REDUCED SKELETAL MUSCLE OXIDATIVE CAPACITY AND IMPAIRED TRAINING ADAPTATIONS IN PEOPLE WITH HEART FAILURE

by

WILLIAM MICHAEL SOUTHERN

(Under the Direction of Kevin McCully)

ABSTRACT

The purpose of this study was to measure muscle oxidative capacity in people with and without heart failure (HF) using near-infrared spectroscopy (NIRS). Participants (aged 45-70 years old) with HF (n = 16) were compared with age-matched controls without HF (n = 20). A subset of participants (HF: n = 7, controls: n = 5) performed 4 weeks of wrist-flexor exercise training. Oxidative capacity was significantly lower in the HF group in both dominant (1.31 ± 0.30 min^{-1} vs. 1.61 ± 0.24 min^{-1}, $p <$ 0.002) and non-dominant arms (1.29 ± 0.24 min^{-1} vs. 1.47 ± 0.25 min^{-1}, $p < 0.039$). After training, the control group exhibited a 51% increase in oxidative capacity, whereas the HF group failed to improve. The reductions in oxidative capacity seen with HF may result from impaired cardiac function or complications of standard pharmacotherapy.

INDEX WORDS: Oxidative metabolism, endurance training, near infrared spectroscopy, heart failure

REDUCED SKELETAL MUSCLE OXIDATIVE CAPACITY AND IMPAIRED TRAINING ADAPTATIONS IN PEOPLE WITH HEART FAILURE

by

WILLIAM MICHAEL SOUTHERN

B.S., North Greenville University, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTERS OF SCIENCE

ATHENS, GEORGIA

2014

REDUCED SKELETAL MUSCLE OXIDATIVE CAPACITY AND IMPAIRED TRAINING ADAPTATIONS IN PEOPLE WITH HEART FAILURE

by

WILLIAM MICHAEL SOUTHERN

Major Professor: Kevin McCully

Committee: Kent Nilsson
Jonathan Murrow
Ellen Evans

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2014

DEDICATION

I would like to dedicate this work to my wonderful wife, Lauren, my parents, Bill and Molly Southern, my older sister Ashley, younger brother Brandon, and my grandparents John and Joanne Putney. Lauren, thank you for your love and support you have given me through the long nights and countless hours of work put into this project. Dad, thank you for instilling in me the value of hard work and determination, your example has been my standard. Mom, your generous love, patience, and support have guided me through the years; thank you for committing so much of your time to teaching me and cultivating my curiosity. Ashley, the high standard you carry in your academic pursuits has always motivated me to work harder to become a better student. Brandon, growing up along side of you, I have always been inspired by your perseverant determination to aspire to new heights in both life and athletics. Papa and Grammy, thank you for all the wisdom and guidance that you have imparted to me through the years, so much of who I am today, I owe to your loving influence.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Kevin McCully. Your passion and enthusiasm for science continuously inspires me to achieve higher and dig deeper. The past two years spent in your lab, have been some of the hardest but most rewarding years of my life. Furthermore, despite your tireless dedication to science, it is obvious that your concern for your students and others around you is always paramount, which is a rare quality among mentors.

I would also like to thank my former fellow graduate student Dr. Terence Ryan, our lab coordinator Jared Brizendine, and my fellow graduate students, Zoe Young, Melissa Erickson, MaryAnn Reynolds, and Kristine Polley. Terence, your friendship and insightful guidance constantly motivate me to aspire to higher standards. Jared, thank you for your steadfast friendship both inside and outside the lab, it is always a joy to discuss both science and philosophy with you. Zoe, Melissa, MaryAnn and Kristine, it has been a pleasure to work with each of you, your lighthearted attitudes always bring a smile to my face. As a special note to both Terence and Jared, thank you for introducing me to the wonderful combination of chocolate and coffee, the mocha. This beverage was instrumental to the success of this project.

I would also like to thank Dr. Kent Nilsson, and Dr. Jonathan Murrow. Your hard work and intellectual insight has made this project possible. Finally, I would like to extend thanks to Dr. Nathan Jenkins who has become a welcome addition to our lab family. Dr. Jenkins, I value your input and desire your enthusiasm.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Heart failure (HF) is a widespread and continually growing epidemic (57, 73, 74). Worldwide, HF is generally present in 1-2% of the adult population, with a much higher prevalence (>10%) in adults over 70 years of age (73). Data from NHANES 2005-2008 reported approximately 2% (5,700,000 Americans) of the United States population self-reported having HF, and projections for 2030 estimate an additional four million Americans (3.2% of population) will develop the disease (74). HF has been defined by functional impairments or structural abnormalities in heart, which limit the heart's capacity to supply adequate oxygen to systemic tissue (73). This condition could result from a number of other health complications. Approximately two-thirds of HF cases have been attributed to coronary artery disease (73), with hypertension and diabetes likely contributing as well (73). Furthermore, HF has been associated with a high mortality, as one in nine deaths were reportedly associated with HF in 2008 (74).

As a result of the widespread prevalence and high mortality associated with HF, it has attracted considerable attention from research communities in order to further understand the pathology of the condition. Reduced exercise capacity has been a central characteristic of HF that has been extensively investigated (28, 76). Originally, cardiac and hemodynamic dysfunction was thought to be the primary cause of exercise intolerance in HF. The effects of chronic HF on the cardiac tissue have been well documented, and it would be natural to assume that abnormalities in the heart were the

source of exercise intolerance in HF. Common HF-mediated alterations in the heart tissue have included left ventricular remodeling (46), decreased ejection fraction, and systolic dysfunction (73). Moreover, changes on the cellular level have also been present such as disruptions in calcium handling, sarcomere function, and oxidative metabolic pathways, which have led to shifts in substrate utilization (53, 117). Interestingly, however, research has shown that reduced exercise capacity in HF does not correlate with cardiac function (31), which suggested that diminished exercise capacity in HF was not due exclusively to cardiac or hemodynamic dysfunction, as previously thought. In fact, exercise intolerance has been shown to be more severe than cardiac function would predict (122), indicating alternative mechanisms might be responsible for the reduction. Research has shown that abnormalities in the skeletal muscle rather than myocardium are largely responsible for reduced exercise capacity in HF (21, 23, 76).

Many skeletal muscle alterations have been found in people with HF, including reduced metabolic oxidative capacity, (23, 68). These findings have caused investigations to focus on skeletal muscle mitochondria. Interestingly, despite multiple investigations related to this topic, there has been no universal consensus regarding the role that mitochondria play in the exercise intolerance in HF (76). Some research has shown that muscular oxidative capacity was impaired due to a HF-induced myopathy (23), whereas other research found that oxidative capacity was not impaired if muscular physical activity was experimentally controlled, (75). Additional investigations are necessary in order to further elucidate the role of skeletal muscle mitochondria in exercise intolerance in HF.

Additionally, while individuals with HF have a low exercise capacity, studies have shown exercise training in HF results in restorative adaptations marked by improvements in both peak VO_2 (17, 59) and skeletal muscle myopathies, including impaired muscle oxidative capacity (8, 78). Few studies, however, have focused specifically on skeletal muscle mitochondria in HF and evaluated their plasticity in response to training. Understanding how mitochondria in HF respond to exercise training may provide more insight into their possible connection reduced exercise capacity.

Previous investigations of skeletal muscle oxidative capacity in HF have been limited to methodologies such as biopsies and magnetic resonance spectroscopy (MRS). While, these methodologies have provided useful information regarding the oxidative health of muscle in HF, extensive use in research is limited. Biopsies are able to provide an in vitro view of the oxidative properties of the muscle, but its invasive nature often makes it unsuitable for testing the HF population. Alternatively, MRS is has the ability to examine the in vivo energetic capacity of muscle, but it is very expensive and often scarcely at the disposal of researchers.

Near-infrared spectroscopy (NIRS) is a useful technology that has been used to noninvasively characterize oxygen levels in both brain and muscle tissue. Recently, a novel application of the NIRS technology has been developed to measure oxidative capacity in the skeletal muscle (11, 98). This method's noninvasive and relatively inexpensive nature, make it an ideal choice for investigation of skeletal muscle oxidative capacity in HF. Additionally, this technique has been successfully validated against the in vivo 'gold standard' MRS (101), which further demonstrates that it is a viable alternative for measuring skeletal muscle oxidative capacity in HF. Development of the

NIRS method has provided researchers with a feasible and effective way to explore skeletal muscle mitochondria. Previously, NIRS has been to track changes in oxidative capacity throughout an endurance training and detraining program in the forearms of young healthy adults (100). The application of NIRS in a similar manner could potentially produce unique valuable insight into the complexities of HF previously unavailable.

Statement of the Problem

Overall, HF is a widespread problem that is linked with reduced exercise capacity. The origin of reduced function in individuals with HF is not related to impairments in the heart, but instead is closely linked to skeletal muscle abnormalities, such as decreased oxidative capacity. Mitochondria are thought to play a key role in this abnormality, but their contribution is still not entirely understood. If skeletal muscle mitochondria are fundamental to the reduction in exercise capacity, then perhaps mitochondria should be a primary target for HF therapy. NIRS provides an effective and inexpensive means of investigating skeletal muscle oxidative capacity, and therefore, could be very useful in exploring these problems. A study designed to specifically investigate oxidative capacity in HF using NIRS is necessary, as it would provide valuable information concerning the pathophysiology of the disease.

Specific Aims

Specific Aim 1: To measure the baseline skeletal muscle oxidative capacity in people with and without HF.

Specific Aim 2: To compare the magnitude of adaptation of skeletal muscle oxidative capacity in people with and without HF in response to 4 weeks of endurance training.

Specific Aim 3: To explore the relationship between oxidative capacity and physical activity level in people with and without HF.

Hypotheses

I. Oxidative capacity will be reduced in participants with HF compared to participants without HF.

II. The magnitude (% improvement) of training adaptation will be lower in individuals with HF than those without HF.

III. Reduced oxidative capacity will not be associated with reduced physical activity.

Significance of the Study

HF is a widespread condition, frequently associated with impaired skeletal muscle function in addition to impaired cardiac dysfunction. Understanding the relationship between skeletal muscle mitochondria and HF is important in designing specific therapeutic approaches to increase the quality of life of those living with HF. NIRS is a new noninvasive approach that offers a unique view of the mitochondrial quality of skeletal muscle. This study will be the first to make direct comparisons regarding

mitochondria capacity between people with and without HF using NIRS. The information obtained from this study will potentially lead to better clinical treatments and improvements in the quality of life of people who have HF.

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

Prevalence and Epidemiology of Heart Failure

Heart failure is a complex clinical syndrome comprised of signs and symptoms related to inadequate oxygenated blood delivery to the organs of the body (74, 81). The most common form of HF is known as diastolic HF or preserved left ventricular ejection fraction. In this form of HF, left ventricular function is maintained, while diastolic filling is limited resulting in reduced cardiac output. Left ventricular systolic dysfunction or reduced left ventricular ejection fraction is a less common form of HF, where functional and structural alterations in the myocardium result in diminished left ventricular function. Coronary artery disease (CAD) is presumed to be responsible for approximately two-thirds of all HF cases, but hypertension, diabetes and possibly other structural or rhythmic abnormalities in the heart could contribute as well (74).

This condition is a growing concern world-wide, as approximately 1-2% of the adult population in the western world have been diagnosed with HF (81). Affecting over 6 million individuals in the US alone, with 825,000 new diagnoses occurring every year, this condition has generated a massive economic burden amassing over $30.7 billion in HF-related expenses in 2012 (35). Moreover, prevalence of HF rapidly escalades with age, increasing from 0.7% at 45 years of age to 8.4% in individuals over the age of 75 (95). Recent reports indicate that HF is also the leading cause of hospital discharge in persons over the age of 65 (37).

A critical yet challenging aspect of HF is diagnosis. Signs and symptoms associated with HF are often used to diagnose patients with HF. Some signs of HF include edema, tachycardia, heart murmurs, displaced apex beat, and cachexia. Common symptoms, on the other hand, may include orthopnea, nocturnal cough, dyspnea, reduced exercise tolerance, and fatigue. In conjunction with a patient's signs and symptoms, each patients can be assigned a HF class reflective of their disease severity. The New York Heart Association (NYHA) defined a functional classification system formulated from severity of HF symptoms and physical activity (74). The NYHA classes of HF are outlined below:

- Class I – No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.
- Class II – Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
- Class III – Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in undue breathlessness, fatigue, or palpitations
- Class IV – Unable to carry on any physical activity without discomfort. Symptoms at rest can be present. If any physical activity is undertaken, discomfort is increased.

Overall, it is clear that HF is a complex clinical condition affecting many people worldwide. Furthermore, HF has many detrimental effects on an individual's health, and

as discussed in the following sections, the effects of this condition extend far beyond the functional impairments in the heart.

Quality of Life of People with Heart Failure

It has been consistently demonstrated that people with HF have reduced quality of life (QOL) (25). Both Hoekstra et al. (48) and Dracup et al. (20) reported reduced QOL in people with HF. Juenger et al. used the SF-36 survey to compare the QOL of healthy individuals to those living with HF (58). The authors found that individuals living with HF were significantly lower than controls in all aspects of QOL. Not surprisingly, the reductions in QOL were greater with worsening disease symptoms (i.e. higher NYHA HF classes). Furthermore, the authors found two important factors that may interact with or contribute to lower QOL in HF: reduced functional capacity and reduced exertional capacity.

Many previous studies have reported diminished functional capacity in HF. Sparrow et al. performed a study in which patients with HF performed a 9-minute walking test followed by a VO_2 max test (110). The authors reported that the walking distance in HF, for all disease severity levels, were lower than healthy controls, clearly illustrating a deficit in functional capacity. These findings are consistent with previous studies that have linked HF with reduced functional capacity (10, 20, 48, 56).

Concurrent with decreased functional capacity, people with HF also have reduced exertional or exercise capacity. Lower peak VO_2 values, decreased walking distance, and early onset of fatigue during exercise have been consistently reported throughout the literature (15, 45, 119, 120). In a study performed by Weber et al., VO_2, stroke volume,

and cardiac output were assessed in HF patients (119). The authors found that increasing severity of HF corresponded with greater exercise intolerance, which was indicated by reduced exercise time, peak VO_2, cardiac output, and stroke volume. Clark et al. reported that fatigue and dyspnea during exercise are primary symptoms of and contributors to exercise intolerance in people with HF (15). Using a standard Bruce protocol to assess peak VO_2, Harrington et al. found significant reductions in peak VO_2 in patients with HF (45). Additionally, they also found greater muscle fatigue and weakness in HF compared to healthy controls.

Exercise Intolerance Unrelated to Cardiac Function in Heart Failure

Interestingly, there is a discrepancy between the severity of exertional intolerance and the extent of cardiovascular impairments in people with HF. Conventional hypotheses proposed that exercise intolerance in HF was purely the result of underperfusion in the skeletal muscle due to decreased cardiac output. A study performed by Wilson et al., measured leg blood flow and oxygen extraction in patients with HF while performing exercising on a cycle ergometer (122). The authors concluded that impaired blood flow to the working muscle was the primary contributor to exercise intolerance in HF.

However, further research has demonstrated that reduced cardiac output and impaired hemodynamics during exercise do not entirely account for the exertional intolerances found in HF. Francosa et al. found that exercise intolerance in people with HF did not correlate with functional cardiac outcome measures (31). Using ^{31}P magnetic resonance spectroscopy to evaluate muscle metabolism during aerobic and ischemic

exercise, Massie et al. found that muscle metabolism was impaired (72). The authors concluded that the irregular skeletal muscle metabolism could not be explained by diminished blood flow alone. Similarly, Wilson et al. performed a study and reported that 26% of the HF patients had significant reductions in exercise capacity despite normal blood flow (121). The authors of this study concluded that exercise intolerance in these patients could not be completely explained by underperfusion of the working muscle. Instead, the authors proposed that impaired skeletal muscle is an important contributor to exercise intolerance in HF.

Altogether, HF-induced reductions in exercise capacity seem to be linked with extreme deconditioning of the skeletal muscle rather than cardiovascular dysfunction. A review published by Coats et al. proposed that the functional and exertional intolerances seen in HF originate in the skeletal muscle (18). Additionally, Drexler and colleagues several years later published an extensive review, which attributed skeletal muscle dysfunction to be the primary cause of exercise intolerance in people with HF (22). The authors discussed vascular and metabolic impairments in skeletal muscle, while disparaging the conventional cause of fatigue in HF, namely cardiac and hemodynamic dysfunction. In a more recent review, Nilsson et al. proposed the term "hemodynamic paradox" to explain how alterations in skeletal muscle rather than compromised myocardium or hemodynamics contribute to function-limiting symptoms (83). Similarly, Maurer et al. published a review in 2012, aiming to bring the focus of research to from the heart to the skeletal muscle (73).

Reduced exercise capacity presents a conundrum for people with HF, as exercise is very strenuous for patients with HF, but it is essential to reverse the detrimental effects

of physical inactivity and HF on the skeletal muscle. Thus, research has focused on investigating the mechanism driving the impairments found in HF. Originally, exertional intolerances were hypothesized to be the result of cardiac dysfunction, reducing blood flow to the working muscle. However, further research has shifted the focus from the heart to alterations in skeletal muscle as the primary contributors to exercise intolerance in HF.

Skeletal Muscle Abnormalities in Heart Failure

There is a considerable volume of research that has investigated the role of skeletal muscle in functional and exercise intolerance in people with HF. Collectively, this research has discovered a number of alterations that occur in the skeletal muscle of people with HF, including skeletal muscle atrophy, change in fiber type, reduced blood flow, reduced capillary density, and reduced muscle metabolism. Each alteration is briefly discussed in the following sections.

One possible symptom HF is muscle wasting, which has been termed cardiac cachexia (4, 5, 33, 69). In an early study, using magnetic resonance imaging, Mancini et al. found ~68% of the participants with HF had reduced skeletal muscle mass compared to normal individuals (69). Furthermore, Anker et al. established that cardiac cachexia in HF is a strong independent risk factor of mortality in HF (4). More recently, in a study of 200 participants with HF, Fulster et al. found that ~20% met the criteria for muscle wasting (33). These patients were found to have lower body weight, BMI, handgrip strength, and quadriceps strength compared to patients not in a cachectic state.

In addition to muscle atrophy, skeletal muscle in people with HF has been shown undergo a shift in fiber type from type I to type IIb. Mancini found evidence of increased percentage of type IIb and atrophy of type IIa fibers in the calf muscle (68). Similarly, Drexler et al. obtained biopsies of the vastus lateralis and found that the fiber type ratio in normal participants was ~55% Type I to 45% Type II, whereas in HF, the distribution was ~40% Type I to 60% Type II (23). These findings are consistent with other similar studies (26, 104, 112).

Research has also found that peripheral skeletal muscle blood flow is reduced in people with HF. Zelis et al. found a ~43% reduction in resting blood flow in the forearms of people with HF (126). Sullivan et al. reported similar results in the legs of people with HF (113). In addition to group differences in blood flow at rest, both studies (113, 126) found that during exercise differences between groups were heightened as exercise intensity progressed. Several similar studies have reported diminished peripheral blood flow during exercise as well (62, 64). It should be noted that some studies did not find any differences in blood flow at rest (62, 116) or during exercise (71). This is not surprising, however, as blood flow can be influenced by many factors such as skin and room temperature, breathing rate, peripheral resistance, etc.…

Skeletal muscle capillary beds are critical junctures in the delivery of oxygen to the working muscle. A reduction in microvasculature density could limit oxygen delivery to the muscle during exercise. Duscha et al. showed ~21% reduction in the number of endothelial cells per muscle fiber in skeletal muscle of people with HF (26). Similar results have been found in animal models of HF (85). Interestingly, a link may exist between the level of severity of HF and the amount of microvascular rarefaction in

the muscle (52). Houben et al. (52) discovered that severe HF (i.e. NYHA III-IV) had significantly greater microvascular rarefaction of the eye and skin nailfold than mild HF. This alteration could be a key factor involved with exercise intolerance in people with HF.

A critical alteration found in the skeletal muscle of HF is reduced muscle metabolism. Research has consistently found evidence pointing to a reduction in the muscle's capacity for oxidative energy production. Several in vivo studies of people with HF have found greater phosphocreatine depletion and early acidification during all levels of exercise when compared to healthy controls (70, 71). Using magnetic resonance spectroscopy, Massie et al. found an imbalance between energy usage and energy production within the skeletal muscle, as evidenced by higher Pi/PCr levels during exercise (70). Recovery of phosphocreatine (PCr) after exercise is directly proportional to oxidative capacity of the muscle, and Mancini et al. reported that PCr recovery kinetics in HF was ~68% slower than healthy controls, indicating impaired skeletal muscle oxidative capacity (69).

In vitro studies have also found evidence of reduced oxidative capacity in the skeletal muscle of people with HF. Research has shown that activity of oxidative enzyme was diminished in HF (23, 76, 104, 112). Drexler et al. clearly showed that volume density of mitochondria in the skeletal muscle of HF was abated compared to sedentary controls (23). Furthermore, these authors also found that the mitochondrial density was positively correlated with peak VO_2 in HF. Taken together, this research points to impairment in mitochondrial function in skeletal muscle of HF.

All the factors mentioned in this section are important contributors to skeletal muscle complications in HF. Not surprisingly, each alteration has been correlated with diminished exercise capacity in HF. Therefore, it is likely that the combination of these factors creates the skeletal muscle myopathy associated with HF, thereby reducing exercise capacity in HF.

Controversial Role of Muscle Oxidative Capacity in Heart Failure

It is clear from previous literature that skeletal muscle metabolism is reduced in HF and most likely contributes to exercise intolerance in HF. A plausible mechanism for this reduction is diminished oxidative capacity in the skeletal muscle. Both Drexler et al. and Mettauer et al. demonstrated that muscle oxidative capacity was correlated with peak VO_2. Furthermore, as previously discussed, several studies have shown reductions in skeletal muscle oxidative metabolism. However, despite these findings, there is not universal agreement in the literature concerning the extent to which muscle oxidative systems may actually contribute to reduced exercise tolerance in HF. Mettauer et al. discovered that while peak VO_2 and skeletal muscle mitochondrial enzymes were reduced in HF, the intrinsic function of the mitochondria was no different than sedentary controls (76). Toth et al. reported similar results (115). When HF patients were compared to physical activity matched controls, no differences between HF and controls in expression of mitochondrial transcription regulators, cytochrome oxidase subunits, or activity of mitochondrial and cystolic enzymes were found. Results by Chati et al. further support this hypothesis (14). The authors found PCr depletion was not different between sedentary controls and HF implying that physical deconditioning could a

primary contributor to decreased oxidative capacity in HF. Collectively, these results suggest that mitochondrial disruption in the skeletal muscle of HF does not directly cause exercise intolerance in HF, but rather is a byproduct of muscular inactivity and deconditioning. Nevertheless, further research is needed in this area to better understand the relationship between muscle oxidative capacity and exercise intolerance in HF.

Exercise Training in Heart Failure

Until the late 1980's, exercise training in HF was not universally considered to be a safe or beneficial treatment for people with HF. However, in recent decades, exercise training in HF has been shown to be an effective and feasible approach to aid in treating HF patients (8, 16, 17, 59, 90). Studies have found that exercise training in HF is beneficial for the myocardium and left ventricular function (24, 34, 42), capable of reversing metabolic and vascular abnormalities in skeletal muscle (2, 40, 50, 79), and can potentially improve QOL and reduce mortality in HF (8, 86).

A meta-analysis of exercise training in HF (ExTraMATCH) found that exercise training was safe and effective means of reducing mortality in HF (90). Similarly, in a more recent controlled trial (HF-ACTION), the effects of exercise training were evaluated in 2331 medically stable HF patients (86). This study found that an intervention of 36 supervised training sessions followed by ~30 months additional home-based training resulted in a modest reduction in all-cause mortality in people with HF. QOL and functional capacity can also be significantly improved through exercise training, as demonstrated by Belardinellie et al., through a 14-month training program in

99 HF patients (8). Overall, contrary to historic recommendations, exercise training is safe and can reduce the risk of adverse clinical events.

While exercise training in HF may only lead to modest reductions in mortality, research has shown it to be beneficial in reversing many cardiovascular and skeletal muscle abnormalities. Improvements in peak VO_2 after exercise training have been consistently observed (59). A meta-analysis published by Rees et al. showed an overall improvement in peak VO_2 of 2.16 ml/kg/min after exercise training in HF, with more intense exercise bouts leading to greater improvements in peak VO_2 (96). These results are consistent with a meta-analysis published by Hwang and Marwick who found an increase of 2.86 ml/kg/min in peak VO_2 (54).

Significant ultrastructural and histological changes accompanied with muscle wasting are common in HF, reducing the strength and functionality of the skeletal muscle. However, exercise training has been shown to be effective in reversing HF induced skeletal muscle functional and morphometric alterations. In a study by Magnusson et al., two groups of HF patients were randomized into either a unilateral endurance or a unilateral strength-training program (65). The strength-trained group was found to have increased cross-sectional area, and higher maximal dynamic and isometric strength, whereas the endurance trained group was found to have increased oxidative enzyme activity and increased capillary density per muscle fiber. Interestingly, no changes were detected in peak VO_2 after training in either group, demonstrating improvements in exercise capacity independent of central adaptations. Gordon et al. performed a similar study, and found that overall endurance as well as oxidative enzyme

activity increased independent of peak VO_2 after 8 weeks of strength training (36). The more recent work of Pu et al. has confirmed these results (91).

Furthermore, in conjunction with functional and central adaptations, exercise training has also been responsible for correcting many metabolic and hemodynamic alterations within the skeletal muscle. Hornig et al. has shown that 4 weeks of handgrip training can restore endothelial function, as evidenced by a dramatic improvement in endothelial-mediated, flow-dependent dilation (50). Hambrecht et al. found similar results in the thigh after a six-month home-based training program (40). After training, endothelium-dependent vasodilation of femoral artery blood flow was significantly improved from baseline, confirming a restorative effect of exercise training on skeletal muscle endothelial function in HF.

Metabolic dysfunction in the skeletal muscle of HF is a paramount characteristic of HF and perhaps a key to improvements in exercise capacity following exercise training. Adaptations to exercise training in muscle metabolic capacity have been well documented from both in vivo and in vitro methodologies. Using magnetic resonance spectroscopy, Adamopoulos et al. found that 8 weeks of cycling training can improve skeletal muscle metabolism in HF (2). After training, the authors found less PCr depletion, less ADP accumulation, and smaller changes in pH during exercise compared to untrained controls, indicating clear metabolic adaptations. In another study, HF patients underwent four weeks of forearm exercise, which resulted in significant improvements in muscle metabolism as indicated by reduced PCr depletion during exercise (79). Several in vitro studies have shown comparable improvements in response to endurance training in people with HF. Hambreich et al. published multiple studies

demonstrating the positive effects of exercise training on skeletal muscle mitochondria (41, 43). The first study (43) found a 19% increase in the total volume density of mitochondria and a 41% increase in cytochrome c oxidase after six months of cycling training. The second study (41) yielded a 41% increase in cytochrome c oxidase, 43% increase in surface density of mitochondrial cristae, and a 92% increase in mitochondrial inner border membrane. Similarly, Belardinelli et al. found a 22% increase in in the volume density of mitochondria after two months of low intensity cycling training (8). Taken collectively, these findings indicate that skeletal muscle metabolism is capable of adapting to local or whole body exercise training.

Pharmacotherapies and Muscle Oxidative Capacity

People with heart failure are often prescribed a vast array of medications to help treat their various conditions (61). Interestingly, some of these medications, including statins and metformin, have been associated with oxidative disruption in skeletal muscle. Statins in particular have been linked with multiple muscle abnormalities, including impaired muscular oxidative capacity (88, 89, 106). Less has been reported about metformin, but some evidence has suggested that it may also alter muscle metabolism.

Beta-adrenergic receptor antagonists (β-Blockers) are a class of drugs commonly prescribed for treatment of cardiovascular conditions. These drugs serve as antagonists to circulating catecholemines by blocking β-adrenergic receptor stimulation in the heart, blood vessels, and lungs (32). β-Blockers have been one of the recommended primary prevention drugs to control and treat hypertension (67). In addition, β-Blockers have been shown to be beneficial for individuals with HF, by providing secondary prevention

of additional myocardial infarctions and reducing mortality (47, 105). Although β-Blockers have been widely recommended for both primary and secondary prevention of cardiovascular events, recent evidence has questioned their effectiveness regarding prevention and mortality (6, 128). In addition, some research has suggested that beta-blockers could be toxic to skeletal muscle and may be associated with myopathies, including impaired skeletal muscle oxidative capacity and mitochondrial biogenesis. Wolfel et al. investigated the effects of β-Blockers on six weeks of aerobic training and found that VO_2 peak and oxidative enzyme activity was significantly attenuated in individuals on β-Blocker therapy compared to controls (125). Interestingly, Miura et al. found that when β-Blockers were administered to mice prior to exercise, proliferator-activated receptor-γ coactivatior-1α (PGC-1α) expression was diminished following exercise (80). This data suggested that PGC-1a expression was adversely affected by β-Blocker treatment. Taken together, this evidence has suggested that mitochondrial biogenesis could be detrimentally affected by administration of β-Blockers.

HMG-CoA reductase inhibitors or statins are a class of drugs used to treat high cholesterol, which has been associated with increased risk of cardiovascular disease. Statins target and inhibit the enzyme 3-hydroxy-3methyl-glutaryl-coenzyme A reductase, which a key enzyme in the cholesterol production pathway in the liver (55, 111). Statins have been shown to be beneficial in people with HF, as statin use was associated with a reduction in mortality (49, 51, 82, 94), as well as other important outcomes (107, 108, 116, 127). Statin use in the United States has been surging over the past several decades, increasing from 2.4% of the population in the years 1988-1994 to 25% in the years 2005-2008 (1). Therefore, not surprisingly, prevalence of statin therapy in HF has also been

increasing (93). While statins have multiple beneficial effects and are generally well tolerated, evidence has shown that they were associated with adverse effects on muscle health in some users. Several studies reported reduced muscle strength and altered muscle oxidative capacity in statin users (88, 89, 106). Paiva et al. (88) found that eight weeks of high dose statin treatment reduced activity of respiratory complexes II-IV as well as citrate synthase activity in skeletal muscle. The authors note that no difference from baseline was found if complex activity was normalized to citrate synthase activity. The authors concluded that mitochondrial function was unaltered, and instead proposed that statins reduced muscle mitochondrial number or volume. In a detailed review, Robinson et al. (97) discussed how statin therapy was linked to mitochondrial-induced cellular apoptosis (9), reductions in mitochondrial content (88), and muscle fiber atrophy (44). Furthermore, in a recent investigation, Mikus et al. showed that statin use impaired adaptations to exercise in overweight and sedentary subjects (78). After 12-weeks of treadmill walking/jogging, individuals on statins exhibited impaired adaptations in both peak VO_2 and citrate synthase activity. The authors concluded that statins were responsible for attenuating muscle oxidative capacity and cardiorespiratory fitness. Overall, statins therapy could be cause detrimental to the muscle, resulting in muscle weakness, atrophy, and oxidative disruption through diminished mitochondrial number or volume.

Other medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and metformin have the potential to interfere with skeletal muscle oxidative capacity as well. NSAIDs are a class of drugs commonly used to reduce pain and inflammation. The efficacy of these drugs in people with cardiovascular disease has been challenged, as

some data has suggested the NSAIDs can actually augment an individual's risk for myocardial infarction or stroke (114). Furthermore, these drugs have been linked with muscle myopathies, including increased mitochondrial-induced apoptosis, uncoupled oxidative phosphorylation, and reduced post-exercise protein synthesis (97). Investigations that have, and adverse effects may only be associated with unnaturally high doses of NSAIDs. Further investigations are needed to confirm the role of NSAIDs in human myopathies, as the majority of studies focusing on the effects of NSAIDS are limited to animal models.

Metformin is a medication commonly prescribed in order to help treat Type II diabetes, through increased insulin sensitization and blood-glucose control (60). Metformin use in HF has been controversial in the past, as the medication was contraindicated for individuals with HF. However, more recent evidence has reversed this determination and found positive outcomes individuals who concurrently have diabetes and HF (3, 29). Little research has focused on adverse musculoskeletal effects from metformin, but evidence has suggested that it could impair muscle oxidative capacity. For example, metformin therapy was shown to inhibit complex I of the oxidative respiratory chain (87), as well as potentially impair oxidative capacity in response to exercise (63). Further research is needed to better understand how metformin may interfere with muscle oxidative function.

Measuring Skeletal Muscle Oxidative Capacity with Near Infrared Spectroscopy

Near infrared spectroscopy (NIRS) is an evolving technology that has been used to measure oxygen levels in both the brain and muscle (39, 124). Near infrared light can

be sent into the tissue at wavelengths specific to the absorption properties of two chromophores: hemoglobin and myoglobin. Once inside the tissue, the light is scattered and absorbed. Light not absorbed by the hemogloblin and myoglobin is reflected out of the tissue and detected by the NIRS device. Changes in this signal represent relative changes in oxygen content within the tissue. NIRS has been used to measure many physiological aspects of skeletal muscle including, skeletal muscle blood flow (19, 84, 109), skeletal muscle oxygen consumption (19, 38, 66, 103, 117), oxidative capacity (13, 39, 99), and flow kinetics of reactive hyperemia (7, 30, 92).

More recently, NIRS has been used to investigate the oxidative capacity of skeletal muscle (99). Using repeated arterial occlusions after a brief bout of exercise, NIRS is able to measure the exponential recovery of mitochondrial oxygen consumption. The recovery data can be fit to a monoexponential curve to produce a rate constant, which is directly proportional to the oxidative capacity of the muscle.

This novel method of investigating oxidative capacity in skeletal muscle has demonstrated excellent reproducibility (99, 109) and has been validated against the 'gold standard', magnetic resonance spectroscopy PCr recovery (102). In a recent study, Ryan et al. demonstrated that the NIRS method was sensitive to change, as adaptations in oxidative capacity were successfully tracked over the course of a four-week endurance training program (100). The method has been shown to effectively measure oxidative capacity in several muscle groups, including the gastrocnemius, vastus lateralis, and forearm (12, 27, 100, 102, 109). The noninvasive nature of this approach provides an excellent advantage to research studies, affording an easy and painless way to measure oxdidative capacity. Moreover, this method provides a unique comprehensive in vivo

view of muscle metabolism that is not possible with in vitro methods, and is far less expensive than other in vivo techniques.

CHAPTER 3

REDUCED SKELETAL MUSCLE OXIDATIVE CAPACITY AND IMPAIRED TRAINING ADAPTATIONS IN PEOPLE WITH HEART FAILURE[1]

[1] Southern, W.M., Ryan, T.E., Nilsson, K.R., Murrow, J.R., and McCully, K.K. To be submitted to *American Journal of Physiology – Heart and Circulatory Physiology*

Abstract

Heart failure (HF) has been associated with exercise intolerance attributed in part to skeletal muscle dysfunction that is not fully accounted for by reduced cardiac output. The purpose of this study was to compare skeletal muscle oxidative capacity and mitochondrial training adaptations in people with and without HF using near-infrared spectroscopy (NIRS). Participants (aged 45-70 years old) with HF and implanted cardioverter-defibrillators (ICDs) (n = 16) were compared with age-match controls without HF (n = 20). A subset of participants (HF: n = 7, controls: n = 5) performed 4 weeks of wrist-flexor exercise training. Oxidative capacity was measured by performing repeated arterial occlusions to measure recovery kinetics of mVO_2 following a brief bout of wrist-flexor exercise using NIRS. Oxidative capacity was significantly lower in the HF group in both dominant (1.31 ± 0.30 min-1 vs. 1.61 ± 0.24 min-1, p = 0.002) and nondominant arms (1.29 ± 0.24 min-1 vs. 1.47 ± 0.25 min-1, p = 0.039). After four weeks of endurance training, controls showed a 51% improvement in oxidative capacity with training. Patients with HF did not show improvements in oxidative capacity with endurance exercise training, suggesting impairments in mitochondrial biogenesis. Further studies should investigate whether the blunted exercise adaptations are associated primarily with heart failure, or a complication of statin use.

KEYWORDS: oxidative capacity, endurance training, near infrared spectroscopy

Introduction

Heart failure (HF) is a complex clinical condition characterized by functional or structural alterations within the heart that lead to an inadequate supply of blood to the body's organs. This condition is widespread in the United States, affecting over six million individuals (38), and is also the leading hospital discharge diagnosis for individuals over the age of 65 (16). A well-known characteristic of HF is exercise intolerance, which interestingly does not correlate with cardiac function (12). Alterations in the skeletal muscle are frequently seen in HF (9, 40) suggesting that peripheral abnormalities in addition to central hemodynamic parameters contribute to reduced exercise capacity in HF. Examples of muscle alterations seen in people with HF include, muscle fiber atrophy (19, 30), shift in fiber type from type I to type IIb (29, 51, 53), reduced muscle metabolism (31-34), and reduced oxidative capacity (9, 30). Originally, muscle oxidative capacity was considered to be a primary contributor to reduced exercise capacity in HF. However, more recent research has challenged the role of mitochondria in exercise intolerance in people with HF (9, 39). Further investigations are needed to confirm the role of skeletal muscle mitochondria in exercise intolerance in HF, as this link could have important implications regarding recommendations of exercise rehabilitation in HF.

In addition to determining how mitochondria are involved in exercise intolerance in HF, it is important to also understand how mitochondria in HF might respond to exercise training. Mitochondrial biogenesis is a complex process that can be activated through multiple intracellular pathways. Repeated bouts of exercise induce cellular stress and ultimately result in up-regulation of mitochondrial transcription factors that promote

mitochondrial protein synthesis as well as increased activity and concentrations of mitochondrial enzymes (21, 59). While exercise has been shown to yield muscular oxidative improvements (1, 42), the capacity of improvement in people with HF has not been assessed. In fact, to our knowledge, no study has directly compared the magnitude of mitochondrial adaptations to endurance exercise training between people with and without HF. Exercise training has been recommended in people with HF as a safe and beneficial means of improving clinical, functional, and physiological outcomes (46). Therefore, it is important to understand how muscle mitochondria in people with HF may respond to exercise training, as deficits in the exercise response could reshape guidelines for exercise rehabilitation in people with HF.

Recently, near infrared spectroscopy (NIRS) has been used to measure skeletal muscle oxidative capacity (48, 50). NIRS has the ability to detect differences in oxidative capacity between groups (4, 49), and could be a useful tool to provide new and unique insight concerning mitochondrial dysfunction in people with HF. The primary aim of this study was to measure and compare baseline skeletal muscle oxidative capacity of people with and without HF. The second aim of this study was to measure the differences in the magnitude of oxidative adaptations to endurance exercise in people with and without HF. If reductions in oxidative capacity are linked to functional and exertional impairments in people with HF, mitochondria could be an important target for exercise and pharmaceutical therapies.

Methods

Participants

Sixteen patients with HF (13 male, 3 female), aged 45-70 years were recruited from a local cardiology clinic. Twenty healthy controls (3 male, 17 female), aged 52-67 years were recruited from the surrounding community. A subset of participants volunteered to participate in an eight-week long training program. One HF participant only completed the first five weeks of the program. Six controls completed the entire eight weeks of the training program, and one completed seven of the eight weeks of the program.

Individuals with HF were excluded from participation in the study if they had stable HF for less than three months, their initial ejection fraction was above 35%, and if they were considered New York Heart Association Functional Class I or IV. General excluding criteria for both groups included uncontrolled Type I or Type II diabetes, individuals who currently smoke, and those currently engaging in wrist-flexor training. Table 1 contains the participant characteristics for both the cross-sectional and training cohorts. This study was conducted with the approval of the Institutional Review Board at the University of Georgia (Athens, GA). All participants gave written, informed consent before being enrolled in the study.

Experimental Design

This study included both a cross-sectional and an intervention component. A subset of cross-sectional participants volunteered to participate in an eight-week exercise training study. NIRS measurements were performed on the wrist-flexor muscles of both the dominant (DOM) and non-dominant (nDOM) arms of all individuals (one HF participant

was under a time constraint and only one arm was measured). The wrist-flexor muscles were selected because they are not involved in locomotion, and, in contrast to other musculature of the body such as the calf or thigh, should be untrained and independent of physical activity levels. Participants enrolled in the cross-sectional study were required to only come in for one testing session. Participants enrolled in the longitudinal training study performed four weeks of wrist-flexor endurance training, followed by four weeks of detraining. The training was performed on the nDOM arm, which was considered to be the least active of the two arms, while the dominant (DOM) arm served as the control. NIRS measurements were performed every 7-8 days on both the nDOM and the dominant DOM wrist-flexors of all participants. Participants performed maximal voluntary isometric contractions (MVIC) every week to track changes in strength. Each individual was prescribed a training weight of ~15% of their MVIC.

Experimental Procedures

Participants were asked to lie supine on a padded table, with the arm to be tested extended 90 degrees from their body. The wrist-flexors (i.e. palmaris longus, flexor carpi ulnaris, and flexor carpi radialis) were located by palpation approximately 5-7 cm from the medial epicondyle of the humerus, and the NIRS device was placed directly over the musculature. For individuals in the training study, measurements from the medial epicondyle were made every testing session to aid in accurate device positioning from week to week. A blood pressure cuff (Hokanson SC5, Bellevue, WA) was placed on the upper arm, proximal to the elbow joint. The blood pressure cuff was attached to a rapid cuff inflating system (Hokanson E20 cuff inflator, Bellevue, WA) powered by a 30-

gallon commercial air compressor (Husky VT6315, Kenosha, WI). The NIRS testing sessions lasted for 20-25 minutes for each arm.

Exercise Training

The wrist-flexor training consisted of 16 total training sessions (four days/week for four weeks) on the nDOM arm only. Participants performed 30 minutes of continuous wrist-flexor exercise. During the first training session, a tolerable training frequency was determined for each individual. Every week, the training frequency increased as was tolerated by each individual. One supervised training session was performed each week following the NIRS testing session. During this session the new training frequency was determined. Each individual was then equipped with a fixed metronome and hand-weight in order to perform the remaining three training sessions at their home. After completing each home-based training session, each individual was required to call or email a report of the training to the study supervisor. After the 16 training sessions were completed, the participants were not allowed to engage in any wrist-flexor training for the remainder of the study.

NIRS Measurements

NIRS signals were obtained using a continuous wave NIRS device (Oxymon MK III, Artinis Medical Systems, The Netherlands). The device provides signals corresponding to the absorption wavelengths of oxyhemoglobin (O2Hb) and deoxyhemoglobin (HHb).

Resting blood flow measurements were made as previously described (57). Three venous occlusions (~65-70 mmHg) were performed for 10 seconds. Each occlusion was

separated by 10 seconds. Simple linear regression was used to calculate the slope of tHB during 7 seconds of venous occlusion (70 data points). The slope was used in the following equation to calculate absolute blood flow according the NIRS manufacturer recommendations:

$$BF = (((\Delta tHb \times 60)/(([Hb]\times 1000)/4))\times 1000)/10 \qquad \text{(Equation 1)}$$

In this equation, [Hb] was assumed for both males and females to be 15 g/100 ml, which was based on average hemoglobin concentrations of healthy individuals reported previously (2, 58). For the final analysis, the values from the three occlusions were averaged. Resting muscle oxygen consumption (resting mVO_2) was measured as the rate of decrease in $Hb_{difference}$ during an arterial occlusion (~250-275 mmHg) as previously described (48). Two 30-second occlusions with a 30-second rest between each were performed. Simple linear regression was used to calculate the slope using the first 20-25 seconds (200-250 data points). For the final analysis, the values from the two occlusions were averaged.

To normalize the NIRS signals, an ischemic/hyperemia calibration was performed as previously described (37, 52). The calibration included a brief bout of exercise (~3-5 seconds), followed by a ~3-5 minute arterial occlusion. Once the NIRS signals plateaued, the cuff was released and a ~1-3 minute period of hyperemia occurred. A 'physiological' range was calculated as the difference between the minimum (0% tissue oxygenation) and maximum (100% tissue oxygenation) NIRS values during the ischemia/hyperemia calibration. All NIRS signals were expressed as a percentage of this calibration range.

A measure of maximal oxygen reperfusion capacity was calculated from the hyperemia following the ischemic calibration occlusion. The elapsed time was calculated from when the occlusion was released to when the percent of O_2 saturation reached half of the peak hyperemic response. The time to half recovery was inversely related to the muscle's maximal reperfusion capacity. This measurement has been shown to correlate with other measures of reperfusion capacity as well as used as an index of vascular health in patients with peripheral vascular disease (6, 25).

To measure the oxidative capacity, a short duration exercise was used increase skeletal muscle oxygen consumption followed by repeated arterial occlusions to measure the rate of recovery of mVO_2, as previously described (5, 17, 44, 48). The series of arterial occlusions were performed in the following manner: occlusions 1-5 (3-5 seconds on/3-5 seconds off), occlusions 5-10 (7 seconds on/7 seconds off), occlusions 10-15 (10 seconds on/10 seconds off), and occlusions 15- (10 seconds on/20 seconds off). The occlusion protocol was designed to maximize our ability to characterize the kinetic changes in mVO_2, while minimizing any discomfort to the participants.

The post-exercise mVO_2 measurements, calculated as the slope of change in the $Hb_{Difference}$ signal during the post-exercise arterial occlusions, were fit to a mono-exponential curve according to the formula below:

$$y = End - Delta \times e^{-k \cdot t} \qquad \text{(Equation 2)}$$

In this equation, the y represents relative mVO_2 during the occlusion, *End* is the mVO_2 immediately after the cessation of exercise, *Delta* is the change in mVO_2 from rest to end exercise, *t* is time, and *k* is the fitting rate constant. The rate constant (k) for the recovery

of mVO_2 is directly related to the muscle's maximal oxidative capacity, while the time constant (Tc = 1/k) is inversely related to the muscle's maximal oxidative capacity (50).

Strength Testing

To determine each participant's wrist flexor training weight and to track any changes in wrist-flexor strength during the training protocol, MVICs were performed each week. Participants were seated upright, arms at 90 degrees and resting on the arms of the chair. Participants were given a calibrated hand dynamometer and instructed to perform three consecutive MVICs. Participants were allowed to rest for 20 seconds between each contraction.

Measurement of Physical Activity

Physical activity was assessed in each group using the International Physical Activity Questionnaire (IPAQ) long form. The total amount of activity per week was calculated and used for analysis. This questionnaire has been validated in similar populations (7, 55).

Statistical Analysis

Data are presented as means ± SD. Statistical analyses were performed using SPSS 19.0 (IBM®, Armonk, NY). T-tests were used to compare between groups for resting O_2 consumption, O_2 reperfusion, and baseline oxidative capacity. The analysis of the training data consisted of a two-way (time*group) mixed model repeated measures ANOVA with a within subjects factor of time and a between subject factors of group. Oxidative capacity was different between HF and control groups at baseline (1.27 ± 0.25 min^{-1} vs. 1.32 ± 0.15 min^{-1}, $p = 0.69$), and between DOM and nDOM arms in both HF and control groups. Therefore, percent change from baseline was used to quantify

relative changes in oxidative capacity in each group. The ANOVA model was applied to the percent change data, where the time factor was baseline through week four of wrist-flexor training and the group factor was HF and control. Between arm analyses within each group were performed on the percent change data with the same statistical model, where the time factor was baseline through week four of wrist-flexor training and the group factor was the DOM and nDOM arms. The ANOVA model was also applied to the exercise training progression and strength measurements data. If a significant interaction was found, post hoc analysis with a Bonferroni correction was performed on the main effect. Significance was accepted at $p < 0.05$.

Results

All participants with the exception of one were able to complete the study with no adverse events. One participant in the HF cohort could not complete the 4 weeks of training due to inflammation and arthritis in the hand. Table 3.1 displays the participant characteristics for both the cross-sectional and training studies.

Exercise Training

Adherence to the home-based training was very high in both groups. In both groups, all participants reported training for the entire 30 minutes for all 16 sessions, with the exception of two participants in the control group who reported completing only 20 of the 30 minutes for one training session each. All participants increased the training contraction frequency each week. The weekly average number of contractions performed during each training session was calculated for each participant in both HF and control groups. The ANOVA revealed the factor of time was significant ($p < 0.001$) indicating an effect of training progression in both groups. The group*time interaction was not

significant indicating no difference in the training progression between HF and controls (p = 0.125). Both HF and control groups significantly increased, from week 1, the number of contractions performed every week ($p < 0.001$) (Figure 3.1).

Resting Measurements

No difference in resting mVO_2 was found between HF and controls (0.31 ± 0.09 vs. 0.34 ± 0.11 %/sec, for HF and control respectively, p = 0.23) (Figure 3.2A). Resting blood flow was not different between HF (5.1 ± 2.9 ml/min/100 ml) and controls (4.8 ± 3.3 ml/min/100 ml, p = 0.63) (Figure 3.2B). The time course of maximal oxygen reperfusion capacity was significantly slower in people with HF (22.5 ± 4.8 seconds) compared with controls (18.0 ± 7.2 seconds), $p < 0.01$ (Figure 3.2C).

NIRS Measurements of Oxidative Capacity

Oxidative capacity in the DOM arm was significantly reduced in HF compared to controls (1.31 ± 0.30 min^{-1} vs. 1.61 ± 0.24 min^{-1}, p = 0.002). Oxidative capacity was also significantly reduced in the nDOM arm in HF compared to controls (1.29 ± 0.24 min^{-1} vs. 1.47 ± 0.25 min^{-1}, p = 0.039). There was no difference between the DOM and nDOM in the HF group (1.33 ± 0.30 min^{-1} vs. 1.29 ± 0.24 min^{-1}, p = 0.40). However, in the control group, the DOM arm had higher oxidative capacity than the nDOM arm (1.61 ± 0.24 min^{-1} vs. 1.47 ± 0.25 min^{-1}, p = 0.03). The oxidative capacity data for each arm and each group are displayed in Figures 3.3.

Among the training cohorts, analysis revealed a significant interaction of time*group $F(4,36) = 6.15$, $p < 0.01$, indicating a difference in the training magnitude between HF and control. Post hoc comparisons revealed a 53.27% increase from baseline in the control group compared to the -1.20% decrease in the HF group after 4

weeks for training ($p < 0.005$). Tests three, four, and five of the control group were significantly different from the HF group ($p < 0.05$). Further comparisons showed that tests two through five in HF group did not change from baseline ($p > 0.05$). However, in the control group, tests three through five were significantly different from the baseline ($p < 0.05$). During detraining, the oxidative capacity decreased and was not different from baseline at the last test point. Figure 3.4 displays the actual change in oxidative capacity (expressed as rate constants) throughout the training and detraining portions of the study.

Between arm analysis in the control group revealed a significant interaction of time*group, indicating a significant difference between arms over time [$F(4,32) = 4.47$, $p < 0.01$]. Further comparisons revealed that oxidative capacity of the training arm was significantly higher than the control arm at tests two, four, and five ($p < 0.05$), confirming a training effect in the nDOM arm of the control group. The same analysis was performed in the HF group, but the time*group interaction was not significant [$F(4,48) = 0.76$, $p = 0.56$], confirming that no training effect was found in the nDOM arm of the HF group.

Strength Measurements

The statistical analysis of the strength measurements revealed that the interaction (group*time) was not significant in either the DOM [$F(4,36) = 0.20$, $p = 0.94$] or the nDOM [$F(4,36) = 0.99$, $p = 0.43$] arms. This indicated that strength was not different between HF and controls, and did not change during the course of the training intervention.

Physical Activity

Physical activity was collected on 14 of the 15 HF participants and 24 of the 26 controls. Physical activity was higher in the control group than HF (3360 ± 3034 met-minutes/week vs. 1804 ± 1944 met-minutes/week, $p = 0.07$). No correlation was found between self-reported physical activity and oxidative capacity in either HF or control groups (HF: r = 0.17 $p = 0.55$; Control: r = 0.025, $p = 0.91$). No difference was found between control and HF among the participants enrolled in the training study (1896 ± 2155 met-minutes/week vs. 1490 ± 2363 met-minutes/week, $p = 0.76$).

Discussion

One of the primary findings of this study was that skeletal muscle oxidative capacity was reduced in both the DOM and nDOM arms of people with HF. In addition, this study found that oxidative capacity in the wrist-flexors of people with HF did not respond to four weeks of exercise training. In contrast, the control group demonstrated a linear increase in oxidative capacity, resulting in a ~53% improvement in oxidative capacity by the end of training. The improvements elicited in the control group have been supported by numerous in vivo and in vitro studies that have documented similar training adaptations in oxidative capacity using in vivo and in vitro methodologies (11, 15, 20, 36, 37). Furthermore, a NIRS study using a similar design, Ryan et al. reported a ~64% linear oxidative adaptation to endurance exercise in a young healthy population (49).

Unlike the control group, and contrary to previous research (1, 42), this study found a lack of adaptation to endurance training in oxidative capacity in people with HF. No significant fluctuations from baseline were observed at any point during the training,

with the end-training result being a -1% change from baseline. These results do not agree with previous findings. Comparable to the current study, Minotti et al. investigated the influence of a 28-day wrist-flexor training program on the oxidative capacity of skeletal muscle in people with HF (42). Following the training, the authors found a reduction in the rate of PCr depletion during exercise, reflecting an improvement (~40% and ~56% in the dominant and nondominant arms respectively) in the oxidative capacity of the muscle. Adamopoulos et al. also found that eight weeks of exercise training reversed impairments (~45% improvement) in oxidative capacity in the skeletal muscle in people with HF (1). In vitro studies have also shown improvements in response to endurance training in people with HF. Hambreich et al. (18) found a 19% increase in the total volume density of mitochondria after six months of cycling training. Similarly, Belardinelli et al. found a 22% increase in in the volume density of mitochondria after two months of low intensity cycling training (3). Therefore, our results clearly demonstrate a difference in magnitude of oxidative training response in people with HF compared to controls.

The lack of mitochondrial plasticity seen in the HF group was unexpected, but several potential explanations could be offered as to the reason of this response. Previously, it has been purported that HF could have detrimental effects on mitochondrial biogenesis in people with HF, and thus could impair oxidative adaptations to exercise training (47). Animal models of HF have supported this idea, as an several studies showed HF was associated with downregulation of all main mitochondrial transcription factors in both cardiac and skeletal muscle tissue (13). However, these results have not been found in human muscle, as Garnier et al. found that mitochondrial transcription

factors were not reduced in humans (14). Additionally, the lack of training response could be due to factors outside of HF, such as pharmacological therapies. In this study, the control group were not taking medications known to interfere with mitochondrial biogenesis, but in the HF group, all participants were on a combination of statin, β-blockers, or metformin therapies. Recently, statins, a class of drugs used to lower cholesterol, were shown to attenuate oxidative adaptations in overweight and sedentary subjects on statin therapy following 12-weeks of treadmill walking/jogging (41). In animal models, β-blockers have been shown to interfere with a key mitochondrial transcription factor, PGC-1α, suggesting that β-blockers could also impede oxidative adaptations to exercise (43). Furthermore, metformin has been prescribed to help insulin resistance in diabetes. Little focus has been put on the interaction between metformin and mitochondria, but a recent study found that metformin impairs oxidative adaptations to exercise in rats (28). Overall, we were not able to distinguish between and the influence of HF from the possible influence of pharmacotherapy on the blocked oxidative adaptations. Unfortunately, this might be a difficult task for future studies, as many people with HF have addition conditions, such as hypercholesterolemia, hypertension, and diabetes, which are commonly treated with pharmacotherapy.

This study found that oxidative capacity was reduced (~21% and ~13% in DOM and nDOM arms respectively) in the skeletal muscle of people with HF compared to controls. Interestingly, there has not been universal consensus in the literature regarding the contribution of skeletal muscle oxidative capacity to exercise intolerance in HF. Several studies have reported diminished markers of oxidative capacity in HF and concluded that it was a primary contributor to exercise intolerance. Sullivan et al. (53)

and Schaufelberger et al. (51) reported reductions in oxidative enzyme activity, and Drexler et al. (9) reported reductions in volume density of mitochondria and surface density of cristae. However, more recent research has shown that oxidative capacity in HF was not reduced if muscle physical activity was experimentally controlled. Mettauer et al. recruited controls that were unfit and physically inactive (peak VO_2 ~30 ml/kg/min). In this study, the authors found mitochondrial function, as assessed by in situ measurements of maximal ADP-stimulated respiration rates, was not different between HF and controls. More recently, Toth et al. matched and monitored physical activity between HF and controls using accelerometry. The authors reported no difference in oxidative enzymes between the groups, but did find a trend toward fewer mitochondria per muscle fiber. This new evidence has brought into question the conventional understanding of the link between oxidative capacity and exercise intolerance in HF. These new findings have suggested that previous results of reduced oxidative capacity may have been due to deconditioning rather than HF. For example, Sullivan et al. (53) reported that over half of the control group was active and engaging in regular exercise, and Schaufelberger et al. (51) did not report physical activity or fitness level of controls. In this study, the physical activity of the controls was not matched to the HF group, these results were from the wrist flexor muscles, which are not involved in locomotor activity and thus should represent a relatively untrained state in both groups, independent of habitual physical activity levels. This assumption has been made in previous studies (45, 49). Overall, the results of our in vivo assessment showed a reduction in oxidative capacity independent of physical activity, suggesting the presence

of a small but significant HF myopathy on skeletal muscle oxidative capacity. (13, 14, 24, 28, 43, 46, 47)

No difference in the resting blood flow was found between HF and control in this study. This is in agreement with previous research (27, 56). Using plethysmography to measure forearm blood flow, Tousoulis et al. (56) found similar values and no difference in the blood flow at rest between HF and control. Massie et al. found no difference in resting flow or flow during exercise (32). In contrast, Zelis et al. found forearm blood flow at rest was reduced by ~43% in people with HF (60). Similarly, Sullivan et al. reported a small reduction in single leg blood flow in people with HF at rest (54). It is possible that high variability in the blood flow measurements in this study hindered our ability to detect a difference between groups.

While no difference was found between HF and controls in resting blood flow, we found a difference between groups in the maximal reperfusion capacity. A ~21% slower time to half recovery after ischemia was detected in the HF group, which indicated possible vascular dysfunction. Previous studies have used plethysmography to measure blood flow after ischemia and characterize reactive hyperemia in people with HF (22). In agreement with our results, Hornig et al. (22) found a ~23% reduction in the magnitude of hyperemic response in forearms of people with HF. Similarly, Zelis et al. found a ~78% difference between HF and controls in peak hyperemic blood flow response following five minutes of ischemia (61). Previous studies have suggested endothelial dysfunction as a possible explanation for the slower reactive hyperemic kinetics in HF (8, 10, 26). Our results were consistent with previous research, which demonstrated a

possible impairment in endothelial function, and thus supported the need for further research to be conducted in this area.

Limitations

Reductions in skeletal muscle oxidative capacity could be attributed to two primary alterations, 1) reduction in mitochondrial number and 2) reduction in intrinsic mitochondrial function. The strength of the NIRS method was that it measured in vivo oxidative capacity of the muscle, but this might be considered a limitation, as it could not distinguish between mitochondrial number and function. Therefore, while we could detect a reduction in oxidative capacity, we could not attribute the reduction to diminished intramuscular mitochondrial density or function.

Unfortunately, due to limited funding and resources, this study was not able to recruit a control group to match the sex of the HF group, as the HF group had 80% males and the control group had just 15% males. While this could have introduced a confounding variable into the study design, it should not have influenced the interpretation of our main outcomes. All the females enrolled in this study were post-menopausal, which eliminated potential estrogen mediated differences between groups in oxidative capacity. Furthermore, previous research has suggested there are no differences in oxidative capacity between males and females (23, 34, 54).

Further limitations might include the small sample size in the training study. Despite the clear difference between the two groups in the training response, further research should be performed to replicate this data. Also, the training program was home-based, which, due to the unsupervised sessions, could lead to inconsistencies in the training stimulus, potentially having an effect on the training response in the HF group.

This is unlikely, however, as both groups were unsupervised and given the same training instructions. Furthermore, each group was able to successfully increase the contraction frequency each week, a task that would be difficult without previous training.

Conclusions

This study found reduced skeletal muscle oxidative capacity in a deconditioned muscle in people with HF. This finding indicates that deconditioning may not be entirely responsible for reductions in oxidative muscle metabolism in HF. This study also found evidence suggesting that HF may interfere with skeletal muscle oxidative adaptations to endurance exercise training. These results could be significant regarding exercise training guidelines and therefore, future studies should be performed to understand the mechanism behind the impaired training response. Together, these results support many previous studies that have outlined the extensive alterations in the skeletal muscle of people with HF. NIRS can be an effective tool in evaluating several aspects of skeletal muscle health in HF, and should be used in future studies to further investigate the complexities of HF and skeletal muscle.

Acknowledgements

This study was funded in part by the University of Georgia Department of Kinesiology's Louise E. Kindig Award. We would like to thank Kirsten Kepple for her assistance with participant recruitment. We would like to thank all the volunteers who participated in this study for their time, effort, and desire to help advance our understanding of heart failure.

Conflict of Interest Statement

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

References

1. **Adamopoulos S, Coats AJ, Brunotte F, Arnolda L, Meyer T, Thompson CH, Dunn JF, Stratton J, Kemp GJ, Radda GK, and et al.** Physical training improves skeletal muscle metabolism in patients with chronic heart failure. *Journal of the American College of Cardiology* 21: 1101-1106, 1993.

2. **Åstrand P-O, Cuddy TE, Saltin B, and Stenberg J**. Cardiac output during submaximal and maximal work. *Journal of Applied Physiology* 19: 268-274, 1964.

3. **Belardinelli R, Georgiou D, Cianci G, and Purcaro A**. Randomized, Controlled Trial of Long-Term Moderate Exercise Training in Chronic Heart Failure: Effects on Functional Capacity, Quality of Life, and Clinical Outcome. *Circulation* 99: 1173-1182, 1999.

4. **Brizendine JT, Ryan TE, Larson RD, and McCully KK**. Skeletal muscle metabolism in endurance athletes with near-infrared spectroscopy. *Med Sci Sports Exerc* 45: 869-875, 2013.

5. **Buchheit M, Ufland P, Haydar B, Laursen PB, and Ahmaidi S**. Reproducibility and sensitivity of muscle reoxygenation and oxygen uptake recovery kinetics following running exercise in the field. *Clinical physiology and functional imaging* 31: 337-346, 2011.

6. **Cheatle TR, Potter LA, Cope M, Delpy DT, Coleridge Smith PD, and Scurr JH**. Near-infrared spectroscopy in peripheral vascular disease. *The British journal of surgery* 78: 405-408, 1991.

7. **Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, and Oja P**. International physical activity

questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381-1395, 2003.

8. **Drexler H, and Hornig B**. Importance of endothelial function in chronic heart failure. *Journal of cardiovascular pharmacology* 27 Suppl 2: S9-12, 1996.

9. **Drexler H, Riede U, Münzel T, König H, Funke E, and Just H**. Alterations of skeletal muscle in chronic heart failure. *Circulation* 85: 1751-1759, 1992.

10. **Fischer D, Rossa S, Landmesser U, Spiekermann S, Engberding N, Hornig B, and Drexler H**. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *Eur Heart J* 26: 65-69, 2005.

11. **Forbes SC, Slade JM, and Meyer RA**. **Forbes SC, Slade JM, and Meyer RA**. Short-term high-intensity interval training improves phosphocreatine recovery kinetics following moderate-intensity exercise in humans. *Appl Physiol Nutr Metab* 33: 1124-1131, 2008.

12. **Franciosa JA, Park M, and Levine TB**. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 47: 33-39, 1981.

13. **Garnier A, Fortin D, Deloménie C, Momken I, Veksler V, and Ventura-Clapier R**. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol* 551: 491-501, 2003.

14. **Garnier A, Fortin D, Zoll J, N'Guessan B, Mettauer B, Lampert E, Veksler V, and Ventura-Clapier R**. Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle. *Faseb J* 19: 43-52, 2005.

15. **Gollnick PD, Armstrong RB, Saltin B, Saubert CWt, Sembrowich WL, and Shepherd RE**. Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J Appl Physiol* 34: 107-111, 1973.

16. **Hall MJ, DeFrances CJ, Williams SN, Golosinskiy A, and Schwartzman A**. National Hospital Discharge Survey: 2007 summary. *National health statistics reports* 1-20, 24, 2010.

17. **Hamaoka T, McCully KK, Quaresima V, Yamamoto K, and Chance B**. Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *Journal Of Biomedical Optics* 12: 062105-062105, 2007.

18. **Hambrecht R, Niebauer J, Fiehn E, Kälberer B, Offner B, Hauer K, Riede U, Schlierf G, Kübler W, and Schuler G**. Physical training in patients with stable chronic heart failure: Effects on cardiorespiratory fitness and ultrastructural abnormalities of leg muscles. *Journal of the American College of Cardiology* 25: 1239-1249, 1995.

19. **Harrington D, Anker SD, Chua TP, Webb-Peploe KM, Ponikowski PP, Poole-Wilson PA, and Coats AJ**. Skeletal muscle function and its relation to exercise tolerance in chronic heart failure. *Journal of the American College of Cardiology* 30: 1758-1764, 1997.

20. **Henriksson J**. Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J Physiol* 270: 661-675, 1977.

21. **Hood DA**. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *Journal of applied physiology (Bethesda, Md : 1985)* 90: 1137-1157, 2001.

22. **Hornig B, Maier V, and Drexler H**. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 93: 210-214, 1996.

23. **Kent-Braun JA, and Ng AV**. Skeletal muscle oxidative capacity in young and older women and men. *Journal of applied physiology (Bethesda, Md : 1985)* 89: 1072-1078, 2000.

24. **Kragelj R, Jarm T, Erjavec T, Prešern-Štrukelj M, and Miklavčič D**. Parameters of Postocclusive Reactive Hyperemia Measured by Near Infrared Spectroscopy in Patients with Peripheral Vascular Disease and in Healthy Volunteers. *Annals of biomedical engineering* 29: 311-320, 2001.

25. **Lam CS, and Brutsaert DL**. Endothelial dysfunction: a pathophysiologic factor in heart failure with preserved ejection fraction. *Journal of the American College of Cardiology* 60: 1787-1789, 2012.

26. **LeJemtel TH, Maskin CS, Lucido D, and Chadwick BJ**. Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. *Circulation* 74: 245-251, 1986.

27. **Linden MA, Fletcher JA, Morris EM, Meers GM, Kearney ML, Crissey JM, Laughlin MH, Booth FW, Sowers JR, Ibdah JA, Thyfault JP, and Rector RS**. Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. *Am J Physiol Endocrinol Metab* 306: E300-310, 2014.

28. **Mancini DM, Coyle E, Coggan A, Beltz J, Ferraro N, Montain S, and Wilson JR**. Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. *Circulation* 80: 1338-1346, 1989.

29. **Mancini DM, Walter G, Reichek N, Lenkinski R, Mccully KK, Mullen JL, and Wilson JR**. Contribution of Skeletal-Muscle Atrophy to Exercise Intolerance and Altered Muscle Metabolism in Heart-Failure. *Circulation* 85: 1364-1373, 1992.

30. **Mancini DM, Wilson JR, Bolinger L, Li H, Kendrick K, Chance B, and Leigh JS**. In vivo magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failure. Demonstration of abnormal muscle metabolism despite adequate oxygenation. *Circulation* 90: 500-508, 1994.

31. **Massie B, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, and Rajagopalan B**. Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. *Circulation* 76: 1009-1019, 1987.

32. **Massie BM, Conway M, Rajagopalan B, Yonge R, Frostick S, Ledingham J, Sleight P, and Radda G**. Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. Evidence for abnormalities unrelated to blood flow. *Circulation* 78: 320-326, 1988.

33. **Massie BM, Conway M, Yonge R, Frostick S, Sleight P, Ledingham J, Radda G, and Rajagopalan B**. 31P nuclear magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with congestive heart failure. *Am J Cardiol* 60: 309-315, 1987.

34. **Mattei JP, Bendahan D, Roussel M, Lefur Y, and Cozzone PJ**. Gender modulates the energy cost of muscle contraction in untrained healthy subjects. A 31P magnetic resonance spectroscopy analysis. *FEBS Lett* 450: 173-177, 1999.

35. **McCully KK, Fielding RA, Evans WJ, Leigh JS, Jr., and Posner JD**. Relationships between in vivo and in vitro measurements of metabolism in young and old human calf muscles. *Journal Of Applied Physiology (Bethesda, Md: 1985)* 75: 813-819, 1993.

36. **McCully KK, Halber C, and Posner JD**. Exercise-induced changes in oxygen saturation in the calf muscles of elderly subjects with peripheral vascular disease. *Journal of gerontology* 49: B128-134, 1994.

37. **Members WG, Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, and Turner MB**. Heart Disease and Stroke Statistics—2012 Update: A Report From the American Heart Association. *Circulation* 125: e2-e220, 2012.

38. **Mettauer B, Zoll J, Sanchez H, Lampert E, Ribera F, Veksler V, Bigard X, Mateo P, Epailly E, Lonsdorfer J, and Ventura-Clapier R**. Oxidative capacity of skeletal muscle in heart failure patients versus sedentary or active control subjects. *Journal of the American College of Cardiology* 38: 947-954, 2001.

39. **Middlekauff HR**. Making the Case for Skeletal Myopathy as the Major Limitation of Exercise Capacity in Heart Failure. *Circulation: Heart Failure* 3: 537-546, 2010.

40. **Mikus CR, Boyle LJ, Borengasser SJ, Oberlin DJ, Naples SP, Fletcher J, Meers GM, Ruebel M, Laughlin MH, Dellsperger KC, Fadel PJ, and Thyfault JP**. Simvastatin impairs exercise training adaptations. *Journal of the American College of Cardiology* 62: 709-714, 2013.

41. **Minotti JR, Johnson EC, Hudson TL, Zuroske G, Murata G, Fukushima E, Cagle TG, Chick TW, Massie BM, and Icenogle MV**. Skeletal muscle response to exercise training in congestive heart failure. *J Clin Invest* 86: 751-758, 1990.

42. **Miura S, Kawanaka K, Kai Y, Tamura M, Goto M, Shiuchi T, Minokoshi Y, and Ezaki O**. An increase in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) mRNA in response to exercise is mediated by beta-adrenergic receptor activation. *Endocrinology* 148: 3441-3448, 2007.

43. **Motobe M, Murase N, Osada T, Homma T, Ueda C, Nagasawa T, Kitahara A, Ichimura S, Kurosawa Y, Katsumura T, Hoshika A, and Hamaoka T**. Noninvasive monitoring of deterioration in skeletal muscle function with forearm cast immobilization and the prevention of deterioration. *Dyn Med* 3: 2, 2004.

44. **Park JH, Brown RL, Park CR, Cohn M, and Chance B**. Energy metabolism of the untrained muscle of elite runners as observed by 31P magnetic resonance spectroscopy: evidence suggesting a genetic endowment for endurance exercise. *Proc Natl Acad Sci U S A* 85: 8780-8784, 1988.

45. **Piña IL, Apstein CS, Balady GJ, Belardinelli R, Chaitman BR, Duscha BD, Fletcher BJ, Fleg JL, Myers JN, and Sullivan MJ**. Exercise and Heart Failure: A Statement From the American Heart Association Committee on Exercise, Rehabilitation, and Prevention. *Circulation* 107: 1210-1225, 2003.

46. **Rosca M, and Hoppel C**. Mitochondrial dysfunction in heart failure. *Heart Fail Rev* 1-16, 2012.

47. **Ryan TE, Erickson ML, Brizendine JT, Young H-J, and McCully KK**. Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. *Journal of Applied Physiology* 113: 175-183, 2012.

48. **Ryan TE, Southern WM, Brizendine JT, and McCully KK**. Activity-induced changes in skeletal muscle metabolism measured with optical spectroscopy. *Med Sci Sports Exerc* 45: 2346-2352, 2013.

49. **Ryan TE, Southern WM, Reynolds MA, and McCully KK**. A cross-validation of near infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *Journal of applied physiology (Bethesda, Md : 1985)* 2013.

50. **Schaufelberger M, Eriksson BO, Grimby G, Held P, and Swedberg K**. Skeletal muscle alterations in patients with chronic heart failure. *Eur Heart J* 18: 971-980, 1997.

51. **Southern WM, Ryan TE, Reynolds MA, and McCully K**. Reproducibility of near infrared spectroscopy measurements of oxidative function and post-exercise

recovery kinetics in the medial gastrocnemius muscle. *Applied Physiology, Nutrition, and Metabolism* 2013.

52. **Sullivan MJ, Green HJ, and Cobb FR**. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation* 81: 518-527, 1990.

53. **Sullivan MJ, Knight JD, Higginbotham MB, and Cobb FR**. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation* 80: 769-781, 1989.

54. **Thompson JR, Swanson SA, Casale GP, Johanning JM, Papoutsi E, Koutakis P, Miserlis D, Zhu Z, and Pipinos, II**. Gastrocnemius mitochondrial respiration: are there any differences between men and women? *J Surg Res* 185: 206-211, 2013.

55. **Tomioka K, Iwamoto J, Saeki K, and Okamoto N**. Reliability and validity of the International Physical Activity Questionnaire (IPAQ) in elderly adults: the Fujiwara-kyo Study. *Journal of epidemiology / Japan Epidemiological Association* 21: 459-465, 2011.

56. **Toth MJ, Miller MS, Ward KA, and Ades PA**. Skeletal muscle mitochondrial density, gene expression, and enzyme activities in human heart failure: minimal effects of the disease and resistance training. *Journal of Applied Physiology* 112: 1864-1874, 2012.

57. **Tousoulis D, Antoniades C, Bosinakou E, Kotsopoulou M, Pitsavos C, Vlachopoulos C, Panagiotakos D, and Stefanadis C**. Effects of atorvastatin on

reactive hyperemia and inflammatory process in patients with congestive heart failure. *Atherosclerosis* 178: 359-363, 2005.

58. **Van Beekvelt MC, Colier WN, Wevers RA, and Van Engelen BG**. Performance of near-infrared spectroscopy in measuring local O(2) consumption and blood flow in skeletal muscle. *Journal of Applied Physiology* 90: 511-519, 2001.

59. **Wolfel EE, Groves BM, Brooks GA, Butterfield GE, Mazzeo RS, Moore LG, Sutton JR, Bender PR, Dahms TE, McCullough RE, and et a**. Oxygen transport during steady-state submaximal exercise in chronic hypoxia. *Journal of Applied Physiology* 70: 1129-1136, 1991.

60. **Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R, and Williams RS**. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 296: 349-352, 2002.

61. **Zelis R, Longhurst J, Capone RJ, and Mason DT**. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 50: 137-143, 1974.

62. **Zelis R, Mason DT, and Braunwald E**. A comparison of the effects of vasodilator stimuli on peripheral resistance vessels in normal subjects and in patients with congestive heart failure. *J Clin Invest* 47: 960-970, 1968.

Figure Legends

Figure 3.1: Progression of wrist-flexor contractions performed during each training session for both heart failure (open circles) and control groups (solid squares). * $p < 0.001$ for weekly average of each group different from baseline. No differences were found between groups at any training level ($p = 0.13$). Data are presented as mean ± SD.

Figure 3.2: (A) Resting muscle oxygen consumption (mVO_2) in wrist-flexors of HF and control groups. (B) Resting blood flow in wrist-flexors of HF and control groups. (C) Maximal reperfusion capacity following ischemic occlusion in wrist-flexors of HF and control groups. Each dataset represents the combined mean from both arms. * $p < 0.005$ for difference between HF and control.

Figure 3.3: Oxidative capacity of both heart failure and control groups for non-dominant arm (nDOM) at baseline, dominant (DOM) arm at baseline, and non-dominant arm after 4 weeks of wrist-flexor training. Data are expressed as rate constants of mVO_2 recovery and presented as mean ± SD.

Figure 3.4: Change in oxidative capacity expressed as rate constants of mVO_2 recovery for heart failure (circles) and controls (squares) during four weeks of wrist-flexor exercise training (solid markers) and detraining (open markers). Data presented as mean ± SD. ** $p < 0.002$, * $p < 0.05$ for difference from baseline. ## $p < 0.005$, # $p < 0.05$ for difference between HF and control.

Table 3.1 - Participant Characteristics

All Participants	HF (n = 16)	Control (n = 20)
Age (years)	64.5 ± 6.6	60.8 ± 5.0
Sex (male/female)	13/3	3/17
Height (cm)	180.7 ± 8.5	167.8 ± 8.3*
Weight (kg)	98.0 ± 13.1	76.6 ± 20.8*
DOM ATT (cm)	0.67 ± 0.3	0.81 ± 0.4
nDOM ATT (cm)	0.68 ± 0.2	0.84 ± 0.4
Length of HF (years)	10.6 ± 10.6	-
Ejection fraction (%)	28.5 ± 11.5	-
Beta-blockers	13	-
ACE inhibitors	10	-
Digoxin	5	-
Statins	14	-
Metformin	5	-
Training Participants	**(n = 7)**	**(n = 5)**
Age (years)	65.7 ± 4.1	60.8 ± 5.5
Sex (male/female)	6/1	2/3
Height (cm)	184.0 ± 3.6	172.2 ± 12.2
Weight (kg)	101.8 ± 10.6	76.7 ± 20.9
DOM ATT (cm)	0.60 ± 0.3	0.66 ± 0.3
nDOM ATT (cm)	0.71 ± 0.3	0.61 ± 0.2
Beta-blockers	5	-
Statins	5	-
Metformin	2	-

Note: Data are presented at mean ± SD; DOM = dominant arm; nDOM = non-dominant arm; ATT = adipose tissue thickness; ACE = angiotensin converting enzyme; * indicates significance at $p < 0.05$.

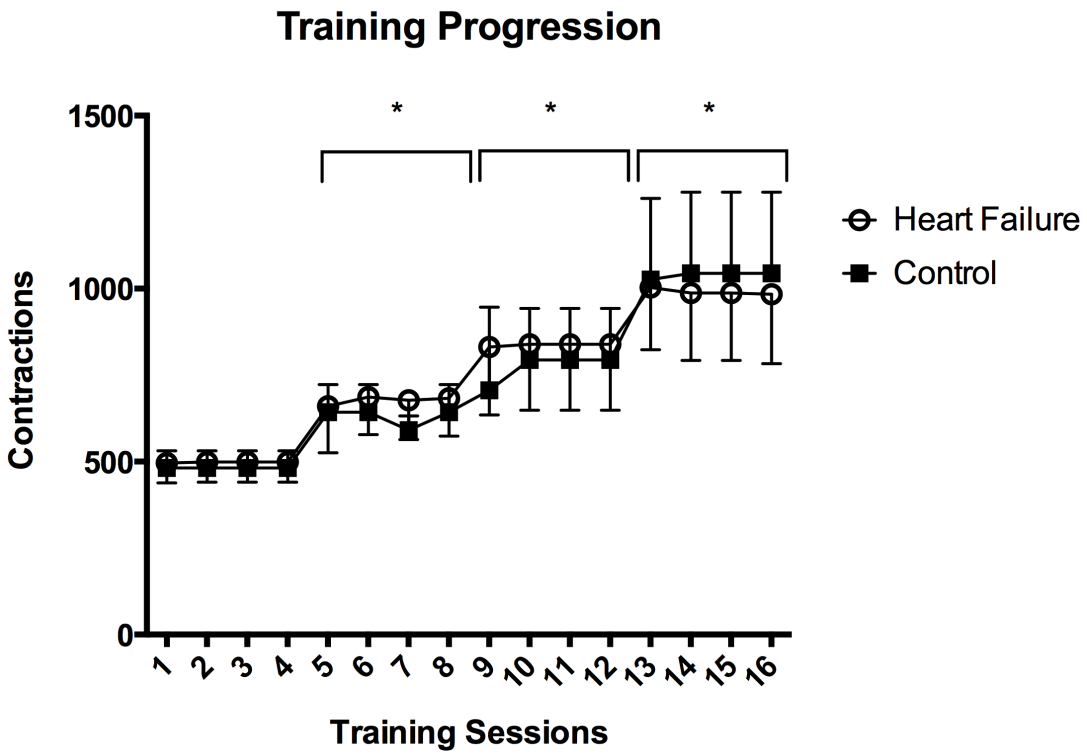

Training Progression
1500
1000
500
0
Contractions
*
*
*
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Training Sessions
Heart Failure
Control

Figure 3.1

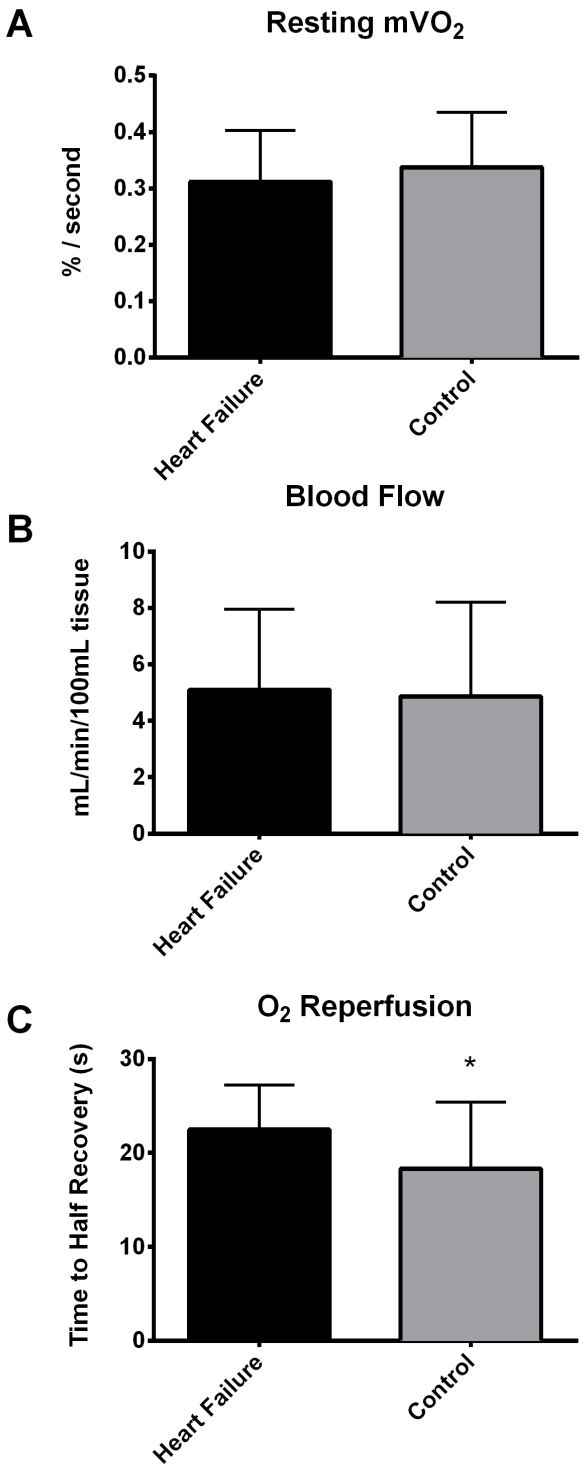

A
Resting mVO2
% / second
0.5
0.4
0.3
0.2
0.1
0.0
Heart Failure
Control
B
Blood Flow
mL/min/100mL tissue
10
8
6
4
2
0
Heart Failure
Control
C
O2 Reperfusion
Time to Half Recovery (s)
30
20
10
0
*
Heart Failure
Control

Figure 3.2

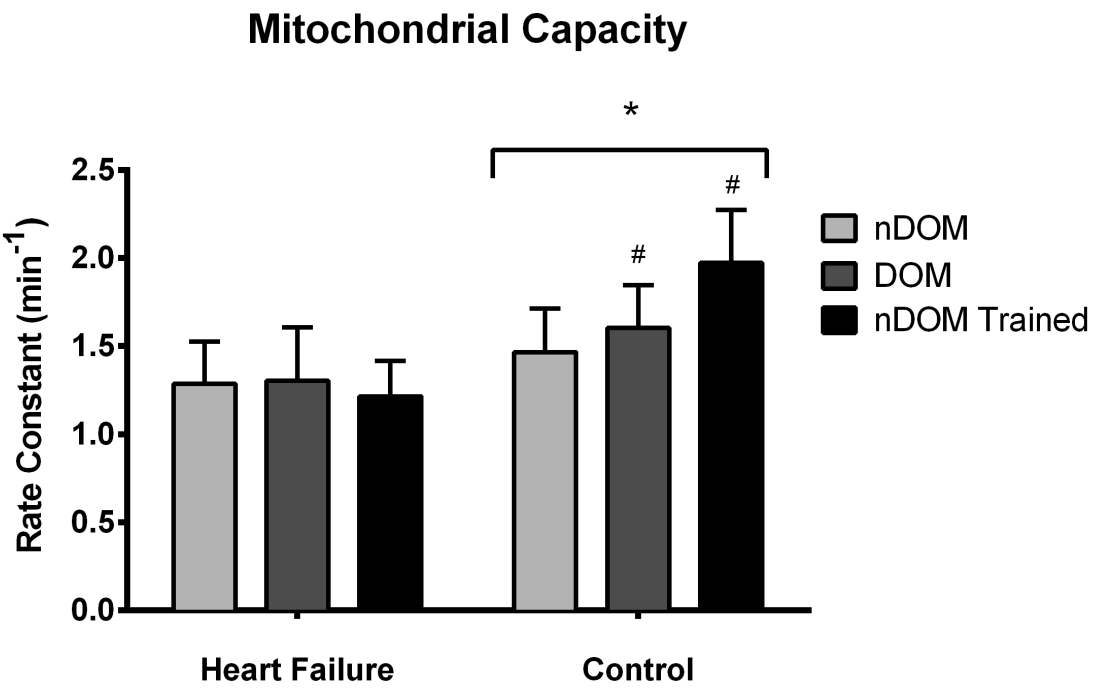
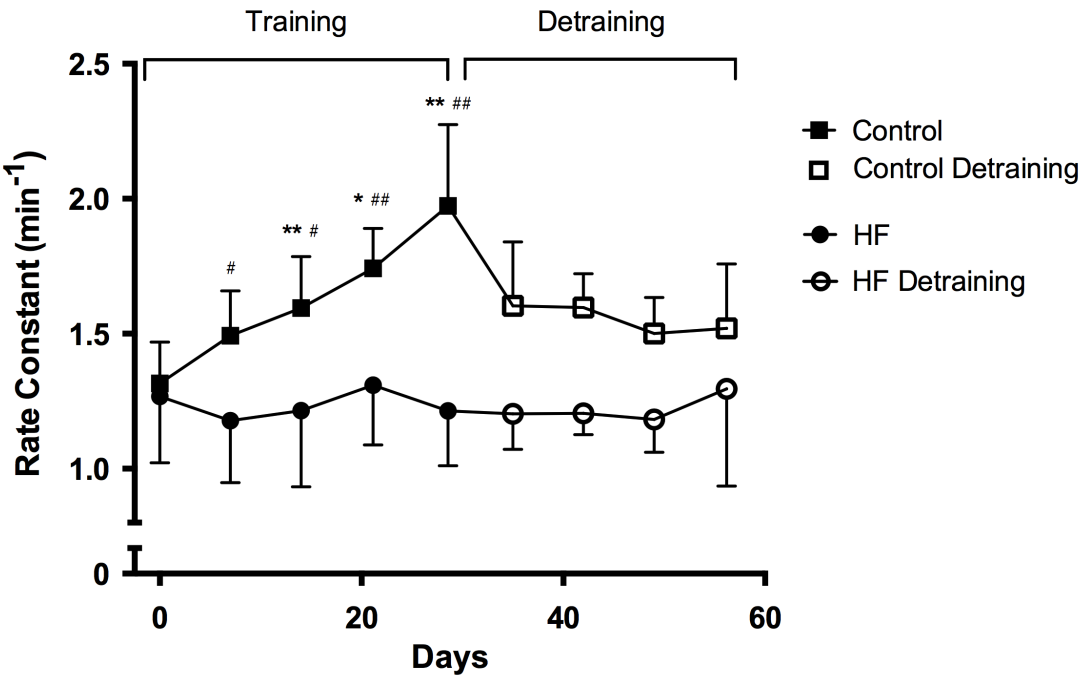

Mitochondrial Capacity
*
#
#
2.5
2.0
1.5
1.0
0.5
0.0
Rate Constant (min-1)
nDOM
DOM
nDOM Trained
Heart Failure
Control

Figure 3.3


Training
Detraining
2.5
2.0
1.5
1.0
0
Rate Constant (min-1)
0
20
40
60
Days

** #
* ##
** ##
Control
Control Detraining
HF
HF Detraining

Figure 3.4

CHAPTER 4

SUMMARY AND CONCLUSION

Major Findings

The primary finding of this study was that skeletal muscle oxidative capacity was reduced at baseline and did not improve with four weeks of exercise training in people with HF. Importantly, these results were found independent of physical activity, as the measurements were performed in deconditioned muscles not involved with locomotion. The clear lack of training response in HF was an unexpected but intriguing result. This finding did not agree with previous exercise training research in HF, suggesting an alternate mechanism outside of the pathology of HF could be responsible for this unique response. Further studies should be performed to examine the interaction of HF, pharmacological therapies, and exercise training.

Reduced Oxidative Capacity in Heart Failure

In this study, individuals with HF had a ~21% reduction in oxidative capacity in the DOM arm as well as a ~13% reduction in the nDOM arm. These findings agreed with previous research that showed oxidative capacity in skeletal muscle of HF was impaired (23, 112). Interestingly, several recent studies (76, 115) have disparaged prior findings, claiming that muscle oxidative capacity in HF was normal as long as physical activity was experimentally controlled. These studies concluded that previous findings were the result of differences in physical activity, and therefore, reflective of muscle

deconditioning rather than a myopathy of HF. In this study, however, oxidative capacity was measured in the wrist flexor muscles, which are relatively inactive and deconditioned. Importantly, our results revealed that oxidative capacity was lower in both the DOM and nDOM arms of HF compared to controls.

Interestingly, no difference was found between the DOM and nDOM arms in HF, while a small but significant 9% difference was found between arms in the control group. The slightly higher oxidative capacity in the DOM arm of the control group was expected given that the DOM arm is generally recruited for more tasks and could be considered a slightly more active muscle. Therefore, the nDOM arm was the more inactive of the two muscles and perhaps was the most suitable muscle for an activity-independent comparison of oxidative capacity between HF and controls. As stated earlier, a significant difference was still found in this group, which suggested that oxidative capacity might actually be reduced in HF irrespective of physical activity levels.

The lack of difference between arms in the HF group is also a significant finding. It appeared from the results of this study, that muscle physical activity levels mediated the difference in oxidative capacity between HF and controls. The difference between HF and controls was minimal unless the muscle was more active, in which case the difference was amplified. These results demonstrate a lack of oxidative plasticity in response to a physical activity stimulus.

In this study, we could only speculate as to the mechanism that could have caused reduced oxidative capacity in HF, as this topic is not well understood in humans. Animal research has demonstrated that cardiac and skeletal muscle oxidative capacity was compromised in models of HF (98). However, these results have not been duplicated in

human studies, warranting the need for more research in this area. Moreover, some research suggests that the intrinsic function of the mitochondria in people with HF was completely normal when compared to sedentary individuals (76, 115). These findings have suggested that deconditioning rather than the failing heart was responsible for oxidative impairments in HF. In addition, the NIRS technology allows the user to measure the entire oxidative capacity of the muscle of interest. However, the downside of this measurement is that it cannot separately evaluate the health and function of the components that constitute the oxidative system. Therefore, while we detected a reduction in oxidative capacity, we cannot attribute the reduction to mitochondrial dysfunction, diminished mitochondrial density, or any other link in the oxidative chain. In the end, our study confirmed a difference in oxidative capacity between HF and control, but was not able to identify the cause. Future studies should be conducted to elucidate the mechanism resulting in oxidative complications in HF.

Blocked Training Adaptations in Heart Failure

In this study, wrist-flexor oxidative capacity was tracked over the course of an exercise training program, in people with and without HF. Unexpectedly, individuals with HF showed no improvement in oxidative capacity from the exercise training, while the participants without HF increased ~53% from baseline. Following the four weeks of exercise training, oxidative capacity in the control group returned to baseline levels, confirming the oxidative adaptations to exercise. Interestingly, the lack of oxidative plasticity in the HF group was not consistent with previous research, as several studies have documented substantial adaptations in oxidative capacity in people with HF (2, 40,

41, 79). Thus, the results of this study have raised an important question regarding what mechanism, if not of HF, could have been responsible for attenuating the oxidative adaptations.

Further analysis of the participants who performed the training study revealed another noteworthy difference between the HF and control groups. Unfortunately, all of the participants in the HF group were taking medications known to interfere with adaptations to endurance exercise, while none of the individuals in the control group were on said medications. Six of the seven HF participants in the training cohort were taking statins. Previously, statins been shown to cause adverse effects on the skeletal muscle, including blunted oxidative adaptations to endurance exercise (78). In addition to statin therapy, five of the seven HF participants were known to have been taking β-Blockers. β-Blockers have been associated with attenuations in peak VO_2 adaptations (125) as well as down-regulation of the mitochondrial transcription factor PGC-1 following endurance exercise (80). Furthermore, two out of the seven HF participants were taking metformin, a drug commonly used to control blood sugar levels in Type II diabetics. Less is known about metformin and how it may interact with skeletal muscle mitochondria. However, some evidence has shown that metformin directly inhibits the oxidative respiratory chain complex I (87), and possibly interferes with oxidative adaptations to exercise training (63).

While this was a significant oversight in the experimental design of this study, several important conclusions can still be extracted from the data. First, our results demonstrated that the combined effect of HF, exercise, and statins and/or metformin impaired oxidative adaptations to exercise training. Unfortunately, we could not

determine whether the impairment was the result of the medications or a systemic effect of HF. Regardless, this finding was still of value considering that the combination of HF and these particular drugs is not uncommon. As HF and pharmacological therapies gradually grow more inseparable, research involving individuals who have HF and take these drugs will become more valuable. Overall, this evidence suggested that patient's pharmacological regimens should be taken into consideration before recommending exercise training as a rehabilitative therapy for people with HF.

Oxidative Capacity and Physical Activity

We found no relationship between the oxidative capacity and the self-reported physical activity, which confirmed our assumption that the oxidative capacity of wrist-flexor muscles was independent of physical activity levels. Although the HF participants had lower self-reported activity values than the controls, the values varied considerably within each group, which made detecting relationships or differences between groups difficult. Future studies should consider employing accelerometers to more accurately measure the physical activity levels.

Evaluation of Individuals on Statin Therapy

In this study, we did not anticipate the complication of myotoxic pharmacological therapies. In the end, seven participants including two from the training study were excluded from the final analysis due to statin and/or metformin use. An a priori power analysis estimated adequate power to detect a 20% difference between the controls taking myotoxic mediations (med) (n = 7) and controls not taking myotoxic medications

(nonmed) (n = 20). Importantly, there was no difference between groups in either the DOM or nDOM arms (p = 0.43 vs. p = 0.21). Also, the participants in the med group had a ~13% higher oxidative capacity in the DOM arm compared to the nDOM arm. Although underpowered to detect a statistical significance (p = 0.11), this finding demonstrates a similar effect of muscle activity in the DOM arm in the med group as was found in the nonmed group. This result indicated that statin and/or pharmacotherapy was not the cause of the tantamount oxidative capacity between the DOM and nDOM arms in the HF group. Interestingly, it would appear that an alternative mechanism, possibly linked with the pathology of HF, was the source of the effect.

Two participants from the training study were also excluded from the data analysis. One participant was on statin therapy and the other was on metformin therapy. Interestingly, neither participant showed an increase in oxidative capacity over the course of the wrist-flexor training program. While it was statistically impractical to form any solid conclusions, this evidence does lend credibility to the hypothesis that statins or metformin, rather than HF, were the cause of the impaired training response in the HF group. A figure comparing the training response in the nonmed group to each excluded participant is included in Appendix A.

NIRS Testing in Heart Failure

The NIRS methodology proved to be a simple and yet effective way to obtain a measurement of oxidative capacity in individuals with HF. Successful results from the NIRS method are often dependent on subcutaneous adipose tissue thickness (ATT). However, despite higher adiposity in other regions of the body, the forearm generally had

lower ATT values, which made it an ideal testing site. Overall, the NIRS testing success rate in both HF and controls was high. In addition, both HF and controls responded well to the testing, with no adverse events occurring during the testing sessions. A majority of participants were even able to fall asleep during the test. Therefore, NIRS is an ideal methodology to measure in oxidative capacity in both HF and healthy individuals, as it provides an inexpensive and noninvasive alternative to other assessments of oxidative capacity.

Wrist-Flexor Exercise Training Program

The wrist-flexor exercise training program employed in this study was adapted from Ryan et al. (100). Unique to this study, however, the majority of training sessions were performed at home, with only one supervised session per week. Immediately following each home-based training session, the participants were required to contact and report the details of the training session to the study coordinator. Initial concerns with using the home-based exercise training model were alleviated as the adherence to the training protocol was very successful, and the control group successfully demonstrated a training response. Therefore, the home-based model of wrist-flexor exercise training proved to be an effective means to increase oxidative capacity in the wrist-flexor muscles.

Future Directions

This study demonstrated that muscle oxidative capacity in HF, as assessed by NIRS, was reduced in the wrist-flexor muscles. NIRS cannot assess individual

components of oxidative capacity such as mitochondrial function and number, or oxidative enzyme activity and concentration. Therefore, future investigations should combine NIRS with in vitro methodologies to elucidate the mechanism causing reduced oxidative capacity in HF. Furthermore, future NIRS investigations could measure other muscles in people with HF such as the gastrocnemius and quadriceps. These measurements could provide additional insight into the role of skeletal muscle oxidative capacity in exercise intolerance in people with HF.

The results of the training portion of this study have raised many critical questions regarding oxidative adaptations to exercise training in people with HF. In the current study, we did not anticipate an impaired training response, therefore the control group was inadequate to evaluate whether HF or medications were responsible for the lack of training. Furthermore, two participants that were excluded from the final analysis provided evidence that statins and metformin could cause the impaired adaptations. Therefore, future investigations could improve upon this study by incorporating the appropriate control group, designed to isolate the mechanism causing the impaired training response. These investigations could use an experimental design similar to this study to evaluate the effects of statin or metformin therapy on local muscle oxidative capacity in either healthy or HF. In addition, future research could investigate the effects of myotoxic drugs on whole-body exercise, both resistance and endurance, in people with HF.

Overall Conclusion

This study was conducted using a novel methodology to measure oxidative capacity in people with heart failure. Before the exercise intervention, oxidative capacity was reduced in both arms of individuals with HF. Moreover, after four weeks of wrist-flexor exercise training, the oxidative capacity did not improve in individuals with HF. Overall, oxidative capacity was reduced in the inactive and deconditioned wrist-flexor muscles of people with HF and therefore was considered independent of whole-body physical activity. We concluded that an alternate mechanism, possibly linked to the pathology of HF, was responsible for the reduced oxidative capacity. Furthermore, the lack of training response exhibited by the HF group could also have been an effect of HF, but prior research and preliminary evidence from this study suggested that pharmacological therapies instead might be to blame. In the end, this study was an important first step toward filling a gap in the literature regarding the relationship between physical activity, oxidative capacity, and HF. In addition this study has drawn attention to a potentially critical conflict between pharmacological and exercise therapies in HF. Currently, however, little research has focused on this topic. On the one hand, exercise training has been shown to be effective in reversing detrimental effects of HF. However, if drugs are administered that counterbalance the oxidative effects of the exercise, then the time and energy of a patient with HF may be better spent elsewhere. Future studies should build upon the findings of this study and strive to more fully understand these interactions.

CHAPTER 5

REFERENCES

1. National Center for Health Statistics. In: *Health, United States, 2010: With Special Feature on Death and Dying*. Hyattsville (MD): National Center for Health Statistics, 2011.

2. **Adamopoulos S, Coats AJ, Brunotte F, Arnolda L, Meyer T, Thompson CH, Dunn JF, Stratton J, Kemp GJ, Radda GK, and et al.** Physical training improves skeletal muscle metabolism in patients with chronic heart failure. *Journal of the American College of Cardiology* 21: 1101-1106, 1993.

3. **Aguilar D, Chan W, Bozkurt B, Ramasubbu K, and Deswal A**. Metformin use and mortality in ambulatory patients with diabetes and heart failure. *Circulation Heart failure* 4: 53-58, 2011.

4. **Anker SD, Ponikowski P, Varney S, Chua TP, Clark AL, Webb-Peploe KM, Harrington D, Kox WJ, Poole-Wilson PA, and Coats AJ**. Wasting as independent risk factor for mortality in chronic heart failure. *Lancet* 349: 1050-1053, 1997.

5. **Anker SD, and Sharma R**. The syndrome of cardiac cachexia. *Int J Cardiol* 85: 51-66, 2002.

6. **Bangalore S, Steg G, Deedwania P, Crowley K, Eagle KA, Goto S, Ohman EM, Cannon CP, Smith SC, Zeymer U, Hoffman EB, Messerli FH, Bhatt DL, and Investigators RR**. beta-Blocker use and clinical outcomes in stable outpatients with and without coronary artery disease. *Jama* 308: 1340-1349, 2012.

7. **Belardinelli R**. Muscle oxygenation kinetics measured by near-infrared spectroscopy during recovery from exercise in chronic heart failure. *G Ital Cardiol* 28: 866-872, 1998.

8. **Belardinelli R, Georgiou D, Cianci G, and Purcaro A**. Randomized, Controlled Trial of Long-Term Moderate Exercise Training in Chronic Heart Failure: Effects on Functional Capacity, Quality of Life, and Clinical Outcome. *Circulation* 99: 1173-1182, 1999.

9. **Blanco-Colio LM, Villa A, Ortego M, Hernandez-Presa MA, Pascual A, Plaza JJ, and Egido J**. 3-Hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors, atorvastatin and simvastatin, induce apoptosis of vascular smooth muscle cells by downregulation of Bcl-2 expression and Rho A prenylation. *Atherosclerosis* 161: 17-26, 2002.

10. **Blyth FM, Lazarus R, Ross D, Price M, Cheuk G, and Leeder SR**. Burden and outcomes of hospitalisation for congestive heart failure. *The Medical journal of Australia* 167: 67-70, 1997.

11. **Brizendine JT, Ryan TE, Larson RD, and McCully KK**. Skeletal muscle metabolism in endurance athletes with near infrared spectroscopy. *Med Sci Sports Exerc* 2012.

12. **Brizendine JT, Ryan TE, Larson RD, and McCully KK**. Skeletal muscle metabolism in endurance athletes with near-infrared spectroscopy. *Med Sci Sports Exerc* 45: 869-875, 2013.

13. **Buchheit M, Ufland P, Haydar B, Laursen PB, and Ahmaidi S**. Reproducibility and sensitivity of muscle reoxygenation and oxygen uptake recovery

kinetics following running exercise in the field. *Clinical physiology and functional imaging* 31: 337-346, 2011.

14. **Chati Z, Zannad F, Jeandel C, Lherbier B, Escanye J-M, Robert J, and Aliot E**. Physical deconditioning may be a mechanism for the skeletal muscle energy phosphate metabolism abnormalities in chronic heart failure. *American heart journal* 131: 560-566, 1996.

15. **Clark AL, Sparrow JL, and Coats AJ**. Muscle fatigue and dyspnoea in chronic heart failure: two sides of the same coin? *Eur Heart J* 16: 49-52, 1995.

16. **Coats AJ, Adamopoulos S, Meyer TE, Conway J, and Sleight P**. Effects of physical training in chronic heart failure. *Lancet* 335: 63-66, 1990.

17. **Coats AJ, Adamopoulos S, Radaelli A, McCance A, Meyer TE, Bernardi L, Solda PL, Davey P, Ormerod O, and Forfar C**. Controlled trial of physical training in chronic heart failure. Exercise performance, hemodynamics, ventilation, and autonomic function. *Circulation* 85: 2119-2131, 1992.

18. **Coats AJ, Clark AL, Piepoli M, Volterrani M, and Poole-Wilson PA**. Symptoms and quality of life in heart failure: the muscle hypothesis. *British heart journal* 72: S36-39, 1994.

19. **De Blasi RA, Ferrari M, Natali A, Conti G, Mega A, and Gasparetto A**. Noninvasive measurement of forearm blood flow and oxygen consumption by near-infrared spectroscopy. *Journal of Applied Physiology* 76: 1388-1393, 1994.

20. **Dracup K, Walden JA, Stevenson LW, and Brecht ML**. Quality of life in patients with advanced heart failure. *The Journal of heart and lung transplantation : the*

official publication of the International Society for Heart Transplantation 11: 273-279, 1992.

21. **Drexler H**. Skeletal muscle failure in heart failure. *Circulation* 85: 1621-1623, 1992.

22. **Drexler H, and Coats AJ**. Explaining fatigue in congestive heart failure. *Annual review of medicine* 47: 241-256, 1996.

23. **Drexler H, Riede U, Münzel T, König H, Funke E, and Just H**. Alterations of skeletal muscle in chronic heart failure. *Circulation* 85: 1751-1759, 1992.

24. **Dubach P, Myers J, Dziekan G, Goebbels U, Reinhart W, Vogt P, Ratti R, Muller P, Miettunen R, and Buser P**. Effect of exercise training on myocardial remodeling in patients with reduced left ventricular function after myocardial infarction: application of magnetic resonance imaging. *Circulation* 95: 2060-2067, 1997.

25. **Dunderdale K, Thompson DR, Miles JN, Beer SF, and Furze G**. Quality-of-life measurement in chronic heart failure: do we take account of the patient perspective? *Eur J Heart Fail* 7: 572-582, 2005.

26. **Duscha BD, Kraus WE, Keteyian SJ, Sullivan MJ, Green HJ, Schachat FH, Pippen AM, Brawner CA, Blank JM, and Annex BH**. Capillary density of skeletal muscle: a contributing mechanism for exercise intolerance in class II-III chronic heart failure independent of other peripheral alterations. *Journal of the American College of Cardiology* 33: 1956-1963, 1999.

27. **Erickson ML, Ryan TE, Young HJ, and McCully KK**. Near-infrared assessments of skeletal muscle oxidative capacity in persons with spinal cord injury. *Eur J Appl Physiol* 113: 2275-2283, 2013.

28. **Esposito F, Mathieu-Costello O, Shabetai R, Wagner PD, and Richardson RS**. Limited Maximal Exercise Capacity in Patients With Chronic Heart Failure: Partitioning the Contributors. *Journal of the American College of Cardiology* 55: 1945-1954, 2010.

29. **Eurich DT, and McAlister FA**. Wrongfully accused: metformin use in heart failure. *Expert review of cardiovascular therapy* 9: 147-150, 2011.

30. **Ferreira LF, Townsend DK, Lutjemeier BJ, and Barstow TJ**. Muscle capillary blood flow kinetics estimated from pulmonary O2 uptake and near-infrared spectroscopy. *Journal of Applied Physiology* 98: 1820-1828, 2005.

31. **Franciosa JA, Park M, and Levine TB**. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 47: 33-39, 1981.

32. **Frishman WH**. Beta-Adrenergic Blockers. *Circulation* 107: e117-e119, 2003.

33. **Fulster S, Tacke M, Sandek A, Ebner N, Tschope C, Doehner W, Anker SD, and von Haehling S**. Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF). *Eur Heart J* 34: 512-519, 2013.

34. **Giannuzzi P, Temporelli PL, Corra U, Tavazzi L, and Group E-CS**. Antiremodeling effect of long-term exercise training in patients with stable chronic heart failure: results of the Exercise in Left Ventricular Dysfunction and Chronic Heart Failure (ELVD-CHF) Trial. *Circulation* 108: 554-559, 2003.

35. **Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA,**

Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB, American Heart Association Statistics C, and Stroke Statistics S. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation* 129: e28-e292, 2014.

36. **Gordon A, Tyni-Lenne R, Persson H, Kaijser L, Hultman E, and Sylven C**. Markedly improved skeletal muscle function with local muscle training in patients with chronic heart failure. *Clinical cardiology* 19: 568-574, 1996.

37. **Hall MJ, DeFrances CJ, Williams SN, Golosinskiy A, and Schwartzman A**. National Hospital Discharge Survey: 2007 summary. *National health statistics reports* 1-20, 24, 2010.

38. **Hamaoka T, Iwane H, Shimomitsu T, Katsumura T, Murase N, Nishio S, Osada T, Kurosawa Y, and Chance B**. Noninvasive measures of oxidative metabolism on working human muscles by near-infrared spectroscopy. *Journal of Applied Physiology* 81: 1410-1417, 1996.

39. **Hamaoka T, McCully KK, Quaresima V, Yamamoto K, and Chance B**. Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *Journal Of Biomedical Optics* 12: 062105-062105, 2007.

40. **Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, and Schuler G**. Regular physical exercise corrects endothelial

dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 98: 2709-2715, 1998.

41. **Hambrecht R, Fiehn E, Yu J, Niebauer J, Weigl C, Hilbrich L, Adams V, Riede U, and Schuler G**. Effects of endurance training on mitochondrial ultrastructure and fiber type distribution in skeletal muscle of patients with stable chronic heart failure. *Journal of the American College of Cardiology* 29: 1067-1073, 1997.

42. **Hambrecht R GSLA, and et al.** Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure: A randomized trial. *Jama* 283: 3095-3101, 2000.

43. **Hambrecht R, Niebauer J, Fiehn E, Kälberer B, Offner B, Hauer K, Riede U, Schlierf G, Kübler W, and Schuler G**. Physical training in patients with stable chronic heart failure: Effects on cardiorespiratory fitness and ultrastructural abnormalities of leg muscles. *Journal of the American College of Cardiology* 25: 1239-1249, 1995.

44. **Hanai J, Cao P, Tanksale P, Imamura S, Koshimizu E, Zhao J, Kishi S, Yamashita M, Phillips PS, Sukhatme VP, and Lecker SH**. The muscle-specific ubiquitin ligase atrogin-1/MAFbx mediates statin-induced muscle toxicity. *J Clin Invest* 117: 3940-3951, 2007.

45. **Harrington D, Anker SD, Chua TP, Webb-Peploe KM, Ponikowski PP, Poole-Wilson PA, and Coats AJ**. Skeletal muscle function and its relation to exercise tolerance in chronic heart failure. *Journal of the American College of Cardiology* 30: 1758-1764, 1997.

46. **Hein S, Kostin S, Heling A, Maeno Y, and Schaper J**. The role of the cytoskeleton in heart failure. *Cardiovascular Research* 45: 273-278, 2000.

47. **Hernandez AF, Hammill BG, O'Connor CM, Schulman KA, Curtis LH, and Fonarow GC**. Clinical effectiveness of beta-blockers in heart failure: findings from the OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure) Registry. *Journal of the American College of Cardiology* 53: 184-192, 2009.

48. **Hoekstra T, Lesman-Leegte I, van Veldhuisen DJ, Sanderman R, and Jaarsma T**. Quality of life is impaired similarly in heart failure patients with preserved and reduced ejection fraction. *Eur J Heart Fail* 13: 1013-1018, 2011.

49. **Hognestad A, Dickstein K, Myhre E, Snapinn S, Kjekshus J, and Investigators O**. Effect of combined statin and beta-blocker treatment on one-year morbidity and mortality after acute myocardial infarction associated with heart failure. *Am J Cardiol* 93: 603-606, 2004.

50. **Hornig B, Maier V, and Drexler H**. Physical Training Improves Endothelial Function in Patients With Chronic Heart Failure. *Circulation* 93: 210-214, 1996.

51. **Horwich TB, MacLellan WR, and Fonarow GC**. Statin therapy is associated with improved survival in ischemic and non-ischemic heart failure. *Journal of the American College of Cardiology* 43: 642-648, 2004.

52. **Houben AJ, Beljaars JH, Hofstra L, Kroon AA, and De Leeuw PW**. Microvascular abnormalities in chronic heart failure: a cross-sectional analysis. *Microcirculation* 10: 471-478, 2003.

53. **Huss JM, and Kelly DP**. Mitochondrial energy metabolism in heart failure: a question of balance. *J Clin Invest* 115: 547-555, 2005.

54. **Hwang R, and Marwick T**. Efficacy of home-based exercise programmes for people with chronic heart failure: a meta-analysis. *Eur J Cardiovasc Prev Rehabil* 16: 527-535, 2009.

55. **Istvan ES, and Deisenhofer J**. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 292: 1160-1164, 2001.

56. **Jenkinson C, Jenkinson D, Shepperd S, Layte R, and Petersen S**. Evaluation of treatment for congestive heart failure in patients aged 60 years and older using generic measures of health status (SF-36 and COOP charts). *Age and ageing* 26: 7-13, 1997.

57. **Jessup M, Abraham WT, Casey DE, Feldman AM, Francis GS, Ganiats TG, Konstam MA, Mancini DM, Rahko PS, Silver MA, Stevenson LW, and Yancy CW**. 2009 Focused Update: ACCF/AHA Guidelines for the Diagnosis and Management of Heart Failure in AdultsA Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *Journal of the American College of Cardiology* 53: 1343-1382, 2009.

58. **Juenger J, Schellberg D, Kraemer S, Haunstetter A, Zugck C, Herzog W, and Haass M**. Health related quality of life in patients with congestive heart failure: comparison with other chronic diseases and relation to functional variables. *Heart* 87: 235-241, 2002.

59. **Keteyian SJ**. Exercise training in congestive heart failure: risks and benefits. *Progress in cardiovascular diseases* 53: 419-428, 2011.

60. **Kirpichnikov D, McFarlane SI, and Sowers JR**. Metformin: an update. *Annals of internal medicine* 137: 25-33, 2002.

61. **Komajda M, Follath F, Swedberg K, Cleland J, Aguilar JC, Cohen-Solal A, Dietz R, Gavazzi A, Van Gilst WH, Hobbs R, Korewicki J, Madeira HC, Moiseyev VS, Preda I, Widimsky J, Freemantle N, Eastaugh J, Mason J, and Study Group on Diagnosis of the Working Group on Heart Failure of the European Society of C**. The EuroHeart Failure Survey programme--a survey on the quality of care among patients with heart failure in Europe. Part 2: treatment. *Eur Heart J* 24: 464-474, 2003.

62. **LeJemtel TH, Maskin CS, Lucido D, and Chadwick BJ**. Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. *Circulation* 74: 245-251, 1986.

63. **Linden MA, Fletcher JA, Morris EM, Meers GM, Kearney ML, Crissey JM, Laughlin MH, Booth FW, Sowers JR, Ibdah JA, Thyfault JP, and Rector RS**. Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. *Am J Physiol Endocrinol Metab* 306: E300-310, 2014.

64. **Longhurst J, Gifford W, and Zelis R**. Impaired forearm oxygen consumption during static exercise in patients with congestive heart failure. *Circulation* 54: 477-480, 1976.

65. **Magnusson G, Gordon A, Kaijser L, Sylven C, Isberg B, Karpakka J, and Saltin B**. High intensity knee extensor training, in patients with chronic heart failure. Major skeletal muscle improvement. *Eur Heart J* 17: 1048-1055, 1996.

66. **Malagoni AM, Felisatti M, Mandini S, Mascoli F, Manfredini R, Basaglia N, Zamboni P, and Manfredini F**. Resting muscle oxygen consumption by near-infrared spectroscopy in peripheral arterial disease: A parameter to be considered in a clinical setting? *Angiology* 61: 530-536, 2010.

67. **Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, and Task Force M**. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Journal of hypertension* 31: 1281-1357, 2013.

68. **Mancini DM, Coyle E, Coggan A, Beltz J, Ferraro N, Montain S, and Wilson JR**. Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. *Circulation* 80: 1338-1346, 1989.

69. **Mancini DM, Walter G, Reichek N, Lenkinski R, Mccully KK, Mullen JL, and Wilson JR**. Contribution of Skeletal-Muscle Atrophy to Exercise Intolerance and Altered Muscle Metabolism in Heart-Failure. *Circulation* 85: 1364-1373, 1992.

70. **Mancini DM, Wilson JR, Bolinger L, Li H, Kendrick K, Chance B, and Leigh JS**. In vivo magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failure. Demonstration of abnormal muscle metabolism despite adequate oxygenation. *Circulation* 90: 500-508, 1994.

71. **Massie B, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, and Rajagopalan B**. Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. *Circulation* 76: 1009-1019, 1987.

72. **Massie BM, Conway M, Rajagopalan B, Yonge R, Frostick S, Ledingham J, Sleight P, and Radda G**. Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. Evidence for abnormalities unrelated to blood flow. *Circulation* 78: 320-326, 1988.

73. **Maurer MS, and Schulze PC**. Exercise intolerance in heart failure with preserved ejection fraction: shifting focus from the heart to peripheral skeletal muscle. *Journal of the American College of Cardiology* 60: 129-131, 2012.

74. **Members ATF, McMurray JJV, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Køber L, Lip GYH, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Rønnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A, Guidelines ECfP, and Reviewers D**. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 33: 1787-1847, 2012.

75. **Members WG, Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, and Turner MB**. Heart

Disease and Stroke Statistics—2012 Update: A Report From the American Heart Association. *Circulation* 125: e2-e220, 2012.

76. **Mettauer B, Zoll J, Sanchez H, Lampert E, Ribera F, Veksler V, Bigard X, Mateo P, Epailly E, Lonsdorfer J, and Ventura-Clapier R**. Oxidative capacity of skeletal muscle in heart failure patients versus sedentary or active control subjects. *Journal of the American College of Cardiology* 38: 947-954, 2001.

77. **Middlekauff HR**. Making the Case for Skeletal Myopathy as the Major Limitation of Exercise Capacity in Heart Failure. *Circulation: Heart Failure* 3: 537-546, 2010.

78. **Mikus CR, Boyle LJ, Borengasser SJ, Oberlin DJ, Naples SP, Fletcher J, Meers GM, Ruebel M, Laughlin MH, Dellsperger KC, Fadel PJ, and Thyfault JP**. Simvastatin impairs exercise training adaptations. *Journal of the American College of Cardiology* 62: 709-714, 2013.

79. **Minotti JR, Johnson EC, Hudson TL, Zuroske G, Murata G, Fukushima E, Cagle TG, Chick TW, Massie BM, and Icenogle MV**. Skeletal muscle response to exercise training in congestive heart failure. *J Clin Invest* 86: 751-758, 1990.

80. **Miura S, Kawanaka K, Kai Y, Tamura M, Goto M, Shiuchi T, Minokoshi Y, and Ezaki O**. An increase in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) mRNA in response to exercise is mediated by beta-adrenergic receptor activation. *Endocrinology* 148: 3441-3448, 2007.

81. **Mosterd A, and Hoes AW**. Clinical epidemiology of heart failure. *Heart* 93: 1137-1146, 2007.

82. **Mozaffarian D, Nye R, and Levy WC**. Statin therapy is associated with lower mortality among patients with severe heart failure. *Am J Cardiol* 93: 1124-1129, 2004.

83. **Nilsson KR, Duscha BD, Hranitzky PM, and Kraus WE**. Chronic heart failure and exercise intolerance: the hemodynamic paradox. *Current cardiology reviews* 4: 92-100, 2008.

84. **Nioka S, Kime R, Sunar U, Im J, Izzetoglu M, Zhang J, Alacam B, and Chance B**. A novel method to measure regional muscle blood flow continuously using NIRS kinetics information. *Dynamic Medicine: DM* 5: 5-5, 2006.

85. **Nusz DJ, White DC, Dai Q, Pippen AM, Thompson MA, Walton GB, Parsa CJ, Koch WJ, and Annex BH**. Vascular rarefaction in peripheral skeletal muscle after experimental heart failure. *American journal of physiology Heart and circulatory physiology* 285: H1554-1562, 2003.

86. **O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F, Pina IL, and Investigators H-A**. Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial. *Jama* 301: 1439-1450, 2009.

87. **Owen MR, Doran E, and Halestrap AP**. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J* 348 Pt 3: 607-614, 2000.

88. **Paiva H, Thelen KM, Van Coster R, Smet J, De Paepe B, Mattila KM, Laakso J, Lehtimaki T, von Bergmann K, Lutjohann D, and Laaksonen R**. High-

dose statins and skeletal muscle metabolism in humans: a randomized, controlled trial. *Clinical pharmacology and therapeutics* 78: 60-68, 2005.

89. **Phillips PS, Haas RH, Bannykh S, Hathaway S, Gray NL, Kimura BJ, Vladutiu GD, England JD, and Scripps Mercy Clinical Research C**. Statin-associated myopathy with normal creatine kinase levels. *Annals of internal medicine* 137: 581-585, 2002.

90. **Piepoli MF, Davos C, Francis DP, Coats AJ, and ExTra MC**. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). *Bmj* 328: 189, 2004.

91. **Pu CT, Johnson MT, Forman DE, Hausdorff JM, Roubenoff R, Foldvari M, Fielding RA, and Singh MAF**. Randomized trial of progressive resistance training to counteract the myopathy of chronic heart failure. *Journal of Applied Physiology* 90: 2341-2350, 2001.

92. **Puente-Maestu L, Tena T, Trascasa C, Pérez-Parra J, Godoy R, García J, and Stringer W**. Training improves muscle oxidative capacity and oxygenation recovery kinetics in patients with chronic obstructive pulmonary disease. *European Journal of Applied Physiology* 88: 580-587, 2003.

93. **Raina A, Pickering T, and Shimbo D**. Statin use in heart failure: a cause for concern? *American heart journal* 152: 39-49, 2006.

94. **Ray JG, Gong Y, Sykora K, and Tu JV**. Statin use and survival outcomes in elderly patients with heart failure. *Archives of internal medicine* 165: 62-67, 2005.

95. **Redfield MM, Jacobsen SJ, Burnett JC, Jr., Mahoney DW, Bailey KR, and Rodeheffer RJ**. Burden of systolic and diastolic ventricular dysfunction in the

community: appreciating the scope of the heart failure epidemic. *Jama* 289: 194-202, 2003.

96. **Rees K, Taylor RS, Singh S, Coats AJ, and Ebrahim S**. Exercise based rehabilitation for heart failure. *Cochrane database of systematic reviews* CD003331, 2004.

97. **Robinson MM, Hamilton KL, and Miller BF**. The interactions of some commonly consumed drugs with mitochondrial adaptations to exercise. *Journal of applied physiology (Bethesda, Md : 1985)* 107: 8-16, 2009.

98. **Rosca M, and Hoppel C**. Mitochondrial dysfunction in heart failure. *Heart Fail Rev* 1-16, 2012.

99. **Ryan TE, Erickson ML, Brizendine JT, Young H-J, and McCully KK**. Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. *Journal of Applied Physiology* 113: 175-183, 2012.

100. **Ryan TE, Southern WM, Brizendine JT, and McCully KK**. Activity-induced changes in skeletal muscle metabolism measured with optical spectroscopy. *Med Sci Sports Exerc* 45: 2346-2352, 2013.

101. **Ryan TE, Southern WM, and McCully KK**. Near-infrared spectroscopy measured changes in skeletal muscle mitochondrial function in response to endurance exercise training and detraining. University of Georgia, 2013.

102. **Ryan TE, Southern WM, Reynolds MA, and McCully KK**. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with

phosphorus magnetic resonance spectroscopy. *Journal of applied physiology (Bethesda, Md : 1985)* 115: 1757-1766, 2013.

103. **Sako T, Hamaoka T, Higuchi H, Kurosawa Y, and Katsumura T**. Validity of NIR spectroscopy for quantitatively measuring muscle oxidative metabolic rate in exercise. *Journal of Applied Physiology* 90: 338-344, 2001.

104. **Schaufelberger M, Eriksson BO, Grimby G, Held P, and Swedberg K**. Skeletal muscle alterations in patients with chronic heart failure. *Eur Heart J* 18: 971-980, 1997.

105. **Sin DD, and McAlister FA**. The effects of beta-blockers on morbidity and mortality in a population-based cohort of 11,942 elderly patients with heart failure. *Am J Med* 113: 650-656, 2002.

106. **Sinzinger H, and O'Grady J**. Professional athletes suffering from familial hypercholesterolaemia rarely tolerate statin treatment because of muscular problems. *British journal of clinical pharmacology* 57: 525-528, 2004.

107. **Sola S, Mir MQ, Lerakis S, Tandon N, and Khan BV**. Atorvastatin improves left ventricular systolic function and serum markers of inflammation in nonischemic heart failure. *Journal of the American College of Cardiology* 47: 332-337, 2006.

108. **Sola S, Mir MQ, Rajagopalan S, Helmy T, Tandon N, and Khan BV**. Statin therapy is associated with improved cardiovascular outcomes and levels of inflammatory markers in patients with heart failure. *Journal of cardiac failure* 11: 607-612, 2005.

109. **Southern WM, Ryan TE, Reynolds MA, and McCully K**. Reproducibility of near infrared spectroscopy measurements of oxidative function and post-exercise

recovery kinetics in the medial gastrocnemius muscle. *Applied Physiology, Nutrition, and Metabolism* 2013.

110. **Sparrow J, Parameshwar J, and Poole-Wilson PA**. Assessment of functional capacity in chronic heart failure: time-limited exercise on a self-powered treadmill. *British heart journal* 71: 391-394, 1994.

111. **Stancu C, and Sima A**. Statins: mechanism of action and effects. *Journal of cellular and molecular medicine* 5: 378-387, 2001.

112. **Sullivan MJ, Green HJ, and Cobb FR**. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation* 81: 518-527, 1990.

113. **Sullivan MJ, Knight JD, Higginbotham MB, and Cobb FR**. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation* 80: 769-781, 1989.

114. **Taubert KA**. Cardiology patient pages. Can patients with cardiovascular disease take nonsteroidal antiinflammatory drugs? *Circulation* 117: e322-324, 2008.

115. **Toth MJ, Miller MS, Ward KA, and Ades PA**. Skeletal muscle mitochondrial density, gene expression, and enzyme activities in human heart failure: minimal effects of the disease and resistance training. *Journal of Applied Physiology* 112: 1864-1874, 2012.

116. **Tousoulis D, Antoniades C, Bosinakou E, Kotsopoulou M, Pitsavos C, Vlachopoulos C, Panagiotakos D, and Stefanadis C**. Effects of atorvastatin on reactive hyperemia and inflammatory process in patients with congestive heart failure. *Atherosclerosis* 178: 359-363, 2005.

117. **Van Beekvelt MCP, Colier WNJM, Wevers RA, and Van Engelen BGM**. Performance of near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal muscle. *Journal of Applied Physiology* 90: 511-519, 2001.

118. **van Bilsen M, Smeets PJH, Gilde AJ, and van der Vusse GJ**. Metabolic remodelling of the failing heart: the cardiac burn-out syndrome? *Cardiovascular Research* 61: 218-226, 2004.

119. **Weber KT, Kinasewitz GT, Janicki JS, and Fishman AP**. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 65: 1213-1223, 1982.

120. **Welsch MA, Alomari M, Parish TR, Wood RH, and Kalb D**. Influence of venous function on exercise tolerance in chronic heart failure. *Journal of cardiopulmonary rehabilitation* 22: 321-326, 2002.

121. **Wilson JR, Mancini DM, and Dunkman WB**. Exertional fatigue due to skeletal muscle dysfunction in patients with heart failure. *Circulation* 87: 470-475, 1993.

122. **Wilson JR, Martin JL, Schwartz D, and Ferraro N**. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation* 69: 1079-1087, 1984.

123. **Wilson JR, Rayos G, Yeoh TK, Gothard P, and Bak K**. Dissociation Between Exertional Symptoms and Circulatory Function in Patients With Heart Failure. *Circulation* 92: 47-53, 1995.

124. **Wolf M, Ferrari M, and Quaresima V**. Progress of near-infrared spectroscopy and topography for brain and muscle clinical applications. *Journal Of Biomedical Optics* 12: 062104, 2007.

125. **Wolfel EE, Hiatt WR, Brammell HL, Carry MR, Ringel SP, Travis V, and Horwitz LD**. Effects of selective and nonselective beta-adrenergic blockade on mechanisms of exercise conditioning. *Circulation* 74: 664-674, 1986.

126. **Zelis R, Longhurst J, Capone RJ, and Mason DT**. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 50: 137-143, 1974.

127. **Zhou Q, and Liao JK**. Pleiotropic effects of statins. - Basic research and clinical perspectives. *Circulation journal : official journal of the Japanese Circulation Society* 74: 818-826, 2010.

128. **Zuckerman IH, Yin X, Rattinger GB, Gottlieb SS, Simoni-Wastila L, Pierce SA, Huang TY, Shenolikar R, and Stuart B**. Effect of exposure to evidence-based pharmacotherapy on outcomes after acute myocardial infarction in older adults. *J Am Geriatr Soc* 60: 1854-1861, 2012.

APPENDIX A

ADDITIONAL FIGURES

This section presents additional figures that were nonessential in the final form of Chapter 3.

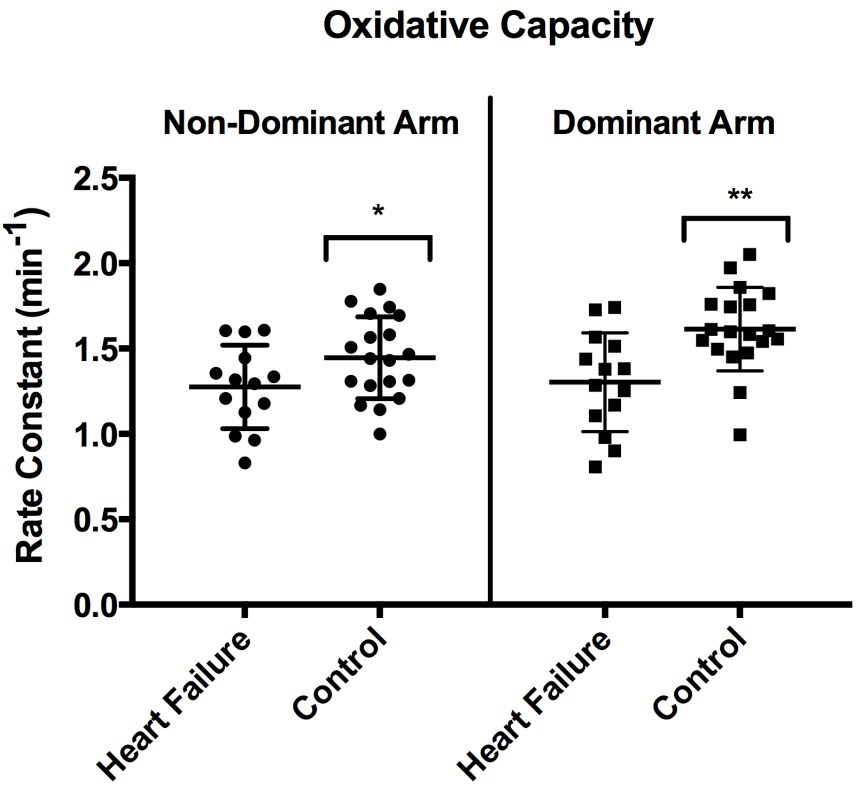


Figure A.1 Baseline oxidative capacity of dominant (squares) and non-dominant (circles) arms for both heart failure (HF) and control groups. Data are expressed as rate constants of mVO_2 recovery and presented as mean ± SD. ** $p = 0.002$, * $p = 0.04$ for difference between HF and control.

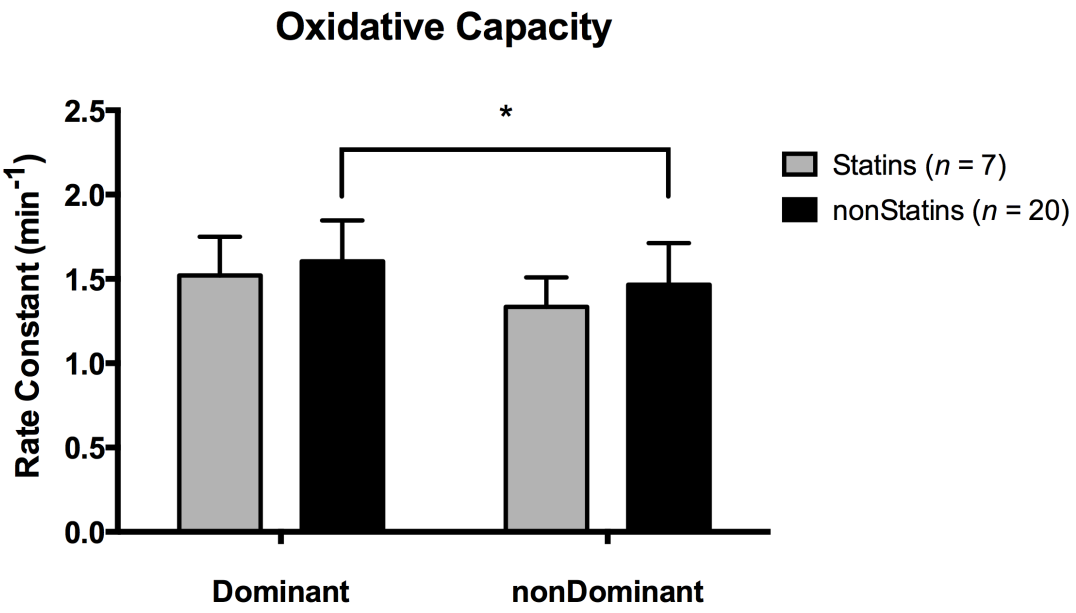


Figure A.2 Oxidative capacity between the DOM and nDOM arms of control participants on and not on statin therapy. Participants on statin therapy were excluded from the final analysis. * $p = 0.03$ for difference between nonStatin DOM and nonStatin nDOM arms.

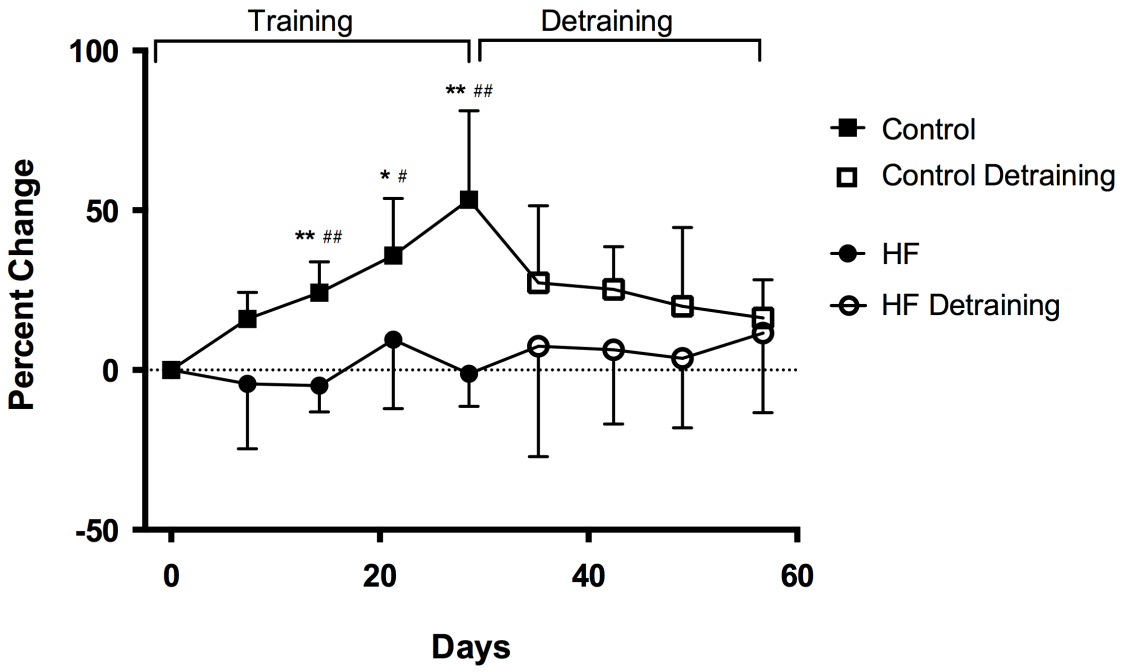


Figure A.3 Percent change in oxidative capacity for heart failure (circles) and controls (squares) during four weeks of wrist-flexor exercise training (solid markers) and detraining (open markers). Data presented as mean ± SD. ** $p < 0.002$, * $p < 0.05$ for difference from baseline. ## $p < 0.002$, # $p < 0.05$ for difference between HF and control.

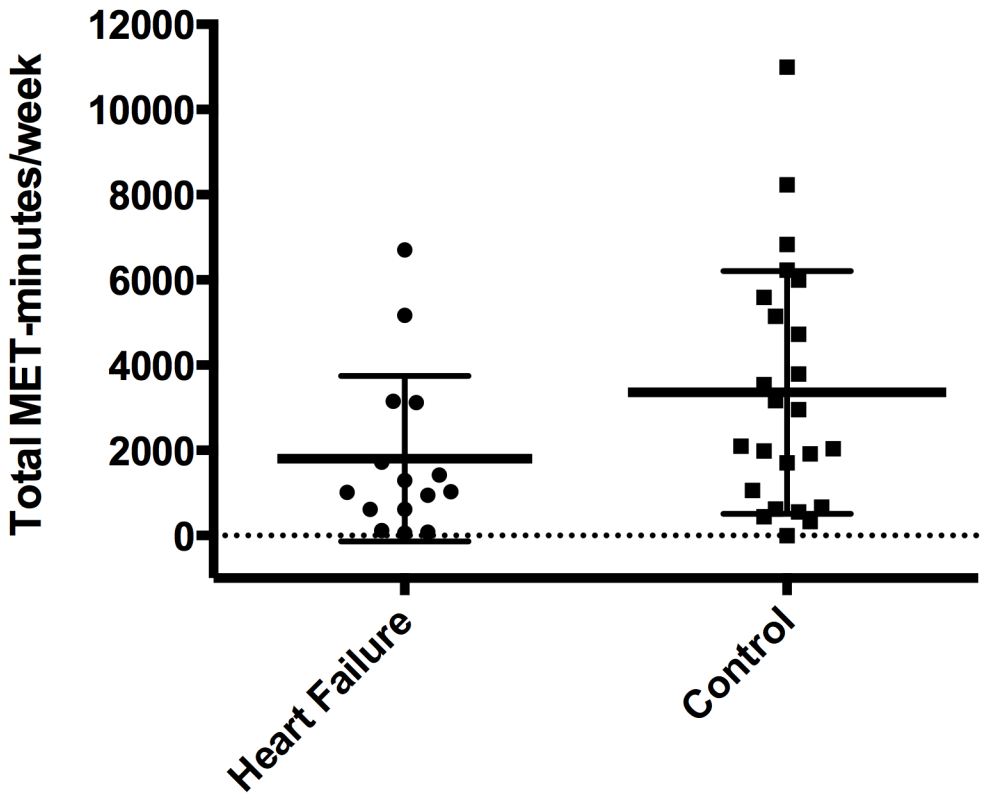


Figure A.4 Individual self-reported physical activity for all participants in both heart failure and control groups. Data expressed as total MET-minutes per week. Data are presented with mean ± SD.

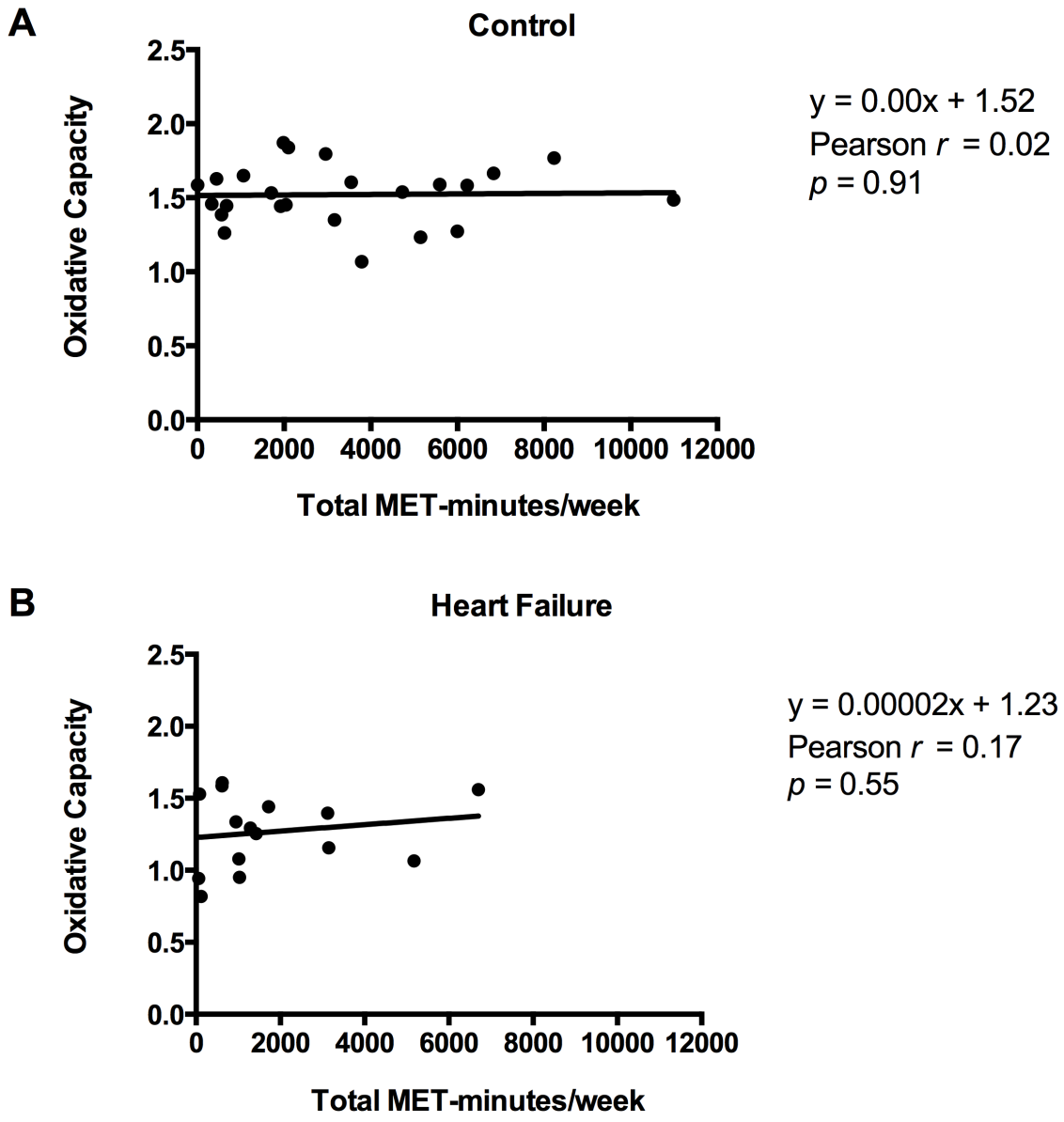


Figure A.5 Relationship between self-reported physical activity and oxidative capacity for heart failure and control groups.