasing to monthly again in year 4 when data analysis begins.

Pittsburgh: Dr. Forman and Dr. Lemieux; Dr. Forman is the overall PI; Dr. Lemieux is head of the VAPHS heart failure program and will work closely with Dr. Forman to achieve recruitment goals at the University Campus and surrounding VA programs.

Boston: Drs. Gottlieb, Aragam, Vokonas, Joseph, Martin, Lazari. Dr. Gottlieb is VABHS site PI. Drs. Aragam and Vokonas will join the study to help in recruitment and facilitation of exercise testing/training. Dr. Martin will complete muscle biopsies and advance study of muscle in relation to human performance. Dr. Lazarri will conduct DXA. Drs. Joseph and Lazarri will participate in overall analyses.

Other investigators

- Dr. Cahalin (based in Miami) remains a key study investigator in relation to the IMT. Dr. Schultz (based in NYC) is similarly important as he is completing complementary work on skeletal muscle and HF at University of Miami. Both will participate by phone. The pilot study demonstrated the enormous value of regularly scheduled meetings.
- Drs. Forman and Aragam visited Dr. Lindner's lab in Seattle at the onset of the investigation. As Dr. Aragam's work is initiated, both Dr. Forman and she will meet monthly with Dr. Lindner (by phone) over the first 2 years of the trial. It is anticipated that meetings will slow to quarterly thereafter.

All study staff hired as part of this protocol will be trained in the importance of data security and patient privacy. Data will be collected using standardized forms and will only be identified using the participant's ID number (no names or identifying information will be on the forms). The codes that link the names of participants and their ID numbers will be kept confidential by the PI in a secured cabinet located within his office.

At each campus there will be exercise therapists and research assistants to facilitate study goals. The study staff in charge of pre- and post-exercise training assessments will be separate from the study staff in charge of the exercise training to eliminate any potential for tester bias.

Statistical Analysis

General considerations: The analyses described below are of continuous outcome measures, primarily changes in function outcomes and gene expression and either continuous or categorical predictors. All analyses will be performed as general linear models. Potential confounders of each analysis will be outlined below and a 10% rule for evaluating confounders will be used, namely including a potential confounder if a 10% or greater change is seen in the main predictor effect. In all instances, outcome variables will be evaluated for violation of regression assumptions and transformation of these variables will be considered as a remedy if needed. Analyses will generally be performed with an intent-to-treat approach, with sensitivity analyses performed to assess the role of drop-outs on the results.

This grant examines a complex interplay of exercise regimens, gene expression and functional measures, resulting in a number of hypotheses that require testing. We are cognizant of the role of type I error in this and in interpreting results, focus will be placed on the overall results of these analyses and not on the results of individual tests. Thus, for example, significant relationships among gene expression outcomes and treatment categories as a whole will be considered as support for these hypotheses and isolated significant associations will not be unduly weighted in the interpretation of results.

Randomization will be generated using a SAS program based upon permuted random blocks of variable size to assure approximate balance over time and will be stratified by sex and statin use.

Validation of Study Measurements: At the start of the study, there will be two assessments of physical function battery. Intraclass correlations will be used to determine the relative repeatability of the various components of the battery. This information will be included in all manuscripts using these measures.

- *Aim 1. To assess differences in functional outcomes (peak VO_2, 1RM) relative to the training therapy: a) Aerobic vs. Strength vs. Aerobic-Strength regimens will be compared to one another b) Inspiratory Training will be compared to Aerobic-Strength:* In this aim, we compare the combined aerobic-strength training regimen to the single-therapy regimens of aerobic training and strength training, as well as the inspiratory training regimen. These comparisons will be made with changes in the functional outcomes (peak VO_2, 1RM) from baseline as the continuous outcome measures in separate models and training regimen as the categorical predictor in a general linear model, with the following factors considered as potential confounder variables: age, sex, and comorbidities. To limit the type I error rate, only the pre-planned comparisons of the combined aerobic-strength training to the other regimens will be considered. In addition, secondary analyses will consider the following aerobic, strength and inspiratory variables as outcome variables in similar analyses to those outlined above: 6MWT, sit-to-stand, and hand grip strength.

- *Aim 2. To assess gene expression and skeletal muscle perfusion in relation to the different training regimens:* In this aim, we examine the change from baseline to post-exercise regimen in the expression of proteolytic (FoxO and Ubiquitin) and anti-proteolysis (IGF-1 and PGC-1α) genes as predictors of the change in functional outcomes (peak VO_2, 1RM) over the same period. Separate general linear models for each functional outcome will be performed with gene expression changes as the primary predictor and the following variables considered as potential confounders: age, sex, and comorbidities. As above, a 10% rule for introducing confounding variables and the transformation of outcome variables to deal with regression assumption violations will be considered.

Treatment regimens will also be categorized as those with and without direct skeletal muscle stimulation and this categorical variable will be considered as a predictor of a change in the expression of proteolytic and anti-proteolysis genes in general linear models in a similar fashion to that described above. In addition, similar general linear models will be used with changes in skeletal muscle perfusion as a continuous outcome and aerobic and inspiratory training vs. strength-only training as a categorical predictor with similar potential confounders as noted above.

Secondary analyses will include assessment of the relative impact of changes in skeletal gene expression vs. changes in perfusion dynamics as predictors of changes in function and quality of life as outcomes. Measures of changes from baseline in serum inflammation, cytokines, and adipokines, and effects of muscle histology will be included as covariates in these analyses. General linear models will be used (outlined above).

Sample size/Power: In this study, we plan to recruit 210 individuals and assign them to one of 4 treatment arms, i.e., randomized between aerobic, aerobic strength, and IMT in VAPHS and between aerobic and inspiratory muscle training in Boston. Sample size calculations are based on 20% attrition over the course of the study. In general, a sample size of 40 per group, using a t-test of independent samples as a simplified model, results in an effect size of 0.64 SD units for 80% power, using a two-sided alpha of 5%.

- *Aim 1*: For this aim, we present calculations for detecting differences in peak VO_2. Previous work by Becker (108) shows a 2.1 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ change in peak VO_2 in a 6 month aerobic-strength training regimen. Similarly, Tyne-Lenne, et al. shows a 1.1 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ change in a strength-only regimen (109), HF-ACTION (85) shows a 0.6 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ change in an aerobic regimen and the IMT trial by Dall'Ago et al. (12) shows a 4 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ change with inspiratory training. Standard deviations [SD] of the change in peak VO_2 over an exercise regimen are not directly available but using interquartile range values from the largest study [HF-ACTION, n=1442], we obtain a SD value of 2.2 for a 3-month intervention. This would correspond to detecting a difference of 1.26 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ in any comparison with 80% power, assuming a SD=2.2.
- *Aim 2*: In a similar fashion, a simplified comparison of those with gene expression below the median vs. those above the median would be able to detect a difference of 1.26 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ in peak VO_2 with 80% power. Analyses of combined training groups facilitates capacity to detect smaller differences between groups.

Study Data Management and Quality Control: Prior to the initiation of the study, all investigators and research staff involved in clinical activities will meet to review study procedures and will complete all required trainings on data and patient privacy procedures. Documentation of any protocol breaches will be required. Each protocol violation will be evaluated by the principal investigator, and a determination made regarding the validity of any justification for the violation.

The clinical database with all research data will be stored on VA network drives and housed behind the VA firewall on VA owned and maintained servers, which are backed up on a regular schedule. All computers will have Kerberos passwords and come equipped with antivirus software. Data will not be stored on desktops or on non-VA servers. Study data will be coded with a unique study identifier for each participant and stored in a de-identified manner. A study data set without identifiable information will be used for all data analysis. Identifiable information will be collected for patient tracking and safety purposes, but will only be kept for as long as the study is active. De-identified clinical data will be stored separately from the participant's name, contact information, and real SSN. Access to the cross-walk file linking the participant's identifiers and their study data will be restricted to the project manager. This file will be destroyed according to CSP policy after the study ends. Study data will be retained and stored after the study ends according to federal and local VA regulations. Access to the study data is heavily restricted to individuals with approval from the principal investigator to access the data. Individuals must be properly credentialed research staff and must be compliant with VA security trainings (i.e., Research Data Security, HIPAA and VA Privacy Training, Cyber-Security, and Good Clinical Practices).

Dissemination and/or Implementation Plan: The findings of this study will be of key interest in a variety of scientific and clinical venues. Abstracts and associated manuscripts will target the molecular biology, HF, and exercise research communities. Initial presentations/publications will include orientation to molecular scientists with focus on cellular physiology and genetic components in cellular regulation as well as the outcomes of training differences. We will also report on impact of atrogenes, PGC-1α and other regulatory factors to predict outcomes and/or guide management, issues of likely interest to HF and cardiac rehabilitation physicians. Dr. Forman and his team of investigators will participate in related meetings, particularly the American Heart Association (AHA). Drs. Forman, Schulze, Brown, Bhatt, Stevenson, Arena, Cahalin, Lecker, and Joseph, as well as the added investigators (Drs. Gottlieb and Baynard) already regularly participate in the AHA as part of their careers. Likewise, the investigators participate in the American College of Cardiology, the American College of Sports Medicine, the HF Society of America and other laudable venues as part of their careers. Dissemination of the data/conclusions from this study will flow from these preexisting patterns.

Limitations: The goal to recruit 210 systolic HF patients, inclusive of men, women, and minorities is an inherent challenge. We are confident that with our project modification into a multisite trial. A related limitation is the potential attrition of patients over an extended training trial. Illness and/or life stresses can potentially exacerbate attrition. Dr. Forman developed considerable experience with these types of dynamics in HF-ACTION (a large training trial in which there was only 5% attrition), and he will work closely with the study team to maintain subjects' adherence. Financial incentives will be helpful, but it became clear in HF-ACTION that the Principal Investigator was the most important factor in maintaining patient participation. Steps will also be implemented to sustain participation in the event of illness, accidents, or travel.

Patients can also dilute the training effects if they modify their regimen by doing supplemental exercises at home (e.g., adding aerobic exercises by those in the strength training arm). We will emphasize the priority of maintaining precise training regimens, and will offer patients the chance to cross-train as soon as the formal protocol is completed.

A different type of logistic limitation relates to technical limitations of PCR analysis. We found that some of the initial samples did not produce meaningful data and these subjects had to be removed from analysis. We became extremely proficient in biopsy techniques and tissue processing, minimizing any chance that our handling will contribute to these possibilities.

From a scientific perspective, limitations relate to the fact that many clinical confounders can potentially obfuscate the effects of gene expression on functional capacities. We were keenly aware of this problem in our pilot investigation when the theoretical concept of "healthy controls" was hampered by the high prevalence of comorbidities. Nonetheless, we still showed the significant correlation of FoxO with disease, reinforcing our confidence the signaling differences remain striking aspects of HF. Yet since we still appreciate the potential for confounders to detract from our findings, in this protocol, we have increased the size of the population as a key means to increase the power of analysis. Dr. Gagnon, a senior statistician now working regularly with our team, has been exploring concepts of factor analysis and other sophisticated techniques to transcend some of the inevitable limitations related to confounders.